Review of Literature
2.1 INFLAMMATION

2.1.1 Definition and causes

Inflammation is defined as the local response of living mammalian tissues to injury due to any agent. It is a body defense reaction in order to eliminate or limit the spread of injurious agent, followed by removal of the necrosed cells and tissues.

The agents causing inflammation may be as under:

1. Infective agents like bacteria, viruses and their toxins, fungi, parasites.
2. Immunological agents like cell-mediated and antigen antibody reactions.
3. Physical agents like heat, cold, radiation, mechanical trauma.
4. Chemical agents like organic and inorganic poisons.
5. Inert materials such as foreign bodies.

Thus, inflammation is distinct from infection—while inflammation is a protective response by the body to variety of etiologic agents (infectious or non-infectious), while infection is invasion into the body by harmful microbes and their resultant ill-effects by toxins. Inflammation involves 2 basic processes with some overlapping, viz. early inflammatory response and later followed by healing. Though both these processes generally have protective role against injurious agents, inflammation and healing may cause considerable harm to the body as well e.g. anaphylaxis to bites by insects or reptiles, drugs, toxins, atherosclerosis, chronic rheumatoid arthritis, fibrous bands and adhesions in intestinal obstruction (Harshmohan, 2010).

2.1.2 Signs of inflammation

The Roman writer Celsus in named the famous 4 cardinal signs of inflammation as:

- Rubor (redness);
- Tumor (swelling);
- Calor (heat); and
- Dolor (pain).

To these, fifth sign functio laesa (loss of function) was later added by Virchow. The word inflammation means burning. This nomenclature had its origin in old times but now we know that burning is only one of the signs of inflammation.
2.1.3 Types of inflammation (Harshmohan, 2010)

Depending upon the defense capacity of the host and duration of response, inflammation can be classified as acute and chronic.

A. Acute inflammation

Is of short duration (lasting less than 2 weeks) and represents the early body reaction, resolves quickly and is usually followed by healing.

The main features of acute inflammation are:
1. Accumulation of fluid and plasma at the affected site;
2. Intravascular activation of platelets; and
3. Polymorphonuclear neutrophils as inflammatory cells.

Sometimes, the acute inflammatory response may be quite severe and is termed as fulminant acute inflammation.

Table 1: Mediators of acute inflammation (Harshmohan, 2010)

<table>
<thead>
<tr>
<th>Mediators</th>
<th>Vasodilation</th>
<th>Vascular permeability</th>
<th>Chemotaxis</th>
<th>Pain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histamine</td>
<td>++</td>
<td>+++</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Serotonin</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Bradykinin</td>
<td>+++</td>
<td>+</td>
<td>-</td>
<td>+++</td>
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<tr>
<td>Prostaglandin</td>
<td>+++</td>
<td>+</td>
<td>+++</td>
<td>+</td>
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<tr>
<td>Leukotrienes</td>
<td>-</td>
<td>+++</td>
<td>+++</td>
<td>-</td>
</tr>
</tbody>
</table>

(+++ severe, ++ moderate, + mild, - absent)

B. Chronic inflammation

Is of longer duration and occurs either after the causative agent of acute inflammation persists for a long time, or the stimulus is such that it induces chronic inflammation from the beginning. A variant, chronic active inflammation is the type of chronic inflammation in which during the course of disease there are acute exacerbations of activity.

The characteristic feature of chronic inflammation is presence of chronic inflammatory cells such as lymphocytes, plasma cells and macrophages, granulation tissue formation, and in specific situations as granulomatous inflammation. In some instances, the term subacute inflammation is used for the state of inflammation between acute and chronic.
<table>
<thead>
<tr>
<th>Mediators</th>
<th>Sources</th>
<th>Primary effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1, IL-2, IL-3</td>
<td>Macrophages, T-lymphocytes</td>
<td>Lymphocyte activation, prostaglandin production</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>T-lymphocytes, endothelial cells, fibroblast</td>
<td>Macrophages and granulocyte activation</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Macrophages</td>
<td>Prostaglandin</td>
</tr>
<tr>
<td>Interferons</td>
<td>Macrophages, endothelial cells, T-lymphocytes</td>
<td>Many</td>
</tr>
<tr>
<td>PDGF</td>
<td>Macrophages, endothelial cells, fibroblast, platelets</td>
<td>Fibroblast chemotaxis, proliferation</td>
</tr>
</tbody>
</table>

**Table 3: Comparison between acute and chronic inflammation** (Harshmohan, 2010)

<table>
<thead>
<tr>
<th>Acute inflammation</th>
<th>Chronic inflammation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Causative agent</strong></td>
<td>Pathogens, injured tissues</td>
</tr>
<tr>
<td><strong>Major cells involved</strong></td>
<td>Neutrophils, mononuclear cells (monocytes, macrophages)</td>
</tr>
<tr>
<td><strong>Primary mediators</strong></td>
<td>Vasoactive amines, eicosanoids</td>
</tr>
<tr>
<td><strong>Onset</strong></td>
<td>Immediate</td>
</tr>
<tr>
<td><strong>Duration</strong></td>
<td>Few days</td>
</tr>
<tr>
<td><strong>Outcomes</strong></td>
<td>Healing, abscess formation.</td>
</tr>
</tbody>
</table>
A. Acute inflammation

Acute inflammatory response by the host to any agent is a continuous process but for the purpose of discussion, it can be divided into following two events:

I. Vascular events.

II. Cellular events.

1. Vascular events

Alteration in the microvasculature (arterioles, capillaries and venules) is the earliest response to tissue injury. These alterations include

1. Haemodynamic changes

2. Changes in vascular permeability

1. Haemodynamic changes

The earliest features of inflammatory response result from changes in the vascular flow and calibre of small blood vessels in the injured tissue. The sequence of these changes is as under:

1. Irrespective of the type of injury, immediate vascular response is of transient vasoconstriction of arterioles. With mild form of injury, the blood flow may
be re-established in 3-5 seconds while with more severe injury the vasoconstriction may last for about 5 minutes.

2. Next follows persistent progressive vasodilatation which involves mainly the arterioles, but to a lesser extent, affects other components of the microcirculation like venules and capillaries. This change is obvious within half an hour of injury. Vasodilatation results in increased blood volume in microvascular bed of the area, which is responsible for redness and warmth at the site of acute inflammation.

3. Progressive vasodilatation, in turn, may elevate the local hydrostatic pressure resulting in transudation of fluid into the extracellular space. This is responsible for swelling at the local site of acute inflammation.

4. Slowing or stasis of microcirculation follows which causes increased concentration of red cells, and thus, raised blood viscosity.

5. Stasis or slowing is followed by leucocytic margination or peripheral orientation of leucocytes (mainly neutrophils) along the vascular endothelium. The leucocytes stick to the vascular endothelium briefly, and then move and migrate through the gaps between the endothelial cells into the extravascular space. This process is known as emigration.

The features of haemodynamic changes in inflammation are best demonstrated by the Lewis experiment. Lewis induced the changes in the skin of inner aspect of forearm by firm stroking with a blunt point. The reaction so elicited is known as triple response or red line response consisting of the following.

i) Red line appears within a few seconds following stroking and is due to local vasodilatation of capillaries and venules.

ii) Flare is the bright reddish appearance or flush surrounding the red line and results from vasodilatation of the adjacent arterioles.

iii) Wheal is the swelling or oedema of the surrounding skin occurring due to transudation of fluid into the extravascular space.
These features thus elicit the classical signs of inflammation—redness, heat, swelling and pain.

**Figure 2:** ‘Triple response’ elicited by firm stroking of skin of forearm with a pencil (A). Diagrammatic view of microscopic features of triple response of the skin (B) (Harshmohan, 2010)

2. **Changes in vascular permeability**

**Pathogenesis**

In and around the inflamed tissue, there is accumulation of oedema fluid in the interstitial compartment which comes from blood plasma by its escape through the endothelial wall of peripheral vascular bed. In the initial stage, the escape of fluid is due to vasodilatation and consequent elevation in hydrostatic pressure. This is transudate in nature. But subsequently, the characteristic inflammatory oedema, exudate, appears by increased vascular permeability of microcirculation.

The appearance of inflammatory oedema due to increased vascular permeability of microvascular bed is explained on the basis of Starling’s hypothesis. In normal circumstances, the fluid balance is maintained by two opposing sets of forces:

   i) Forces that cause outward movement of fluid from microcirculation are intravascular hydrostatic pressure and colloid osmotic pressure of interstitial fluid.
ii) Forces that cause inward movement of interstitial fluid into circulation are intravascular colloid osmotic pressure and hydrostatic pressure of interstitial fluid.

*Mechanisms of increased vascular permeability*

In acute inflammation, normally non-permeable endothelial layer of microvasculature becomes leaky.

1) *Contraction of endothelial cells*

This is the most common mechanism of increased leakiness that affects venules exclusively while capillaries and arterioles remain unaffected. The endothelial cells develop temporary gaps between them due to their contraction resulting in vascular leakiness. It is mediated by the release of histamine, bradykinin and other chemical mediators. The response begins immediately after injury, is usually reversible, and is for short duration (15-30 minutes). Example of such immediate transient leakage is mild thermal injury of skin of forearm.

2) *Retraction of endothelial cells*

In this mechanism, there is structural re-organisation of the cytoskeleton of endothelial cells that causes reversible retraction at the intercellular junctions. This change too affects venules and is mediated by cytokines such as interleukin-1 (IL-1) and tumour necrosis factor (TNF)-α. The onset of response takes 4-6 hours after injury and lasts for 2-4 hours or more (somewhat delayed and prolonged leakage). The example of this type of response exists in vitro experimental work only.

3) *Direct injury to endothelial cells*

Direct injury to the endothelium causes cell necrosis and appearance of physical gaps at the sites of detached endothelial cells. Process of thrombosis is initiated at the site of damaged endothelial cells. The change affects all levels of microvasculature (venules, capillaries and arterioles). The increased permeability may either appear immediately after injury or last for several hours or days (immediate sustained leakage), or may occur after a delay of 2-12 hours and last for hours or days (delayed prolonged leakage). The examples of immediate sustained leakage are severe bacterial
infections while delayed prolonged leakage may occur following moderate thermal injury and radiation injury.

4) **Endothelial injury mediated by leucocytes**

Adherence of leucocytes to the endothelium at the site of inflammation may result in activation of leucocytes. The activated leucocytes release proteolytic enzymes and toxic oxygen species which may cause endothelial injury and increased vascular leakiness. This form of increased vascular leakiness affects mostly venules and is a late response. The examples are seen in sites where leucocytes adhere to the vascular endothelium e.g. in pulmonary venules and capillaries.

5) **Leakiness in neovascularisation**

In addition, the newly formed capillaries under the influence of vascular endothelial growth factor (VEGF) during the process of repair and in tumours are excessively leaky.

**Figure 3: The major local manifestations of acute inflammation, compared to normal.** (1) Vascular dilation and increased blood flow (causing erythema and warmth), (2) Extravasation and deposition of plasma fluid and proteins (edema), and (3) Leukocyte emigration and accumulation in the site of injury (Vinay et al., 2007)
II. Cellular events

The cellular phase of inflammation consists of 2 processes:

1. Exudation of leucocytes
2. Phagocytosis

1. Exudation of Leucocytes

The escape of leucocytes from the lumen of microvasculature to the interstitial tissue is the most important feature of inflammatory response. In acute inflammation, polymorphonuclear neutrophils (PMNs) comprise the first line of body defense, followed later by monocytes and macrophages.

1. Changes in the formed elements of blood

In the early stage of inflammation, the rate of flow of blood is increased due to vasodilatation. But subsequently, there is slowing or stasis of bloodstream. With stasis, changes in the normal axial flow of blood in the microcirculation take place. The normal axial flow consists of central stream of cells comprised by leucocytes and RBCs and peripheral cellfree layer of plasma close to vessel wall. Due to slowing and stasis, the central stream of cells widens and peripheral plasma zone becomes narrower because of loss of plasma by exudation. This phenomenon is known as margination. As a result of this redistribution, the neutrophils of the central column come close to the vessel wall; this is known as pavementing.

2. Rolling and adhesion

Peripherally marginated and pavemented neutrophils slowly roll over the endothelial cells lining the vessel wall (rolling phase). This is followed by the transient bond between the leucocytes and endothelial cells becoming firmer (adhesion phase).

3. Emigration

After sticking of neutrophils to endothelium, the former move along the endothelial surface till a suitable site between the endothelial cells is found where the neutrophils throw out cytoplasmic pseudopods. Subsequently, the neutrophils lodged between the endothelial cells and basement membrane cross the basement membrane by damaging it locally with secreted collagenases and escape out into the extravascular space; this is known as emigration. The damaged basement membrane is repaired almost
immediately. As already mentioned, neutrophils are the dominant cells in acute inflammatory exudate in the first 24 hours, and monocyte-macrophages appear in the next 24-48 hours. However, neutrophils are short-lived (24-48 hours) while monocyte-macrophages survive much longer. Simultaneous to emigration of leucocytes, escape of red cells through gaps between the endothelial cells, diapedesis, takes place. It is a passive phenomenon—RBCs being forced out either by raised hydrostatic pressure or may escape through the endothelial defects left after emigration of leucocytes. Diapedesis gives haemorrhagic appearance to the inflammatory exudate.

4. Chemotaxis

The chemotactic factor-mediated transmigration of leucocytes after crossing several barriers (endothelium, basement membrane, perivascular myofibroblasts and matrix) to reach the interstitial tissues is called chemotaxis. The concept of chemotaxis is well illustrated by Boyden’s chamber experiment. In this, a millipore filter (3 µm pore size) separates the suspension of leucocytes from the test solution in tissue culture chamber. If the test solution contains chemotactic agent, the leucocytes migrate through the pores of filter towards the chemotactic agent.

2. Phagocytosis

Phagocytosis is defined as the process of engulfment of solid particulate material by the cells (cell-eating). The cells performing this function are called phagocytes. There are 2 main types of phagocytic cells:

i) Polymorphonuclear neutrophils (PMNs) which appear early in acute inflammatory response, sometimes called as microphages.

ii) Circulating monocytes and fixed tissue mononuclear phagocytes, commonly called as macrophages.

Neutrophils and macrophages on reaching the tissue spaces produce several proteolytic enzymes—lysozyme, protease, collagenase, elastase, lipase, proteinase, gelatinase, and acid hydrolases. These enzymes degrade collagen and extracellular matrix. The microbe undergoes the process of phagocytosis by polymorphs and macrophages and involves the following 3 steps,

1. Recognition and attachment
2. Engulfment
3. Killing and degradation

Outcomes of acute inflammation

The acute inflammatory process can culminate in one of the following outcomes,

1. Resolution: It means complete return to normal tissue following acute inflammation. This occurs when tissue changes are slight and the cellular changes are reversible e.g. resolution in lobar pneumonia.

2. Healing: Healing by fibrosis takes place when the tissue destruction in acute inflammation is extensive so that there is no tissue regeneration. But when tissue loss is superficial, it is restored by regeneration.

3. Suppuration: When the pyogenic bacteria causing acute inflammation result in severe tissue necrosis, the process progresses to suppuration. Initially, there is intense neutrophilic infiltration. Subsequently, mixture of neutrophils, bacteria, fragments of necrotic tissue, cell debris and fibrin comprise pus which is contained in a cavity to form an abscess. The abscess, if not drained, may get organised by dense fibrous tissue, and in time, get calcified.

4. Chronic inflammation: Persisting or recurrent acute inflammation may progress to chronic inflammation in which the processes of inflammation and healing proceed side by side (Harshmohan, 2010).

Figure 4: Outcomes of acute inflammation: resolution, healing by fibrosis, or chronic inflammation (Vinay et al., 2007)
B. Chronic inflammation

Definition and causes

Chronic inflammation is defined as prolonged process in which tissue destruction and inflammation occur at the same time.

Chronic inflammation can be caused by one of the following 3 ways:

1. **Chronic inflammation following acute inflammation:**

When the tissue destruction is extensive, or the bacteria survive and persist in small numbers at the site of acute inflammation e.g. in osteomyelitis, pneumonia terminating in lung abscess.

2. **Recurrent attacks of acute inflammation:**

When repeated bouts of acute inflammation culminate in chronicity of the process e.g. in recurrent urinary tract infection leading to chronic pyelonephritis, repeated acute infection of gallbladder leading to chronic cholecystitis.

3. **Chronic inflammation starting de novo:**

When the infection with organisms of low pathogenicity is chronic from the beginning e.g. infection with Mycobacterium tuberculosis.

General features of chronic inflammation

Though there may be differences in chronic inflammatory response depending upon the tissue involved and causative organisms, there are some basic similarities amongst various types of chronic inflammation. Following general features characterise any chronic inflammation:

1. **Mononuclear cell infiltration:**

Chronic inflammatory lesions are infiltrated by mononuclear inflammatory cells like phagocytes and lymphoid cells. Phagocytes are represented by circulating monocytes, tissue macrophages, epithelioid cells and sometimes, multinucleated giant cells. The macrophages comprise the most important cells in chronic inflammation. These may appear at the site of chronic inflammation from:

   i) Chemotactic factors and adhesion molecules for continued infiltration of macrophages;
ii) Local proliferation of macrophages; and  
iii) Longer survival of macrophages at the site of inflammation.

The blood monocytes on reaching the extravascular space transform into tissue macrophages. Besides the role of macrophages in phagocytosis, they may get activated in response to stimuli such as cytokines (lymphokines) and bacterial endotoxins. On activation, macrophages release several biologically active substances e.g. acid and neutral proteases, oxygen-derived reactive metabolites and cytokines. These products bring about tissue destruction, neovascularisation and fibrosis.

Other chronic inflammatory cells include lymphocytes, plasma cells, eosinophils and mast cells. In chronic inflammation, lymphocytes and macrophages influence each other and release mediators of inflammation.

2. **Tissue destruction or necrosis:**

Tissue destruction and necrosis are central features of most forms of chronic inflammatory lesions. This is brought about by activated macrophages which release a variety of biologically active substances e.g. protease, elastase, collagenase, lipase, reactive oxygen radicals, cytokines (IL-1, IL-8, TNF-α), nitric oxide, angiogenesis growth factor etc.

3. **Proliferative changes:**

As a result of necrosis, proliferation of small blood vessels and fibroblasts is stimulated resulting in formation of inflammatory granulation tissue. Eventually, healing by fibrosis and collagen laying takes place.

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**Systemic effects of chronic inflammation**

Chronic inflammation is associated with following systemic features:

1. **Fever:** Invariably there is mild fever, often with loss of weight and weakness.
2. **Anaemia:** Chronic inflammation is accompanied by anaemia of varying degree.
3. **Leucocytosis:** As in acute inflammation, chronic inflammation also has leucocytosis but generally there is relative lymphocytosis in these cases.
4. **ESR:** ESR is elevated in all cases of chronic inflammation.
5. **Amyloidosis:** Long-term cases of chronic suppurative inflammation may develop secondary systemic amyloidosis.
**Types of chronic inflammation**

Conventionally, chronic inflammation is subdivided into 2 types:

1. **Non-specific:**
   When the irritant substance produces a nonspecific chronic inflammatory reaction with formation of granulation tissue and healing by fibrosis e.g. chronic osteomyelitis, chronic ulcer.

2. **Specific:**
   When the injurious agent causes a characteristic histologic tissue response e.g. tuberculosis, leprosy, syphilis.

However, for a more descriptive classification, histological features are used for classifying chronic inflammation into 2 corresponding types:

1. **Chronic non-specific inflammation:**
   It is characterised by non-specific inflammatory cell infiltration e.g. chronic osteomyelitis, lung abscess. A variant of this type of chronic inflammatory response is chronic suppurative inflammation in which infiltration by polymorphs and abscess formation are additional features e.g. actinomycosis.

2. **Chronic granulomatous inflammation:**
   It is characterised by formation of granulomas e.g. tuberculosis, leprosy, syphilis, actinomycosis, sarcoidosis etc.

**2.1.4 Chemical mediators of inflammation** (Harshmohan, 2010)

1. **Vasoactive amines**
   Two important pharmacologically active amines that have role in the early inflammatory response (first one hour) are histamine and 5- hydroxytryptamine (5-HT) or serotonin; another recently added group is of neuropeptides.

   i) **Histamine**
   It is stored in the granules of mast cells, basophils and platelets. Histamine is released from these cells by various agents as under:
   a) Stimuli or substances inducing acute inflammation e.g. heat, cold, irradiation, trauma, irritant chemicals, immunologic reactions etc.
b) Histamine-releasing factors from neutrophils, monocytes and platelets.
c) Interleukins.

The main actions of histamine are: vasodilatation, increased vascular (venular) permeability, itching and pain. Stimulation of mast cells and basophils also releases products of arachidonic acid metabolism including the release of slowreacting substances of anaphylaxis (SRS-As). The SRS-As consist of various leukotrienes (LTC4, LTD4 and LTE4).

ii) 5-Hydroxytryptamine (5-HT or serotonin)
It is present in tissues like chromaffin cells of GIT, spleen, nervous tissue, mast cells and platelets. The actions of 5-HT are similar to histamine but it is a less potent mediator of increased vascular permeability and vasodilatation than histamine.

iii) Neuropeptides
Another class of vasoactive amines is tachykinin neuropeptides, such as substance P, neurokinin A, vasoactive intestinal polypeptide (VIP) and somatostatin. These small peptides are produced in the central and peripheral nervous systems. The major proinflammatory actions of these neuropeptides is as follows:
a) Increased vascular permeability.
b) Transmission of pain stimuli.
c) Mast cell degranulation.

2. Arachidonic acid metabolites (eicosanoids)
Arachidonic acid metabolites or eicosanoids are the most potent mediators of inflammation, much more than oxygen free radicals. Arachidonic acid is a fatty acid, eicosatetraenoic acid; Greek word ‘eikosa’ means ‘twenty’ because of 20 carbon atom composition of this fatty acid. Arachidonic acid is a constituent of the phospholipid cell membrane, besides its presence in some constituents of diet. Arachidonic acid is released from the cell membrane by phospholipases. It is then activated to form arachidonic acid metabolites or eicosanoids by one of the following 2 pathways: via cyclo-oxygenase pathway and via lipo-oxygenase pathway:
i) *Metabolites via cyclo-oxygenase pathway: Prostaglandins, thromboxane A2, prostacyclin.*

The name ‘prostaglandin’ was first given to a substance found in human seminal fluid but now the same substance has been isolated from a number of other body cells. Prostaglandins and related compounds are also called autocoids because these substances are mainly auto- and paracrine agents. The terminology used for prostaglandins is abbreviation as PG followed by suffix of an alphabet and a serial number e.g. PGG2, PGE2 etc. Cyclo-oxygenase (COX), a fatty acid enzyme present as COX-1 and COX-2, acts on activated arachidonic acid to form prostaglandin endoperoxide (PGG2). PGG2 is enzymatically transformed into PGH2 with generation of free radical of oxygen. PGH2 is further acted upon by enzymes and results in formation of the following 3 metabolites,

a) Prostaglandins (PGD2, PGE2 and PGF2-α). PGD2 and PGE2 act on blood vessels to cause increased venular permeability, vasodilatation and bronchodilatation and inhibit inflammatory cell function. PGF2-α induces vasodilatation and bronchoconstriction.

b) Thromboxane A2 (TXA2). Platelets contain the enzyme thromboxane synthetase and hence the metabolite, thromboxane A2, formed is active in platelet aggregation, besides its role as a vasoconstrictor and broncho-constrictor.

c) Prostacyclin (PGI2). PGI2 induces vasodilatation, bronchodilatation and inhibits platelet aggregation.

d) Resolvins are a newly described derivative of COX pathway. These mediators act by inhibiting production of pro-inflammatory cytokines. Thus, resolvins are actually helpful—drugs such as aspirin act by inhibiting COX activity and stimulating production of resolvins.

It may be mentioned here that some of the major antiinflammatory drugs act by inhibiting activity of the enzyme COX; e.g. non-steroidal anti-inflammatory drugs (NSAIDs), COX-2 inhibitors.

ii) *Metabolites via lipo-oxygenase pathway: 5-HETE, leukotrienes, lipoxins.*

The enzyme, lipo-oxygenase, a predominant enzyme in neutrophils, acts on activated arachidonic acid to form hydroperoxy eicosatetraenoic acid (5-HPETE) which on further peroxidation forms following 2 metabolites,
a) 5-HETE (hydroxy compound), an intermediate product, is a potent chemotactic agent for neutrophils.

b) Leukotrienes (LT) are so named as they were first isolated from leucocytes. Firstly, unstable leukotriene A4 (LTA4) is formed which is acted upon by enzymes to form LTB4 (chemotactic for phagocytic cells and stimulates phagocytic cell adherence) while LTC4, LTD4 and LTE4 have common actions by causing smooth muscle contraction and thereby induce vasoconstriction, bronchoconstriction and increased vascular permeability; hence they are also called as slowreacting substances of anaphylaxis (SRS-As).

c) Lipoxins (LX) are a recently described product of lipooxygenase pathway. Lipooxygenase-12 present in platelets acts on LTA4 derived from neutrophils and forms LXA4 and LXB4. Lipoxins act to regulate and counterbalance actions of leukotrienes.

3. Lysosomal components

The inflammatory cells—neutrophils and monocytes, contain lysosomal granules which on release elaborate a variety of mediators of inflammation. These are as under:

i) Granules of neutrophils. Neutrophils have 3 types of granules: primary or azurophil, secondary or specific, and tertiary.

a) Primary or azurophil granules are large azurophil granules which contain functionally active enzymes. These are myeloperoxidase, acid hydrolases, acid phosphatase, lysozyme, defensin (cationic protein), phospholipase, cathepsin G, elastase, and protease.

b) Secondary or specific granules contain alkaline phosphatase, lactoferrin, gelatinase, collagenase, lysozyme, vitamin-B12 binding proteins, plasminogen activator.

c) Tertiary granules or C particles contain gelatinase and acid hydrolases.

Myeloperoxidase causes oxidative lysis by generation of oxygen free radicals, acid hydrolases act within the cell to cause destruction of bacteria in phagolysosome while proteases attack on the extracellular constituents such as basement membrane, collagen, elastin, cartilage etc.

However, degradation of extracellular components like collagen, basement membrane, fibrin and cartilage by proteases results in harmful tissue destruction.
which is kept in check by presence of antiproteases like α1-antitrypsin and α2-macroglobulin.

ii) Granules of monocytes and tissue macrophages. These cells on degranulation also release mediators of inflammation like acid proteases, collagenase, elastase and plasminogen activator. However, they are more active in chronic inflammation than acting as mediators of acute inflammation.

4. Platelet activating factor (PAF)
It is released from IgE-sensitised basophils or mast cells, other leucocytes, endothelium and platelets. Apart from its action on platelet aggregation and release reaction, the actions of PAF as mediator of inflammation are:

✓ increased vascular permeability;
✓ vasodilatation in low concentration and vasoconstriction otherwise;
✓ bronchoconstriction;
✓ adhesion of leucocytes to endothelium; and
✓ chemotaxis.

5. Cytokines
Cytokines are polypeptide substances produced by activated lymphocytes (lymphokines) and activated monocytes (monokines). These agents may act on ‘self’ cells producing them or on other cells. Although over 200 cytokines have been described, major cytokines acting as mediators of inflammation are: interleukin-1 (IL-1), tumour necrosis factor (TNF)-α and β, interferon (IFN)-γ, and chemokines (IL-8, PF-4).

IL-1 and TNF-α are formed by activated macrophages while TNF-β and IFN-γ are produced by activated T cells. The chemokines include interleukin 8 (released from activated macrophages) and platelet factor-4 from activated platelets, both of which are potent chemoattractant for inflammatory cells and hence their name.
6. Free radicals: oxygen metabolites and nitric oxide

Free radicals act as potent mediator of inflammation:

i) Oxygen-derived metabolites are released from activated neutrophils and macrophages. These oxygen-derived free radicals have the following action in inflammation:

- Endothelial cell damage and thereby increased vascular permeability.
- Activation of protease and inactivation of antiprotease causing tissue matrix damage.
- Damage to other cells.

ii) Nitric oxide (NO) was originally described as vascular relaxation factor produced by endothelial cells. Now it is known that NO is formed by activated macrophages during the oxidation of arginine by the action of enzyme, NO synthase. NO plays the following role in mediating inflammation:

- Vasodilatation
- Anti-platelet activating agent
- Possibly microbicidal action (Harshmohan, 2010).

Figure 5: Chemical mediators of inflammation (Vinay et al., 2007)
2.1.5 Regulation of inflammation

The onset of inflammatory responses outlined above may have potentially damaging influence on the host tissues as evident in hypersensitivity conditions. Such self-damaging effects are kept in check by the host mechanisms in order to resolve inflammation (Harshmohan, 2010). These mechanisms are as follows:

i) Acute phase reactants

A variety of acute phase reactant (APR) proteins are released in plasma in response to tissue trauma and infection. Their major role is to protect the normal cells from harmful effects of toxic molecules generated in inflammation and to clear away the waste material. APRs include the following:

i) Certain cellular protection factors (e.g. α1-antitrypsin, α1- chymotrypsin, α2-antiplasmin, plasminogen activator inhibitor): They protect the tissues from cytotoxic and proteolytic damage.

ii) Some coagulation proteins (e.g. fibrinogen, plasminogen, von Willebrand factor, factor VIII): They generate factors to replace those consumed in coagulation.

iii) Transport proteins (e.g. ceruloplasmin, haptoglobin): They carry generated factors.

iv) Immune agents (e.g. serum amyloid A and P component, C-reactive protein): CRP is an opsonising agent for phagocytosis and its levels are a useful indicator of inflammation in the body.

v) Stress proteins (e.g. heat shock proteins—HSP, ubiquitin): They are molecular chaperons who carry the toxic waste within the cell to the lysosomes.

vi) Antioxidants (e.g. ceruloplasmin are active in elimination of excess of oxygen free radicals.

The APR are synthesised mainly in the liver, and to some extent in macrophages. APR along with systemic features of fever and leucocytosis is termed ‘acute phase response’. Deficient synthesis of APR leads to severe form of disease in the form of chronic and repeated inflammatory responses.
ii) **Glucosteroids**
The endogenous glucocorticoids act as anti-inflammatory agents. Their levels are raised in infection and trauma by self-regulating mechanism.  

iii) **Free cytokine receptors**
The presence of freely circulating soluble receptors for cytokines in the serum correlates directly with disease activity.

iv) **Anti-inflammatory chemical mediators**
PGE2 or prostacyclin has both pro-inflammatory as well as anti-inflammatory actions.

### 2.1.6 Inflammatory cells (Harshmohan, 2010)

1. **Polymorphonuclear Neutrophils (PMNs)**

Commonly called as neutrophils or polymorphs, these cells along with basophils and eosinophils are known as granulocytes due to the presence of granules in the cytoplasm. These granules contain many substances like proteases, myeloperoxidase, lysozyme, esterase, aryl sulfatase, acid and alkaline phosphatase, and cationic proteins. The diameter of neutrophils ranges from 10 to 15 µm and are actively motile. These cells comprise 40-75% of circulating leucocytes and their number is increased in blood (neutrophilia) and tissues in acute bacterial infections. These cells arise in the bone marrow from stem cells.

The functions of neutrophils in inflammation are as follows:

i) Initial phagocytosis of microorganisms as they form the first line of body defense in bacterial infection. The steps involved are adhesion of neutrophils to vascular endothelium, emigration through the vessel wall, chemotaxis, engulfment, degranulation, killing and degradation of the foreign material.

ii) Engulfment of antigen-antibody complexes and nonmicrobial material. iii) Harmful effect of neutrophils in causing basement membrane destruction of the glomeruli and small blood vessels.

2. **Eosinophils**

These are larger than neutrophils but are fewer in number, comprising 1 to 6% of total blood leucocytes. Eosinophils share many structural and functional similarities with neutrophils like their production in the bone marrow, locomotion, phagocytosis, lobed
nucleus and presence of granules in the cytoplasm containing a variety of enzymes, of which major basic protein and eosinophil cationic protein are the most important which have bactericidal and toxic action against helminthic parasites. However, granules of eosinophils are richer in myeloperoxidase than neutrophils and lack lysozyme. High level of steroid hormones leads to fall in number of eosinophils and even disappearance from blood.

The absolute number of eosinophils is increased in the following conditions and, thus, they partake in inflammatory responses associated with these conditions:

i) Allergic conditions;

ii) Parasitic infestations;

iii) Skin diseases; and

iv) Certain malignant lymphomas.

3. **Basophils (Mast Cells)**

The basophils comprise about 1% of circulating leucocytes and are morphologically and pharmacologically similar to mast cells of tissue. These cells contain coarse basophilic granules in the cytoplasm and a polymorphonuclear nucleus. These granules are laden with heparin and histamine. Basophils and mast cells have receptors for IgE and degranulate when cross-linked with antigen.

The role of these cells in inflammation is:

i) in immediate and delayed type of hypersensitivity reactions; and

ii) release of histamine by IgE-sensitised basophils.

4. **Lymphocytes**

Next to neutrophils, these cells are the most numerous of the circulating leucocytes (20-45%). Apart from blood, lymphocytes are present in large numbers in spleen, thymus, lymph nodes and mucosa-associated lymphoid tissue (MALT). They have scanty cytoplasm and consist almost entirely of nucleus.

5. **Plasma cells**

These cells are larger than lymphocytes with more abundant cytoplasm and an eccentric nucleus which has cart-wheel pattern of chromatin. Plasma cells are normally not seen in peripheral blood. They develop from B lymphocytes and are rich
in RNA and γ-globulin in their cytoplasm. There is an interrelationship between plasmacytosis and hyperglobulinaemia. These cells are most active in antibody synthesis.

Their number is increased in the following conditions:

i) Prolonged infection with immunological responses e.g. in syphilis, rheumatoid arthritis, tuberculosis;

ii) Hypersensitivity states; and

iii) Multiple myeloma.

6. Mononuclear-Phagocyte System (Reticuloendothelial System)

This cell system includes cells derived from 2 sources with common morphology, function and origin. These are as under:

Blood monocytes: These comprise 4-8% of circulating leucocytes.

Tissue macrophages: These include the following cells in different tissues:

i) Macrophages in inflammation.

ii) Histiocytes which are macrophages present in connective tissues.

iii) Kupffer cells are macrophages of liver cells.

iv) Alveolar macrophages (type II pneumocytes) in lungs.

v) Macrophages/histiocytes of the bone marrow.

vi) Tingible body cells of germinal centres of lymph nodes.

vii) Littoral cells of splenic sinusoids.

7. Giant cells

A few examples of multinucleate giant cells exist in normal tissues (e.g. osteoclasts in the bones, trophoblasts in placenta, megakaryocytes in the bone marrow). However, in chronic inflammation when the macrophages fail to deal with particles to be removed, they fuse together and form multinucleated giant cells. Besides, morphologically distinct giant cells appear in some tumours also.

A. Giant cells in inflammation:

i) Foreign body giant cells. These contain numerous nuclei (up to 100) which are uniform in size and shape and resemble the nuclei of macrophages. These nuclei are scattered throughout the cytoplasm. These are seen in chronic infective granulomas, leprosy and tuberculosis.
ii) Langhans’ giant cells. These are seen in tuberculosis and sarcoidosis. Their nuclei are like the nuclei of macrophages and epithelioid cells. These nuclei are arranged either around the periphery in the form of horseshoe or ring, or are clustered at the two poles of the giant cell.

iii) Touton giant cells. These multinucleated cells have vacuolated cytoplasm due to lipid content e.g. in xanthoma.

iv) Aschoff giant cells. These multinucleate giant cells are derived from cardiac histiocytes and are seen in rheumatic nodule.

B. Giant cells in tumours:

i) Anaplastic cancer giant cells. These are larger, have numerous nuclei which are hyperchromatic and vary in size and shape. These giant cells are not derived from macrophages but are formed from dividing nuclei of the neoplastic cells e.g. carcinoma of the liver, various soft tissue sarcomas etc.

ii) Reed-Sternberg cells. These are also malignant tumour giant cells which are generally binucleate and are seen in various histologic types of Hodgkin’s lymphomas.

iii) Giant cell tumour of bone. This tumour of the bones has uniform distribution of osteoclastic giant cells spread in the stroma (Harshmohan, 2010).

2.1.7 Pre-clinical screening methods for anti-inflammatory agents (In vivo methods) (Vogel and Vogel, 2002)

Vascular permeability

This test is useful to evaluate inhibitory activity of drugs against increased vascular permeability, which is induced by phlogistic substance. After inflammation, histamine, prostaglandins and other mediators of inflammation are released this leads to dilation of arterioles and increased vascular permeability. Histamine liberator compound 48/80 is used to increase vascular permeability and this can be recognized by infiltration of injected sites of the skin with Evans blue. Evaluation is based on measurement of area, which is penetrated by the dye.
Oxazolone induced ear edema in mice
It is a model of delayed contact hypersensitivity that permits the quantitative evaluation of the topical & systemic anti-inflammatory activity following topical administration. Average values of the increase of the weight are calculated for each treated group and compared statistically with the control group. This method is useful to detect anti-inflammatory activity of both steroidal as well as non-steroidal drugs.

Croton-oil ear edema in rats and mice
This method is useful for assessment of antiphlogistic and thymolytic activities of topically applied steroids. The composition of irritant is 1 part croton oil, 10 parts ethanol, 20 parts pyridine and 69 parts ethyl ether. Evaluation is based on comparison of weight of treated and control animals. Rabbits are also useful for testing the drugs.

Pleurisy test
Pleurisy is well known phenomenon of exudative inflammation in man. Pleurisy can be induced by using histamine, bradykinin, dextran, enzymes, antigen, turpentine, and carrageenan. Carrageenan induced pleurisy is an excellent acute inflammatory model in which fluid extravasation leukocyte migration and various biochemical parameters can be studied which are associated with inflammation. Evaluation is based on following parameter:
· Measurement of WBC
· Determination of lysosomal enzyme activities
· Determination of fibronectin
· Determination of PGE2

Granuloma pouch technique
In this method carrageenan or croton oil are used to produce aseptic inflammation, which results in large volumes of hemorrhage exudate. Evaluation is based on volume of exudate fluid and total leukocyte count in control and test animals.

Urate crystal induced synovitis
Deposition of urate crystal and sodium urate is important in gout and gouty tophi. It has been found that injection of urate crystals in synovial fluid of dog results in severe
pain resembling gout. This is the excellent method to study anti-inflammatory activity and it closely resembles pathological condition. For evaluation a scoring system is adopted in which inflammatory symptoms ranging from tenderness, limping, occasional legged gait to complete 3-legged gait are scored from 1+ to 4+.

**Glass rod granuloma**

This method is used to develop chronic proliferative inflammation. In contrast to other methods, glass rod granuloma measures the late proliferative phase of inflammation. Since, the newly formed connective tissue is not contaminated with the irritant, biochemical analysis can be performed. The peculiar feature is the possibility of newly formed proliferative connective tissue.

**Carrageenan induced rat paw edema** (Winter et al., 1962)

This method was first proposed by Winter et al (1962). They injected carrageenan (an extract of chondrus) 1%, 0.1ml in 0.9% sterile NaCl. They found that carrageenan probably does not release histamine or serotonin since relatively large doses of strong inhibitors of serotonin or histamine are ineffective in this test. Thus, this model has distinct advantage over other edema models which employ brewer’s yeast, formalin, dextran, egg white as phlogistic material. Vinegar et al (1987) found that development of edema after carrageenan injection is biphasic. The 1st phase begins immediately after injection of irritant and diminishes in an hour. The 2nd phase begins immediately at the end of the 1st phase. All the steroidal and non-steroidal anti-inflammatory drugs inhibit the edema formation in dose dependent manner.

**Chronic inflammation (cotton pellet induced granuloma)**

The cotton pellet induced method was first introduced by (Swingle and Shideman, 1972) to study the effect of local and systemic application of cortisone upon developing granulation tissue. Since then this method has been extensively used for evaluation of anti-inflammatory agents. This method can be divided into three phases namely exudation, granulation, and consolidation.

Exudation phase: Three days after implantation, there is marked rise in the wet weight of the normal pellets attributable to accumulation of visible fluid exudate. The fluid is protein rich and contains numerous polymorphnuclear leukocytes largely neutrophils.
Granulation phase: At seventh day, the granulation tissue is loosely adherent to the normal cotton pellets. The granulation layer thickens during the following week and its capillary net work become more and more complex with noticeable budding of new vessels towards the pellet. Fibroblast and macrophage numbers markedly increase with a thickening of the web of collagen fiber.

Consolidation phase: In this phase there is a decrease in dry weight of deposing material. This is accompanied by further decrease in wet weights. Histological changes occurring during this time are further penetration of fibroblast and collagen fiber to the centers of pellets and some giant cell formation around the cotton fibers. There is rapid increase in weight of cotton pellets granulomas between 0-2 days of the implantation.

2.1.8 Plants reported to have anti-inflammatory activity

Recent data from literature demonstrate the anti-inflammatory activity of many plant derived compounds. The mechanism of action is attributed to their ability to inhibit cytokine, chemokine or adhesion molecule synthesis (Calixto et al., 2004). The naturally occurring compounds with anti-inflammatory properties are summarized in Table 4. Plant phytochemicals such as polysaccharides, terpenes, alkaloids, etc. have been reported to be useful in alleviating inflammatory diseases including arthritis, rheumatism, acne skin allergy and ulcers. Plants containing polysaccharides are reported to be the most potent in curing inflammatory diseases.

Table 4: Naturally occurring compounds with anti-inflammatory properties

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Class of compound</th>
<th>Botanical name</th>
<th>Active chemical constituent</th>
<th>Mechanism of action</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Flavonoids</td>
<td><em>Silybum marianum</em> (Asteraceae)</td>
<td>Silymarin</td>
<td>Prevent TNF-α induced NF-κB activation in human histiocytic lymphoma cells</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td><em>Scutellaria baicalensis</em> Georgi (Lamiaceae)</td>
<td>Baicalin, Baicalein, Wogonin</td>
<td>Inhibit LPS induced NO production and iNOS gene expression and antioxidant properties</td>
</tr>
<tr>
<td>No.</td>
<td>Family</td>
<td>Plant Name</td>
<td>Compound</td>
<td>Activity Description</td>
</tr>
<tr>
<td>-----</td>
<td>------------------------</td>
<td>-------------------------------------------------</td>
<td>----------------------------------</td>
<td>--------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>3</td>
<td>Ginkgo biloba L</td>
<td>Quercetin</td>
<td>Suppresses the activation of the transcription factor AP-1</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Hypericum perforatum</td>
<td>Hypericin</td>
<td>Inhibited IL-12 production on LPS activated macrophages</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(hypericaceae)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Curcuma zedoaria L</td>
<td>Procurcumenol</td>
<td>Inhibited TNF-α secretion in LPS activated macrophages</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(Zingiberaceae)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Polyphenols</td>
<td>Curcuma longa L</td>
<td>Curcumin</td>
<td>Block IL-12 mediated T cell proliferation. Down regulate the TNF-α induced NF-κB inhibition</td>
</tr>
<tr>
<td></td>
<td>(Zingiberaceae)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Caesalpinia sappan Linn</td>
<td>Hematein</td>
<td>Effectively reduced TNF-α induced VCAM-1 expression in HUVECs</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(Leguminosae)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>8</td>
<td>Lignans</td>
<td>Coptis japonica Mankino</td>
<td>Pinoresinol, Woorenoside V</td>
<td>Inhibition of TNF-α</td>
</tr>
<tr>
<td></td>
<td>(Ranunculaceae)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Morina chinensis</td>
<td>Morinols A and B</td>
<td>Inhibition of cytokines</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(Dipsacaceae)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Terpenes</td>
<td>Tripterygium wifordii Hook F</td>
<td>Celastrol</td>
<td>Inhibited mRNA synthesis and protein expression of MMP-3 and MMP-13, induced by the proinflammatory cytokines IL-17, TNF-α</td>
</tr>
<tr>
<td></td>
<td>(Cellastraceae)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Panax ginseng</td>
<td>Ginsenosides Rb1 and Rb2</td>
<td>Inhibition of TNF-α</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(Araliaceae)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Terpenic saponins</td>
<td>Kalopanax pictus Nakai</td>
<td>Kalopanaxasapon in A</td>
<td>Inhibition of TNF-α</td>
</tr>
<tr>
<td></td>
<td>(Araliaceae)</td>
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</tbody>
</table>
Thus, it is quite clear that many plant derived compounds present significant anti-inflammatory effects. Plants appear to be a major source for potential molecules for the development of new drugs, especially designed for the treatment or control of chronic inflammatory states such as rheumatism. In fact, pharmaceutical industries are currently making tremendous efforts in order to identify new, relevant therapeutic molecules capable of modulating cytokine activated responses. These agents would be useful not only for the treatment of inflammatory disorders, but also for the control of some other diseases which have an inflammatory origin, such as atherosclerosis and alzheimer’s disease. Thus, this potential of vast medicinal flora needs to be explored. In this context, the development of therapeutic agents based on plant derived compounds that present anti-inflammatory activities would have clear benefits. Plant derived agents could be used alone or in association with other available anti-inflammatory drugs, allowing a reduction in costs and side effects and possibly an increase in effectiveness. Therefore, the plant *Cyathocline purpurea* (Buch-Ham ex D. Don.) Kuntze Fam. Asteraceae was selected after detailed review to establish the relevance of folk claims in developing modern drugs.
2.2 ARTHRITIS: HISTORICAL BACKGROUND & EPIDEMIOLOGY

Rheumatic musculoskeletal disorder (RMSD) more popularly known as “Arthritis” or “Rheumatism” is a primitive bone and joint disorder mostly affecting senior citizens around the world. Arthritis, a pathophysiological conditions of joint most common and major socio-economic problem now days. Arthritis is a general term used to describe many connective tissue disorders that affect bone and joints. The name “Arthritis” derived from Greek ‘arthro’ meaning joints and ‘itis’ meaning inflammation; indicates a group of conditions involving damage to the joints of the body.

Arthritis is the oldest disease of the universe and has been in this world since the beginning of civilization. The first known traces of human arthritis date back as far as 4500 BC in the fossils of native Americans, found in Tennessee, USA. The first written reference on arthritis was found in Indian holistic medicinal book Charaka Samhita, where it was described as swollen painful joints, initially occurring in hands, feet, causing loss of appetite and occasionally related with fever. The ancient classic Ayurvedic text has described painful deforming polyarthritis called “amavata” and “sandhighatvata” that bears resemblance to rheumatoid arthritis and inflammatory arthritis (Chopra and Doiphode, 2000). It is an established fact that the dinosaurs suffered from arthritis and there is ample proof to show that our earliest ancestors also suffered from chronic aches and pains. Remains of a herd of Iguanadons, small (three-ton) dinosaurs, around 85,000,000 BC were found in Brussels, Belgium. From their remains, we find that they had ankle osteoarthritis (OA). This is a rare phenomenon, since not many dinosaurs show symptoms of primary OA, but many show symptoms of secondary OA from injuries or congenital defects. According to researchers, due to difference in joint structure, dinosaurs that weighed several tons did not suffer from OA. Between 30,000 BC and 28,000 BC, a relative of modern man, Neanderthal man, made his first appearance. According to his remains, individuals of this time developed secondary OA due to injuries and the difficulties of daily life. In 4500 BC, arthritis was first discovered in human beings. It was seen in the skeletal remains of native Americans of Tennessee and parts of modern day Olathe, Kansas, America. In fact, arthritis was evidenced in ancient, a mummy in 3000 BC. Â-tzi was the name given to a mummy, popularly known as the Iceman, who crossed the Alps near the border of Italy and Austria. Though he was not successful in his venture, the
mummified remains of his body does, with the pouch of medicinal herbs that he carried with him and his arthritic joints which provide valuable information even 5000 years after he died. Rheumatoid arthritis is a prime paleopathology showed that rheumatoid arthritis was present in the skeletal remains of North American Indians thousands of years old (Rothschild et al., 1992).

2.2.1 Rheumatoid arthritis
Rheumatoid arthritis (RA) is one of the most common inflammatory disorders affecting the population worldwide. It is a systemic inflammatory disease which affects not only the joints but a wide range of extra-articular organs. The disease, if not treated early, will lead to progressive joint deformity and increased morbidity and mortality. RA is a potentially fatal illness, with mortality increased twofold and an average decrease in life expectancy of 7-10 years. Patients with RA have an increased prevalence of other serious illnesses. The predominant conditions leading to this increased co-morbidity and mortality include infections, renal impairment, cardiovascular disease and lymphomas. The incidence of lymphoma is twofold higher than expected before taking into account the disease-modifying immunosuppressant drugs used in treating RA (Roger and Cate, 2012).

2.2.2 Epidemiology and Etiology
RA affects approximately 1% of the population worldwide. RA arises from an immunologic reaction, and there is speculation that it is in response to a genetic or infections antigen. Risk factors associated with the development of RA include:

- Female gender (3:1 females to males)
- The prevalence of RA increases with age in both sexes; nearly 5% of women and 3% of men over the age of 65 years are affected by the disease.
- The peak age of incidence is about 30-50 years in women and slightly older in men.
- RA also affects young children and its classification and treatment differs slightly from adults.
- Current tobacco smoking. Studies have identified a direct relationship between tobacco use and RA disease severity.
Family history of RA. Genetic studies demonstrate a strong correlation between RA and the presence of major histo-compatibility complex class II human leukocyte antigens (HLA), especially HLA-DR1 and HLA-DR4. HLA is a molecule associated with the presentation of antigens to T lymphocytes.

Potential environmental exposures. The number of RA cases has increased during industrialization, although a specific link to environmental factors has not been determined.

Oral contraceptive use and high ingestion of vitamin D and tea are associated with a decreased risk of RA (Marie et al., 2006).

2.2.3 Pathophysiology

The characteristics of a synovium affected by RA are,

- The presence of a thickened, inflamed membrane lining called pannus.
- The development of new blood vessels.
- An influx of inflammatory cells in the synovial fluid, predominantly T lymphocytes.

The pathogenesis of RA is driven by T lymphocytes, but the initial catalyst causing this response is unknown. Understanding specific components of the immune system and their involvement in the pathogenesis of RA will facilitate understanding of current and emerging treatment options for RA. The components of most significance are T lymphocytes, cytokines, and B lymphocytes (Marie et al., 2006).

*T lymphocytes*

The development and activation of T lymphocytes are important to maintain protection from infection without causing harm to the host. Activation of mature T lymphocytes requires two signals. The first is the presentation of antigen by antigen-presenting cells to the T lymphocyte receptor. Second, a ligand receptor complex on antigen presenting cells binds to CD28 receptors on T lymphocytes. Once a cell successfully passes through all the stages, the inflammatory cascade is activated. Activation of T lymphocytes (1) stimulates the release of macrophages or monocytes, which subsequently causes the release of inflammatory cytokines, (2) activates osteoclasts, (3) activates the release of matrix metalloproteinases or enzymes
Cytokines

An imbalance of proinflammatory and inflammatory cytokines in the synovium leads to inflammation and joint destruction. Proinflammatory cytokines including IL-1, TNF-α, IL-6 and IL-12 are found in high concentrations in synovial fluid. Cytokines are low-molecular weight regulatory proteins or glycoprotein’s secreted by white blood cells and various other cells in the body in response to a number of stimuli. These proteins assist in regulating the development of immune effector cells, and some cytokines possess direct effector functions of their own. Cytokines are involved in a staggeringly broad array of biological activities including innate immunity, adaptive immunity, inflammation, and hematopoiesis.

Table 5: Cytokines involved in the pathogenesis of RA (Marie et al., 2006)

<table>
<thead>
<tr>
<th>Cytokines</th>
<th>Secreted by</th>
<th>Targets and effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interleukin 1 (IL-1)</td>
<td>Monocytes, macrophages, endothelial cells, epithelial cells</td>
<td>Vasculature (inflammation); hypothalamus (fever); liver (induction of acute phase proteins)</td>
</tr>
<tr>
<td>Tumor Necrosis Factor-α (TNF-α)</td>
<td>Macrophages</td>
<td>Vasculature (inflammation); liver (induction of acute phase proteins); loss of muscle, body fat (cachexia); induction of death in many cell types; neutrophil activation</td>
</tr>
<tr>
<td>Interleukin-12 (IL-12)</td>
<td>Macrophages, dendritic cells</td>
<td>Natural killer (NK) cells; influences adaptive immunity</td>
</tr>
<tr>
<td>Interleukin-6 (IL-6)</td>
<td>Macrophages, endothelial cells</td>
<td>Liver (induces acute phase proteins); influences adaptive immunity (proliferation and antibody secretion of B cell lineage)</td>
</tr>
<tr>
<td>Interferon-α (IFN-α)</td>
<td>Macrophages</td>
<td>Induces an antiviral state in most nucleated cells; increases MHC class I expression; activates NK cells</td>
</tr>
</tbody>
</table>
**Interferon-β (IFN-β)**

Fibroblasts

Induces an antiviral state in most nucleated cells; increases MHC class I expression; activates NK cells

---

*B Lymphocytes*

In addition to serving as antigen-presenting cells to T lymphocytes, B lymphocytes may produce proinflammatory cytokines and antibodies. Antibodies of significance in RA are rheumatoid factors and antibodies against cyclic citrullinated peptide (CCP). Rheumatoid factors are not present in all patients with RA, but their presence is indicative of disease severity and likelihood of extraarticular manifestations. CCPs are produced early in the course of disease. High levels of anti-CCP antibodies are indicative of aggressive disease and a greater likelihood of poor outcomes. Monitoring anti-CCP antibodies may be useful to predict the severity of disease and match aggressive treatment appropriately.

**2.2.4 Comorbidities associated with RA** (Marie et al., 2006)

RA reduces a patient’s average life expectancy by 3 to 10 years, but RA alone rarely causes death. Instead, specific comorbidities contribute to premature death independent of safety issues surrounding the use of immunomodulating medications. The comorbidities with the greatest impact on morbidity and mortality associated with RA are (1) cardiovascular disease, (2) infections, (3) malignancy, and (4) osteoporosis.

*Cardiovascular*

Half of all deaths in RA patients are cardiovascular related. Because a patient with RA experiences inflammation and swelling in his or her joints, it is likely that there is inflammation elsewhere, such as in blood vessels, termed vasculitis. C-reactive protein (CRP), a nonspecific marker of inflammation, is associated with an increased risk of cardiovascular disease; CRP is elevated in patients with RA. Chronic systemic inflammation may contribute to the relationship between RA and cardiovascular disease, but the exact mechanism is still under investigation.
Infection

RA alone leads to changes in cellular immunity and causes a disproportionate increase in pulmonary infections and sepsis. Because medications that alter the immune system are linked to an increased risk of infections, it is difficult to distinguish between an increased risk of infection secondary to RA and the medications used to treat RA. Patients and clinicians must pay close attention to signs and symptoms of infection because of this increased risk.

Malignancy

Patients with RA have an increased risk of developing lymphoproliferative malignancy (e.g., lymphoma, leukemia, and multiple myeloma) and a decreased risk of developing cancer of the digestive tract. The relationship between RA and cancer is not clear. To confound the issue, medications for the treatment of RA may contribute to cancer risk. Patients presenting with new onset of symptoms (e.g., fevers, night sweats, chills, or anorexia) out of proportion with disease activity and patients not responding to conventional RA treatment should be evaluated further for lymphoproliferative malignancy.

Osteoporosis

Osteoporosis associated with RA follows a multifaceted pathogenesis, but the primary mechanism likely is mediated by osteoclast activity. The cytokines involved in the inflammatory process directly stimulate osteoclast and inhibit osteoblast activity. Additionally, arthritis medications can lead to increased bone loss. Bone mineral density should be evaluated at baseline and routinely using dual-energy x-ray absorptiometry.

2.2.5 Clinical manifestations (Roger and Cate, 2012)

There are different patterns of clinical presentation of rheumatoid arthritis. The disease may start as polyarticular arthritis with a gradual onset, intermittent or migratory joint involvement, or monoarticular manifestations may be present. Extraarticular features occur in approximately 75% of seropositive patients and are often associated with a poor prognosis.
Disease onset is usually insidious with the predominant symptoms being pain, stiffness and swelling. Typically, the metacarpophalangeal and proximal interphalangeal joints of the fingers, interphalangeal joints of the thumbs, the wrists, and metatarsophalangeal joints of the toes are affected during the early stages of the disease. Other joints of the upper and lower limbs, such as the elbows, shoulders and knees, are also affected. Morning stiffness may last for 30 min to several hours, and usually reflects the severity of joint inflammation. Up to one-third of patients also suffer from prominent myalgia, fatigue, low-grade fever, weight loss and depression at disease onset.

Rheumatoid arthritis shows a marked variation of clinical expression in individual patients both in number and pattern of joints affected, disease progression and the rapidity of joint damage. Disease activity may not abate in 10-20% of cases. Remission has been reported in a small proportion of patients but usually is very rare without DMARDs.

**Extra-articular features of RA**

- Amyloidosis
- Carpal tunnel syndrome
- Episcleritis
- Felty’s Syndrome
- Fever
- Lymphadenopathy
- Nodules; may be subcutaneous or within the lungs, eyes or heart
- Osteoporosis
- Pericarditis
- Pleural and pericardial effusions
- Vasculitis

**2.2.6 Diagnosis** (Roger and Cate, 2012)

A clinical diagnosis of RA is made based on the patient’s history, presenting symptoms and clinical findings. Family history is useful, as well as investigations including blood tests, ultrasound for the presence of synovitis and X-rays. The latter is
used to demonstrate joint destruction which indicates a late manifestation of the disease.

Table 6: Summary for diagnosis of RA

<table>
<thead>
<tr>
<th>Arthritis</th>
<th>Patients History</th>
<th>Physical Exam</th>
<th>Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rheumatoid arthritis</td>
<td>-Pain duration &gt; 6 weeks</td>
<td>-Synovitis</td>
<td>Radiologic</td>
</tr>
<tr>
<td></td>
<td>-Morning stiffness (lasting &gt; 30 minutes)</td>
<td>-Joint involvement, symmetrical</td>
<td>-Erosions on X-Ray or MRI</td>
</tr>
<tr>
<td></td>
<td>-Systemic symptoms (e.g. anorexia, fatigue)</td>
<td>-Joint destruction -Extra-articular manifestations</td>
<td>-Synovitis noted by ultrasound</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-ESR or CRP</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>-Anti-CCP</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>-Rheumatoid factor</td>
</tr>
</tbody>
</table>

2.2.7 Investigations

Inflammatory markers, including C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR), are usually but not always elevated in RA and are useful for monitoring response to treatment. Rheumatoid factor (RF) is an autoantibody directed against the host immunoglobulin and is most commonly found in RA. Routinely performed tests only detect immunoglobulin M rheumatoid factor (IgM RF) which is present in 75-80% of patients with RA (termed seropositive disease) and 5% of normal subjects. Those patients who do not have a detectable RF are said to be ‘seronegative’. RF is not specific to RA and is also present in patients with chronic lung and liver disease, other connective tissue diseases, neoplasia, infections (particularly bacterial endocarditis) and cryoglobulinaemia.

Anti-cyclic citrullinated peptide antibodies (anti-CCP antibody) are a more specific of 90-96% compared with the specificity of IgM RF of 85%. They are more useful for the early detection of RA in a patient with inflammatory arthritis. The sensitivity of both anti-CCP antibody and IgM RF is approximately 70%.

Antinuclear antibodies (ANA) and extractable nuclear antigens (ENA) are useful for establishing the differential diagnosis, such as other connective tissue diseases.
presenting or associated with arthritis. ANA is almost universally positive in systemic lupus erythematosus and only positive in 20% of patients with RA.

Other abnormal laboratory tests include an elevated alkaline phosphatase, an elevated platelet count, a decreased serum albumin and a normochromic, normocytic anaemia. White cell count, particularly neutrophils, is elevated in patients with infected joints and is also elevated whilst the patient is on steroids (Roger and Cate, 2012).

2.2.8 Pre-clinical screening methods for antiarthritic agents (In vivo methods)

Rat and mouse models of experimental autoimmune arthritis provide powerful alternative approaches to evaluate potential etiopathogenetic mechanisms in human RA.

The major rat models of experimental erosive arthritis can be classified into three general groups (Wilder et al., 1999; Crofford and Wilder, 1997).

- The first group is induced by hyperimmunization of genetically susceptible rat strains with antigens such as native type II collagen (collagen-induced arthritis, CIA) or cartilage oligomeric matrix protein (COMP-induced arthritis) in incomplete Freund’s adjuvant (IFA).

- The second group is induced by intradermal administration of various oil-based adjuvants, of which heat-killed Mycobacterium tuberculosis emulsified in IFA is the most widely studied (Mtb-adjuvant-induced arthritis, AIA). Chronic erosive arthritis has also been induced with other oil-based adjuvants including avridine in IFA (avridine-induced arthritis), pristane (pristane-induced arthritis, PIA), and IFA alone (oil-induced arthritis [OIA]). Additionally, newer models of adjuvant arthritis have recently been described that are induced by exogenous chemicals such as dioctadecyldiammonium bromide (DDA=C_{38}H_{80}NBr), heptadecane (C_{17}H_{36}) and even endogenous lipids such as the cholesterol precursor squalene (C_{30}H_{50}) (Lorentzen, 1999).

- The third group of rat models includes various forms of bacterial cell wall peptidoglycanpolysaccharide-induced arthritis (Wilder et al., 1999; Simelyte et al., 1999). The streptococcal cell wall (SCW) arthritis model is the best characterized of the third group. Additional arthritogenic cell-wall structures from bacteria and yeast such as beta-glucan, lipopolysaccharide, and
trehalosydimycolate, have been identified that induce arthritis in susceptible strains of rats (Lorentzen, 1999).

In mice, CIA, PIA and proteoglycan-induced arthritis are the major experimentally induced models. Mice are relatively resistant to classic AIA (Yoshino et al., 1998) and SCW arthritis (Wilder et al., 1999).

**Rat models of erosive arthritis**

*Collagen-induced arthritis*

The collagen-induced arthritis (CIA) model in rats is typically induced by intradermal injections of native, heterologous (non-rat) type II collagen (CII) in IFA, followed by a booster injection on day 7. CII is highly arthritogenic in DA and LEW rats, but not in F344 inbred rats. Homologous (rat) CII is also arthritogenic in DA, but not other strains of rats. Autoimmunity to type IX collagen (CIX), in contrast to CII and type XI collagen (CXI), is not directly pathogenic but may contribute to joint injury provided arthritis is initiated by an independent disease process (Cremer et al., 1998). Erosive polyarthritis typically develops 10 to 14 days after the primary immunization. Similar to RA, female DA rats tend to be more susceptible than males (Wilder et al., 1999).

Pathogenesis of CIA depends on autoreactive T cells as well as B cells that produce antibodies to type II collagen. TNF-α is also important for disease progression. A multivalent guanylhydrazone (CNI-1493) that inhibits TNF-α production by suppressing its translational efficiency, when injected intraperitoneally either before the onset of arthritis or after the establishment of clinical disease suppresses the severity of arthritis in DA rats (Kerlund et al., 1999). IL-1 is also of major importance in mediating disease progression in rat CIA. Bendele and coworkers have demonstrated that sustained blood levels of IL-1 receptor antagonist (IL-1Ra) result in dose dependent suppression of joint inflammation, pannus formation, cartilage damage, and bone lesions (Bendele et al., 1999). Neutrophil elastase, among other hydrolytic enzymes induced in CIA, contributes to cartilage degradation in CIA, and elastase inhibitors reduce the clinical scores and joint swelling (Janusz and Durham, 1997).
Cartilage oligomeric matrix protein–induced arthritis

Immunization with both native and denatured rat COMP in IFA induces severe arthritis in susceptible rat strains. Although the peripheral joint arthritis in this newly described model clinically resembles RA, COMP induced arthritis does not result in permanent destruction of the joints. Disease development appears to be dependent on an immune response to autologous COMP and not on cross-reactivity to rat cartilage collagens (Carlson et al., 1998).

Mycobacteria-adjuvant induced arthritis in rats

An intradermal injection at the base of the tail with heat killed mycobacteria (Mtb) in IFA results in destructive arthritis within 14 days in susceptible DA or LEW inbred rats. AIA can also be induced with cell walls from other bacterial types in IFA, although the arthritogenicity varies. The disease progresses rapidly over several weeks in what appears clinically as a monophasic process.

Increased synthesis of TNF-α, IL-1 and IL-6 is detected as early as day 4 after adjuvant injection. As in CIA, IL appears to be very important in mediating the bone resorption that occurs in rat AIA (Wilder et al., 1999). Granulocytes and autoreactive CD4+ cells play major roles in the disease.

Avridine-induced arthritis

Injection of avridine (N, N-dioctadecyl-N, N'-bis (2-hydroxyethyl) propanediamine/CP-20961), emulsified in IFA, at the base of the tail is potentially arthritogenic in susceptible rat strains (DA and LEW) (Wilder et al., 1999). As in RA, females develop more severe disease than males. Experimental data also suggest that sex chromosomes regulate the gender differences in avridine-induced arthritis severity, but gonadal hormones also exert regulatory effects. Since avridine is a synthetic adjuvant devoid of properties that elicit classical T cell or B cell immunological responses, this model has focused investigations on the role of adjuvants and the innate immune system in the development of erosive arthritis. T cells, however, are critical to the development of the disease because avridine-induced arthritis does not develop in athymic nude rats.
Pristane-induced arthritis

Intradermal injection of 2, 6, 10, 14-tetramethylpentadecane (pristane), another synthetic immunological adjuvant, at the base of the tail, induces arthritis in susceptible rats (DA and LEW) that resembles RA (Wilder et al., 1999). PIA develops about 2 to 3 weeks after injection and progresses with a relapsing course that persists for months.

T cells are required for disease development. Bone erosions, beginning subchondrally, are evident about two days after the onset of clinical arthritis. Arthritic (E3 x DA) F2 rats have increased serum concentrations of COMP on days 35 and 49 after pristane injection (Vingsbo-Lundberg et al., 1998). COMP levels correlate with the severity of macroscopically detectable arthritis at both time points ($r > 0.9$). Rats with a chronic disease course are distinguished by higher serum concentrations of COMP during the acute stage than animals with similar early clinical scores but with resolving arthritis. Serum analysis of COMP offers promise for monitoring tissue involvement in experimental arthritis.

Oil-induced arthritis

This unique rat arthritis model develops in DA but not other inbred rat strains, after a single subcutaneous injection of IFA, which is a very weak adjuvant. The onset of OIA is around day 14. Joint inflammation is milder than in other rat arthritis models. Interestingly, autoreactive T cells, expressing high levels of IL-2, interferon-g and TNF-α, mediate OIA. In contrast to CIA, antibodies to type II collagen are not produced in OIA (Wilder et al., 1999).

Streptococcal cell wall-induced arthritis

A single intraperitoneal injection of an aqueous suspension of cell wall peptidoglycan-polysaccharide fragments from group A streptococci and several other types of Eubacteria including *E. aerofaciens, E. contortum*, and *E. lentum*, into susceptible rat strains, such as LEW, induces severe erosive arthritis (Wilder et al., 1999; Simelyte et al., 1999). An acute, thymic-independent, complement-dependent phase develops within 24 hours. This primary acute arthritic phase is followed by a chronic, secondary, thymic-dependent phase, which begins about 14 days after injection and is characterized by a fluctuating course similar to that observed in
patients with RA. In affected joints, this chronic phase is associated with the production of high levels of proinflammatory cytokines, growth factors, metalloproteinases, cyclooxygenase-2 and nitric oxide. Administration of plasmid DNA encoding human TGF-b1 intramuscularly to rats with SCW induced arthritis suppressed progression of inflammation and joint destruction (Song et al., 1998). Intra-articular injection of streptococcal cell wall antigen followed by intravenous challenge (reactivation) results in destructive, lymphocyte-dependent monoarticular arthritis. To define further the role of immune mechanisms in this model, antibodies to Th1- and Th2-related cytokines were evaluated (Schimmer et al., 1998). Anti–IL-4, but not anti-IL-10 or anti-IFN-g, is effective in lowering joint swelling in the reactivation model of streptococcal cell wall-induced arthritic rats, suggesting that Th2 mechanisms, to some extent, may be operative in this model of arthritis (Schimmer et al., 1998). Alternatively, IL-4 may play a role in the development of Th-1 related inflammation. TNF-α plays a major role in the development of joint swelling, whereas IL-1 is dominant in mediating cartilage destruction and inflammatory cell influx (Kuiper et al., 1998). As in RA patients, the transcription factor NF-κB is activated in the synovium of rats with “reactivation” SCW-induced arthritis (Miagkov et al., 1998). In vivo suppression of NF-κB in the synovium of rats with SCW- and pristane-induced arthritis by either proteasomal inhibitors or intra-articular adenoviral gene transfer of super-repressor kBa profoundly enhances apoptosis. Activation of NF-κB protects synovial cells against apoptosis and thus provides a potential link between inflammation and synovial hyperplasia. Intra-articular administration NF-κB decoys not only prevents the recurrence of SCW arthritis in treated joints but in addition reduces the severity of arthritis in the untreated joints (Miagkov et al., 1998).

### 2.2.9 Treatment

The goals of the treatment in RA are

- To reduce or eliminate pain
- To protect articular structures
- To control systemic complications
- To prevent loss of joint functions
- To improve or maintain quality of life.
Non-pharmacologic therapy

All patients should receive education about the non-pharmacologic and pharmacologic measures to help manage RA. Empowered patients take an active role in care by participating in therapy-related decisions. Certain forms of non-pharmacologic therapy benefit all levels of severity, whereas others (i.e. surgery) are reserved for severe cases only.

Occupational and physical therapy may help patients to preserve joint function, extend joint range of motion and strengthen joints and muscles through strengthening exercises. Patients with joint deformities may benefit from the use of mobility or assistive devices that help to minimize disability and allow continued activities of daily living. In situations where the disease has progressed to a severe form with extensive joint erosions, surgery to replace or reconstruct the joint may be necessary (Marie et al., 2006).

Pharmacologic therapy

The pharmacological management of RA is evolving rapidly as more advanced therapies become available. The advent of biological therapies has brought new technologies which target different cytokine pathways involved in the pathogenesis of RA and have revolutionized disease management.

There are four main categories of drugs employed in RA: non-steroidal anti-inflammatory drugs (NSAIDs) including cyclooxygenase (COX)-II inhibitors, glucocorticoids, DMARDs and biological therapies. Simple analgesia also has a small role to play in basic symptom relief and includes paracetamol, codeine and opiate combination products. These analgesics do not have any anti-inflammatory effect and will not aid disease modification. The aim of analgesia is to achieve symptom relief and reduce the need for long term use of NSAIDs, COX-II inhibitors and glucocorticoids (Marie et al., 2006).

Non-steroidal anti-inflammatory drugs (Roger and Cate, 2012)

The analgesic and anti-inflammatory properties of NSAIDs are used to reduce joint pain and swelling. However, as with simple analgesics, these drugs provide only symptomatic relief to improve joint functions, and should always be used in combination with other agents which modify the disease process.
The COX enzyme converts arachidonic acid into prostaglandins and thromboxanes. These prostanoids have a variety of physiological functions, and are also believed to be responsible for causing pain and swelling in inflammatory conditions. There are two main isoforms of the COX enzyme: COX-I produces prostaglandins required for homeostatic functions, such as maintaining the gastric mucosa, support of renal function and platelet function. COX-II is responsible for the production of inflammatory prostanoids.

NSAIDs vary in their selectivity for the COX-I and COX-II isoforms, and are categorized as either non-selective NSAIDs or selective COX-II inhibitors, otherwise known as the coxibs. Non-selective NSAIDs generally block COX-I and COX-II, whereas the coxibs have higher selectivity for the COX-II isoforms. However, COX-II selectivity in NSAIDs varies according to the dose of drug given, which is demonstrated by the dose-related toxicity exhibited by some agents such as ibuprofen. The three most commonly used non-selective NSAIDs have differing levels of COX-I or COX-II selectivity: diclofenac is COX-II ‘preferential’, whereas ibuprofen and particularly naproxen preferentially inhibit COX-I. Originally, inhibition of COX-II was thought to be involved solely with the anti-inflammatory, anti-pyretic and analgesic properties of NSAIDs. However, it is possible that COX-II inhibition may also impair endothelial health, cause a prothrombotic state and promote cardiovascular disease.

Table 7: Common and shared side effects of NSAIDs (Laurence et al., 2011)

<table>
<thead>
<tr>
<th>System</th>
<th>Manifestations</th>
</tr>
</thead>
<tbody>
<tr>
<td>GI (side effects decreased with COX-2–selective drugs)</td>
<td>Abdominal pain</td>
</tr>
<tr>
<td></td>
<td>Nausea</td>
</tr>
<tr>
<td></td>
<td>Anorexia</td>
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<td>Gastric erosions/ulcers</td>
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<td></td>
<td>Anemia</td>
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<td>GI hemorrhage</td>
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<tr>
<td></td>
<td>Perforation</td>
</tr>
<tr>
<td></td>
<td>Diarrhea</td>
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</tbody>
</table>
### Renal
- Salt and water retention
- Edema, worsening of renal function in renal/cardiac and cirrhotic patients
- Decreased effectiveness of antihypertensive medications
- Decreased effectiveness of diuretic medications
- Decreased urate excretion (especially with aspirin)

### CNS
- Headache
- Vertigo
- Dizziness
- Confusion
- Depression
- Lowering of seizure threshold
- Hyperventilation (salicylates)

### Platelets (side effects absent with COX-2–selective drugs)
- Inhibited platelet activation
- Propensity for bruising
- Increased risk of hemorrhage

### Uterus
- Prolongation of gestation
- Inhibit labor

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**Cyclooxygenase enzyme**

Since the discovery of the mechanism of the non-steroidal anti-inflammatory drugs by Vane (1971), it has been widely accepted that the inhibition of prostaglandin (PG) synthesis through cyclooxygenase (COX) blockade is responsible for both their therapeutic and side effects. More recently, a second inducible COX has been characterized as a distinct isoform, named COX-2, and is encoded by a gene different form that producing the constitutive isoform, COX-1 (Trummlitz and Van Ryn, 2002). COX-1, a constitutive enzyme located in most tissues, for example, the platelets, endothelium, stomach, kidney, smooth muscles and lumen of endoplasmic reticulum and performs a housekeeping function to synthesize PGs with normal cell regulatory activity. It is a membrane bound haem and glycoprotein with a molecular
weight of 71 KDa with 599 amino acid residues. The protein contained both the
cyclooxygenase and endoperoxidase activities required to form PGG2 and PGH2
respectively. COX-2 is an immediate early gene product, with a molecular weight of
70 KDa with 604 amino acid residues. It is expressed, as inducible form, considerably
after exposure to inflammatory mediators like fibroblasts, cytokines etc. Levels of
COX-2 protein increase in parallel with overproduction of prostaglandins in many
cells and tissue in chronic inflammation. COX-1 is the only isoform in the normal
gastric mucosa and platelets and is responsible primarily for the biosynthesis of
eicosanoids involved in gastrointestinal mucosal cytoprotection and the maintenance
of platelet function. COX-2, on the other hand, is involved in many physiologic
responses, but mainly in the amplification of inflammation and pain.

Structure and Functions of COX-1 and COX-2 (Willoughby et al., 2000)
The structure of COX protein consists of three different domains the N terminal
epidermal growth factor domain, a membrane binding motif, and a C-terminal
catalytic domain that contains the COX and peroxidase active sites. Both isoforms
consists of cassette of 17 amino acids and 18 amino acids sequence near the N-
terminal of COX-1 and COX-2 respectively. The catalytic domain is a globular
structure containing the cyclooxygenase and peroxidase active sites.

The peroxidase active site
The peroxidase activity has two functions: it reduces the PGG2 produced by the
cyclooxygenase step and activates the cyclooxygenase reaction. This enzyme
collectively termed prostaglandin synthase actually has two different active sites. On
one side, it has the cyclooxygenase active site and on the opposite side, is has an
entirely separate peroxidase site, which is needed to activate the haem group that
participate in the cyclooxygenase reaction. The enzyme complex is a dimmer of
identical subunits; so altogether, there are two cyclooxygenase active sites and two
peroxidase active sites in close proximity. Each subunit has a small carbon-rich knob,
pointing downward in this illustration. These knobs anchor the complex to the
membrane of the endoplasmic reticulum, shown in light blue at the bottom of the
picture. The cyclooxygenase active site is buried deep within the protein, and is
reachable by a tunnel that opens out in the middle of the knob. This acts like a funnel,
guiding arachidonic acid out of the membrane and into the enzyme for processing. In the structure shown here, cyclooxygenase enzyme, a drug (yellow and green) is blocking the active site in both subunits. The haem groups are also shown above the drug in each subunit.

*The cyclooxygenase active site*

The cyclooxygenase active site consists of a long, narrow channels extending from the outer surface of the membrane-binding motif. Tyr-385 is found at the apex of the COX-1 enzymes and Tyr-371 in case of COX-2 represent together with the haem group at the catalytic center. X-ray crystallography of 3D structures of COX-1 and COX-2 enzymes as well as complexes with NSAIDs has provided insight onto the mechanism of action. COX-1 and COX-2 are very similar enzymes consisting of a long narrow channel with a hairpin bend at their end and both are membrane associated. Arachidonic acid released from damaged membranes adjacent to the opening of the enzymes channel, mostly hydrophobic is sucked in twisted around the hairpin bend and subjected to chemical reactions, resulting in the formation of the cyclopenta ring of PGs. Epidermal Growth factor like domain (blue), amphapatic membrane binding motif (red), catalytic globular domain (gray), haem groups (brown) and arachidonic acid in its binding site (yellow).

**Figure 6: Comparison of NSAIDs binding sites of COX-1 and COX-2** (Rang et al., 2007)
Difference between binding sites of COX-1 and COX-2 (Flower, 2003)

After isolation in the early 1990’s of the COX-2 isozyme its genetic structure and regulation of expression were characterized and compared with COX-1. Both enzymes are encoded by separate genes on different chromosomes: COX-1 is on chromosome 9; COX-2 is on chromosome 1. The COX-2 gene contains regions characteristic of early response genes, allowing a rapid up regulation in response to inflammatory stimuli as well as rapid turnover and diminished expression in the absence of continued stimulation. Meanwhile, the COX-1 gene is expressed in almost all normal tissues and regulated by inflammatory stimuli (constitutive expression).

Although both isozymes are 60% homologous there are small differences in the amino acid sequence lining the COX active sites. The active site is preponderantly hydrophobic in nature with two internal hydrophilic pockets I and II, both of which have a valine (val) in COX-2 and an isoleucine in COX-1 (positions 523 and 89) at the opening of the pocket, leading to the constriction of this pocket in COX-1. The accessibility of these pockets is controlled by a valine in COX-2 as against isoleucine in COX-1, at position 523. The side chain of residue at 523 packs against phenylalanine (Phe) 518, which forms a molecular gate that extends to the hydrophilic pocket I. In COX-1, this gate is closed because of the bulkier side chain whereas in COX-2, with the less voluminous Val at 523, the gate has room to swing open, allowing the entry of inhibitor.

**Figure 7: Structural differences in active sites of cyclooxygenase (COX)-1 and COX-2** (Harvey et al, 2009)
Physiological function of COX-1 and COX-2 (Flower, 2003)

Gastrointestinal Tract: - The clearest function is in the gastrointestinal (GI) tract, where prostaglandins clearly play a protective role, as evidenced by the superior safety of COX-2 selective agents and the protective effects of misoprostal on the GI lining. The primary drawback of traditional nonselective agents is in the gastric system where reduction of protective prostaglandins causes ulceration, bleeding and occasionally death.

Cardiovascular systems: - Cardiovascular effects of prostaglandins are more complex. The coagulation system is clearly modulated by platelet derived thromboxanes, which have pro-coagulation effects and the anticoagulative effects of endothelial cell-derived prostacyclin. Thromboxanes are clearly COX-1 derived because platelets do not express COX-2. The source of endothelial cell prostacyclin production is less clear with both enzymes expressed and mixed opinions on the relative contribution of the two enzymes.

Kidney: - Prostaglandins regulate renin-angiotensin secretion and thus glomerular filtration rate and sodium homeostasis. These effects appear to be COX-2 driven. The kidney is a rare organ, one that expresses COX-2 under nonpathological situations. Expression in the loop of Henle apparently drives prostaglandin formation in the kidney and the subsequent physiological responses. Thus a selective agent would likely have similar negative effects on kidney function as those of the nonselective NSAIDs.

Cancer: - COX-2 is over expressed in cancerous lesions in the colon and the degree of its expression has been related to survival. COX-2 is also over expressed in the precancerous lesions of patients with familial adenomatous polyposis (FAP). The precise role of COX-2, and specifically its interplay with COX-1 in carcinogenesis, not clears.

Atherosclerosis: - Expression of both COX isoforms is detectable in atherosclerotic lesions in humans. In summary, COX-2 expressed, along with COX-1 in atherosclerotic plaques. However, the predominant prostaglandins formed vary with cell type and have divergent effects on disease progression and perhaps on plaque stability.

Neurological disease: - The place of COX-2 inhibitors in neurological diseases, such as Alzheimer’s disease, Parkinson’s disease and seizure disorders continues to attract
basic and clinical investigation. Initial studies in Alzheimer’s disease failed to support the hypothesis that COX-2 inhibition would slow the development of the disease. This hypothesis had been configured on the presence of increased expression of COX-2 in Alzheimer’s disease lesions (along with COX-1 in some cases); exacerbation of amyloid deposition following over expression of neuronal COX-2.

**Rheumatoid arthritis:** - Rheumatoid arthritis is an autoimmune disease in which there is joint inflammation, synovial proliferation and destruction of articular cartilage. Immune complexes composed of IgM activate complement and release factors, which are chemotactic for neutrophils. These inflammatory cells release lysosomal enzymes, which damage cartilage and erode bone, while PGs produced in the process cause vasodilatation and pain.

**Safety**

In 2004, rofecoxib, selective COX-II inhibitor, was withdrawn from the worldwide market due to evidence of an increased risk of confirmed serious thrombotic events that included myocardial infarction and stroke, following long term use. In the following years, similar evidence against the other COX-II inhibitors and also against some of the non-selective NSAIDs accumulated.

At present, the exact cardiovascular risk for individual selective COX-II inhibitors and NSAIDs is not known. Evidence from clinical trials of COX-II inhibitors suggests that about 3 additional thrombotic events per 1000 patients/year may occur in the general population.

A dose-dependent increase in cardiovascular risk is associated with use of celecoxib, high-dose diclofenac (150 mg/day) and high-dose ibuprofen (2400 mg/day). There does not appear to be an increased risk of myocardial infarction in association with low-dose ibuprofen (<1200 mg/day). Naproxen is associated with a lower risk of arterial thrombotic events than COX-II inhibitors. There may be some increased cardiovascular risk in all patients receiving any NSAID, irrespective of their baseline risk or duration of therapy. The key message is that patients should use the lowest effective dose and the shortest duration of treatment necessary to control symptoms to minimize the risk of adverse events.

The most common adverse events of NSAIDs are those that predominantly inhibit COX-I and cause adverse gastro-intestinal effects. These range from minor
symptoms, including dyspepsia, nausea and diarrhoea, to more serious events, such as gastric erosion, bleeding and duodenal and gastric ulceration. Patients are at higher risk of serious gastro-intestinal complications if they are over 65 years of age, have a previous history of gastro-intestinal ulceration/bleeding or peptic ulcer disease, or are taking concomitant anti-platelet, anti-coagulation or steroid therapy. There are several gastro-protective agents available who may be used to reduce adverse events, including H₂ antagonists; misoprostol and proton pump inhibitors (PPIs). PPIs, such as omeprazole and lansoprazole, have shown to be particularly effective at preventing gastric and duodenal ulcers with NSAIDs. All patients taking a non-selective NSAID or COX-II inhibitor should receive concomitant treatment with a PPI to minimize gastro-intestinal adverse effects.

Aspirin inhibits the COX enzyme irreversibly through a different mechanism of action to the NSAIDs. Therefore, there is an increased risk of gastro-intestinal toxicity if aspirin and non-selective NSAIDs are used concomitantly, and the gastro-intestinal advantage of using selective COX-II inhibitor is severely reduced. Low-dose aspirin should only be co-prescribed with NSAIDs where absolutely necessary.

All NSAIDs may potentially cause adverse cardio-renal effects such as edema, hypertension and heart failure. The distribution of COX-I and COX-II differs in the kidney, but there is no evidence to suggest differing degrees of isoforms inhibition have an impact on the severity of cardio-renal adverse effects. Pharmacokinetic parameters, such as half-life and metabolism, may affect both thrombotic and cardio-renal properties of NSAIDs (Roger and Cate, 2012).

**Choice of agent**

Evidence suggests that all non-selective NSAIDs and COX-II inhibitors are of similar efficacy, but vary in their toxicity profiles. However, there is individual patient variability in terms of response to a given NSAID, and so some patient may need to try several agents before reaching an effective and well tolerated agent. Non selective NSAIDs or COX-II inhibitors should be used at the lowest effective dose for the shortest possible period of time. There are no recommendations on which agent to use first-line as all NSAIDs have analgesic effects of similar magnitude. However, as these drugs vary in terms of potential gastro-intestinal, liver and cardio-renal toxicity,
it is advised that the choice of drug should take into account the patient’s individual risk factors, including age.

*Disease-modifying anti-rheumatic drugs* (Roger and Cate, 2012)

Joint damage is known to occur early in RA and is largely irreversible. The need for early intervention with DMARDs as part of an aggressive approach to minimize disease progression has become standard practice and is associated with better patient outcome. Early introduction of DMARDs also results in fewer adverse reactions and withdrawals from therapy.

The DMARDs that are commonly used for RA and have clear evidence of benefit are methotrexate, sulphasalazine, leflunomide and intramuscular gold. There is less compelling evidence for the use of hydroxyl-chloroquine, d-penicillamine, oral gold, ciclosporin and azathioprine, although these agents do improve symptoms and some objective measures of inflammation. The exact mechanism of action of all these drugs is unknown. All DMARDs inhibit the release or reduce the activity of inflammatory cytokines, such as TNF-α, IL-1, IL-2 and IL-6. Activated T-lymphocytes have been implicated in the inflammatory process, and these are inhibited by methotrexate, leflunomide and ciclosporin.

Patients should be made aware that the DMARDs all have a slow onset of action. They must be taken for at least 8 weeks before any clinical effect is apparent, and it may be months before an optimal response is achieved. Whilst early initiation of DMARDs is crucial, it is important to ensure the patient is maintained on therapy to maintain disease suppression. This itself is a challenge, due to the toxicity profiles of the majority of these drugs. The majority of the DMARDs require blood monitoring. Guidelines are available on the action to take in the event of abnormal blood results.

Patients with a new diagnosis of RA should be offered combination DMARD therapy as first-line therapy as soon as possible, ideally within 3 months of the onset of persistent disease symptoms. The combination therapy should include methotrexate and at least one DMARD usually sulphasalazine and/or hydroxychloroquine. Evidence suggests that combination therapy appears to be superior in terms of benefits to symptoms, quality of life, remission rates and slowing of joint damage, when compared to monotherapy. Once sustained and satisfactory levels of disease
control have been achieved, the doses of drugs should be cautiously reduced to levels that continue to maintain disease control.

In patients where combination therapy is not appropriate, for example where there are contraindications to a drug due to existing co-morbidities, DMARD monotherapy should be started, placing greater emphasis on fast escalation to a clinically effective dose rather than on choice of agent.

There are many factors that influence the choice of DMARD: relative efficacy, severity of disease, convenience, monitoring requirements, patient co-morbidities, cost, time period to benefit, prescriber’s experience and success rates with the drug, side effects and patient adherence. Studies have shown that methotrexate has the best benefit to toxicity ratio. Both sulphasalazine and hydroxychloroquine alone does not slow radiological damage. Most patients started on a DMARD will not be taking it 3-4 years later because of adverse reactions or lack of efficacy. Despite promising results initially, some patients experience disease reactivation at a later stage and become unresponsive to treatment.

**Methotrexate**

Methotrexate is recognized as the gold standard DMARD in the management of RA. It is given as once weekly dose and can be given orally or parenterally via the intramuscular or subcutaneous routes. Patients usually begin on oral therapy; parenteral methotrexate may be considered in those who do not respond adequately to the maximum tolerated oral dose, or in those who suffer from gastro-intestinal side effects. Doses used, whether administered by the parenteral or oral route, are similar, although bioavailability is greater with parenteral administration. Methotrexate is primarily excreted unchanged by the kidneys and so elderly patients or those with renal impairment may require lower dose. Methotrexate is a folic acid antagonist and acts by reversibly inhibiting dihydrofolate reductase, the enzyme that reduces folic acid to tetrahydrofolic acid. Concomitant administration of oral folic acid reduces adverse effects of methotrexate and improves continuation of therapy and adherence. Doses used range from 5mg weekly to 5mg daily except on the day of methotrexate administration.

Methotrexate is associated with lung, liver and bone marrow toxicities. As a consequence, strict monitoring is advised and alcohol intake should be minimized.
Methotrexate pneumonitis is usually seen within the first year of treatment, but can sometimes occur after long-term therapy. Myelosuppression can cause significant falls in blood cell counts. It is more likely to occur in the elderly, patients with renal impairments or patients who are also taking anti-folate drugs. A clinically significant drop in cell counts calls for immediate withdrawal of methotrexate, and folinic acid rescue therapy. Patients should be counseled to report any of the following warning symptoms immediately to a healthcare professional: blood disorders, for example sore throat, bruising, mouth ulcers, liver toxicity, for example nausea, vomiting, abdominal discomfort, dark urine, and respiratory effects, for example shortness of breath, persistent dry cough. Methotrexate tablets are available as 2.5mg and 10mg strengths; most pharmacies will dispense the 2.5mg strength only for non-chemotherapy indications such as RA.

**Sulphasalazine**

Sulphasalazine has been shown to slow joint erosions and suppress inflammatory activity in RA. Blood dyscrasias usually occur within the first 3-6 months of treatment, therefore necessitating close monitoring in the initiation period. Patients should also be counseled to report warning symptoms of unexplained bleeding, bruising, purpurea, sore throat, fever or malaise. Enteric-coated tablets are available to minimize gastro-intestinal side effects.

**Hydroxychloroquine**

Hydroxychloroquine is significantly less effective than other DMARDs and historically was reserved for milder cases of RA. It still has a place in therapy, particularly in combination with other DMARDs, as it seems to give some symptomatic relief to patients and is the least toxic of the DMARDs. It has also been used relatively safely in pregnancy. Regular visual assessment for retinopathy is recommended as long term use of anti-malarial agents has been linked to ocular toxicity.

**Leflunomide**

Leflunomide has a long half-life of approximately 2 weeks, and consequently a loading dose may be given to achieve therapeutic drug levels more quickly. However,
in practice, the loading dose is often omitted due to intolerable gastro intestinal side effects such as diarrhoea. Leflunomide is associated with hepato- and haematotoxicity, and should be used with caution if co-prescribed with drugs which also cause these adverse effects. Washout procedures using colestyramine or activated charcoal may be necessary when switching to other DMARDs, in the event of a serious adverse effect or before conception in females.

**Gold therapy**
Gold compounds can be given via intramuscular injection as sodium aurothiomalate, or orally as auranofin. Intramuscular gold is more effective than oral. These drugs can be used over a long period of time provided the patient does not experience side effects such as proteinuria, blood disorders, rashes, gastro-intestinal side effects or bleeding.

**Other DMARDs**
D-Penicillamine is less commonly used, as side effects such as rashes, taste loss and vomiting, are common. It can be effective in some patients, but doses above 750mg daily are associated with a high incidence of adverse effects. Azathioprine and ciclosporin can be used in refractory RA, but use is limited due to monitoring requirements and high incidence side effects.

**Glucocorticoids**
Steroids can be given via the oral, intramuscular or intra-articular routes. They act by inhibiting cytokine release and give rapid relief of symptoms and decrease inflammation. Prednisolone is the most commonly used oral steroid. Intra-articular injections, such as triamcinolone or methyl-prednisolone, are administered into inflamed joints for local anti-inflammatory action, pain relief and to reduce deformity. The effects of the injection tend to last for approximately 4 weeks and should generally not be repeated more than three times a year into an affected joint. Intramuscular and, less commonly intravenous, injections are used as high dose pulse therapy to control aggressive disease flares.
Steroids are also used as a bridging therapy and are particularly useful when introducing DMARDs which may take several months to take effect. There are
various studies which demonstrate steroids are disease modifying in slowing radiological damage over 2 years. Doses of prednisolone 7.5 mg daily have been suggested to reduce the rate of joint destruction in moderate to severe RA of less than 2 years duration.

Ideally, steroids should be reserved for short-term use in new-onset RA because of their long-term complications and adverse effects. However, because they exert such a potent anti-inflammatory effect, it may be difficult in some patients to withdraw therapy as the disease tends to flare with dose reductions. Gradual reducing regimens should be used with the aim to reach the lowest possible maintenance dose. Steroids can induce osteoporosis, which is a known complication associated with RA itself. Prophylactic therapy, such as calcium and vitamin D supplementation and bisphosphonates, should be considered in patients on steroids at a high dose or for an extended period of time. Gastroprotection may also be necessary in the form of H₂ antagonist or proton pump inhibitors. Other adverse effects associated with steroids are diabetes, increased risk of infection, hypertension and weight gain.

**Biological therapies**

Over the past decade, there have been significant advances in the treatment of RA due to emerging biological therapies. The so-called biologics in the RA are genetically engineered monoclonal antibodies which selectively target different parts of the inflammatory pathways. Activated T-cells release pro-inflammatory cytokines including TNF-α, IL-1 and IL-6. Adalimumab, etanercept, golimumab, infliximab and certolizumab pegol target TNF-α, anakinra and tocilizumab target the interleukins, whilst abatacept and rituximab act on T-cells and B-cells, respectively.

In current practice, biologics are used after a patient has failed DMARDs, although there is emerging evidence to suggest they should be used earlier in the disease. Combination DMARD therapy is increasingly advocated and may lead to earlier use of biologics. For example, a patient may now be trialed on two DMARDs, including methotrexate, over a period of 6 months and if the response is inadequate could be eligible for an anti-TNF agent within a year of diagnosis.
Anti-TNF agents

There are five anti-TNF agents available: adalimumab, etanercept, golimumab, infliximab and cetrolizumab pegol. All inhibit TNF-α which is an inflammatory cytokine found in high concentrations within the joint synovium of RA patients.

Infliximab was the first anti-TNF agent licensed for the treatment of RA. It is a chimeric human-murine monoclonal antibody that binds with high affinity to TNF-α, thereby neutralizing its activity. Infliximab is the only anti-TNF agent which is given by intravenous infusion and must be given concomitantly with methotrexate. It is usually well tolerated, with the most common adverse effects being mild infusion reactions, such as headache and urticaria. Anaphylaxis and delayed hypersensitivity reactions have also been rarely reported. As infliximab is part murine monoclonal antibody, it is thought to carry a higher risk of developing human anti-chimeric antibodies (HACAs). HACAs are associated with an increased frequency of infusion-related reactions and can be minimized by administering with an immunomodulating therapy.

Adalimumab is a recombinant human monoclonal antibody that binds to and neutralizes TNF-α. Etanercept is a human TNF fusion protein that binds to TNF cell surface receptors, thereby inhibiting interactions of TNF-α with its receptors. Cetrolizumab pegol is a pegylated antibody fragment which binds and neutralizes TNF-α and is thought to have a relatively more rapid onset of action. Golimumab is the most recent addition to this family of agents and has the advantage of having a less frequent dosing schedule. Optimum clinical benefit is achieved when these drugs are used in combination with methotrexate. However, adalimumab, etanercept and cetrolizumab pegol can be used alone as monotherapy in patients for whom methotrexate is not tolerated.

Safety

The anti-TNF agents are generally well tolerated, with the main side effects being injection site reaction with the subcutaneous agents, and infusion-related reactions with infliximab. There are fewer monitoring requirements compared to the DMARDs and less frequent dosing, making these drugs potentially more appealing. However, the long-term safety of these drugs is being monitored in the UK by the British Society of Rheumatology Biologics Registry. This database collects data on efficacy
and safety outcomes of all patients on biologics in a variety of rheumatological conditions including RA.

**Rituximab**

Rituximab is a chimeric human-murine monoclonal anti-body which binds to the C20 antigen on B-lymphocytes to mediate B-cells lysis. It causes depletion of peripheral B-cells which play a vital role in the pathogenesis of RA. Recovery of B-cells appears to occur 6 months after treatment, with some patients showing prolonged B-cell depletion persisting up to 2 years after treatment.

Rituximab in combination with methotrexate is licensed for the treatment of severe active RA in patients who have had an inadequate response or intolerance to other DMARDs including one or more anti-TNF agent. Rituximab is also licensed for non-Hodgkin’s disease and chronic lymphocytic leukemia, both using a different dosing schedule to that of RA. A course of Rituximab consists of two intravenous infusions administered as a day case in hospital: 1000-mg infusion followed by a second 1000-mg infusion 2 weeks later. The course may be repeated every six months depending on patient response. Disease response varies between patients in that some achieve disease remission after one course and do not require re-treatment, whilst others require further repeat infusions every 6-12 months.

Rituximab is generally well tolerated, and the most common adverse effects are infusion-related reactions including fever, changes in blood pressure and rash. Minor infusion related side effects can be managed by reducing the rate of infusion and giving treatment for relief of symptoms such as paracetamol.

As with the anti-TNF agents, Rituximab increases the risk of infections and should not be used in the presence of active or severe infections. Use has been associated with progressive multifocal leukoencephalopathy. It may also exacerbate existing cardiac conditions such as angina pectoris and atrial fibrillation. Patients with known history of cardiac disease should be closely monitored during treatment administration for changes in blood pressure and pulse. Anti-hypertensive may be omitted 12 hr prior to the infusion.
**Abatacept**
Abatacept acts by blocking the full activation of T-cells thereby inhibiting the release of inflammatory cytokines. It is licensed for use in combination with methotrexate in the treatment of moderate to severe active RA in adults who have had an insufficient response or intolerance to other DMARDs including at least one anti-TNF agent and who cannot receive Rituximab, or when Rituximab is withdrawn due to an adverse event.

**Anakinra**
Anakinra blocks the binding of IL-1 to its receptor, thus inhibiting the inflammatory effects of IL-1. The evidence of its benefit in RA is weak and it is considered modestly effective. There are no trials directly comparing Anakinra with other biologic agents. Anakinra is not cost effective in the treatment of RA and availability for routine use in the NHS has not been supported.

**Tocilizumab**
Tocilizumab acts by binding to IL-6 receptors. IL-6 is a pro-inflammatory cytokine produced by a variety of cells including T and B-cells, and has been implicated in the pathogenesis of RA and other inflammatory diseases. Tocilizumab is licensed for use in combination with methotrexate in the treatment of moderate to severe active RA in adults who have had an insufficient response or intolerance to other DMARDs including at least one anti-TNF agent. It is given as a monthly intravenous infusion at a dose of 8 mg/kg and requires regular monitoring of liver function tests and full blood count.

Tocilizumab is recommended for the treatment of RA in patients who fulfill the following criteria:
- The patients has not responded adequately to one or more anti-TNF agents
- The patients cannot receive Rituximab due to contraindication
- The patients has not responded adequately to Rituximab treatment

**2.2.10 RA and pregnancy** (Roger and Cate, 2012)
The management of RA during pregnancy is a common challenge, with disease activity improving in approximately 70-80% of patients. Disease activity usually
decreases in the first trimester, and this lasts for a number of weeks to months into the postpartum period. Subsequently, 90% of patients will then experience a flare usually during the first 3 months.

Although case control studies of pregnancy outcome demonstrate a slight increase in spontaneous abortion in women with RA, most reports have failed to show an increase in fetal morbidity. Medication may potentially increase the risk, for example, steroids may restrict intrauterine growth. Women with active RA or other types of inflammatory arthritis may have children with lower birth weights.

None of the available drug treatments for RA are absolutely safe in pregnancy. A prescriber must carefully assess the risks and benefits of treatment in consultation with the patient. In patients with active RA during pregnancy, prednisolone is recommended at the lowest dose (below 20mg daily if possible) to control the disease. Sulphasalazine and hydroxychloroquine are considered safe to prescribe by most obstetric physicians.
2.3 *Cyathocline purpurea* (Buch-Ham ex D. Don.) Kuntze

![Figure 8: *Cyathocline purpurea* (Buch-Ham ex D. Don.) Kuntze](image)

### 2.3.1 Taxonomic Classification

- **Kingdom:** Plantae
- **Phylum:** Magnoliophyta
- **Class:** Magnoliopsida
- **Order:** Asterales
- **Family:** Asteraceae
- **Genus:** Cyathocline
- **Species:** Cyathocline purpurea
- **Part used:** Whole plant

### 2.3.2 Botanical description and Vernacular name

*Cyathocline purpurea* is one of the most valuable plant in traditional system of medicine from ancient time. Synonyms of *Cyathocline purpurea* (Buch-Ham ex D. Don.) Kuntze are *Tanacelum purpureum*, *Cyathochine lyrata*.

*Cyathocline purpurea* is also called as,

- Bandhaniya in Hindi,
- Gangotra in Marathi,
- Gal Phulle in Nepali,
- Hong Hao Zhi in Chinese.
2.3.3 Morphology (Joshi, 2013)

*Cyathocline* is a genus of flowering plants in the daisy family i.e. Asteraceae. The family Asteraceae includes 1100 genera and 30000 species. In India, the family is represented by 167 genera and 980 species. In majority of the members of the family sesquiterpene lactones are present which are known for their anti-infective property (anticancer, antifungal, anti-inflammatory, antibacterial, antiviral, antimalarial and antiageing activity).

*Cyathocline purpurea* is an erect, strongly aromatic, glandular hairy annual or biennial herb, upto 60 cm high. The leaves are sessile, variable in size and shape. Leaf segments are irregularly serrate. Heads are small, in panicles, dark purple. Stem is branched grooved. Flower heads 0.3 to 0.6 cm across in terminal corymbose panicles; acute, hairy on margins. The stems of this plant are usually reddish or purplish tinged, branched from the base and glandular-pubescent. Whole plant is aromatic and is commonly observed as undergrowth in open spaces especially near wet surface; rice fields or on the banks of river streams.

2.3.4 Occurrence and Distribution

*Cyathocline purpurea* is a rare existence Indian medicinal plant which is not popular among the local inhabitants and commonly found in moist habitats such as along water courses and in rice fields throughout most of peninsular and northern India at an elevation of 1300m. It is also available in the western and central Himalayas. *Cyathocline* is a small genus with two or three species and distributed in tropical Asia. The genus was described in 1829 by Cassini as *Cyathocline purpurea* (Buch – Ham. ex D. Don.) Kuntze. *Cyathocline purpurea* is native to India and south-western China and is a traditional medicine.

2.3.5 Phytoconstituents

*Cyathocline purpurea* (Buch-Ham ex D. Don.) Kuntze is reported to contain guaianolide (Chintalwar et al., 1991), eudesmanolide, sesquiterpene lactones, isoivangustin, santamarine, 9 β-acetoxycostunolide and 9 β-acetoxyparthenolide (Nagasampagi et al., 1981).
Sesquiterpene lactones are secondary metabolites that belong to the group of C15 terpenoids. They are formed from three isoprene units. This is a large group of secondary metabolites. So far, over 90% of identified lactones were isolated from the plant family Asteraceae. Sesquiterpene lactones were also isolated from the more primitive representatives of families such as Magnoliaceae and Lauraceae, but also from the more derivative groups such as Apiaceae. Sesquiterpene lactones were formed in a pathway from isoprene C5 units. All of them are modifications of germacranolide, a compound that forms from germacradien. Germacranolides are precursors for most of the more derivative lactones, such as guaianolides, pseudoguaianolides, eudesmanolides etc. The first identified lactones were artemisinin and costunolide, which do not belong to guaianolides. They were both isolated from the plant family Asteraceae.

2.3.6 Pharmacological activities reported of *Cyathocline purpurea* (Buch-Ham ex D. Don.) Kuntze

**Anticancer** (Guoyi et al., 2009)

*Cyathocline purpurea* is used in traditional Chinese medicine as an herbal remedy for human tuberculosis, malaria and bleeding (Yu et al., 1993). The traditional medicinal practitioners of the Hani ethnic minority in Yunnan, China also commonly use this plant to treat various cancers. Three sesquiterpene lactones, santamarine, 9β-acetoxycostunolide and 9β-acetoxyparthenolide were isolated from *Cyathocline purpurea* by bioactivity-guided fractionation (Li et al., 2006). Sesquiterpene lactones are a class of natural sesquiterpenes which are chemically distinct from other members of the group through the presence of a γ-lactone system and have a wide range of biological activities including mutagenic, genotoxic, cytotoxic and antitumour actions (Rodriguez et al 1976; Picman, 1986). Many sesquiterpene lactones have shown significant antineoplastics effects (Lee et al., 1971). Santamarine and 9β-acetoxycostunolide are sesquiterpene lactones with the α-methylene-γ-lactone moiety in their structure. There have been no reports of cytotoxicity with new compounds of 9β-acetoxycostunolide and 9β-acetoxyparthenolide.

Santamarine and 9 β-acetoxycostunolide inhibited mitosis and reduced thymidine uptake in L1210 cells. The mechanism of action of santamarine and 9 β-
acetoxycostunolide might be related to suppression of microtubular protein formation and activation of caspase 3, induction of cell cycle blockage and apoptosis. It has been mentioned that *Cyathocline purpurea* has been traditionally used to treat various diseases related to inflammation including cancers for many years without any reports of toxicity to humans (Yu et al., 1993), suggesting that it is not harmful to humans. This investigation provides pharmacological support to its use in cancers.

**Antibacterial** (Joshi, 2013)

The essential oils from the roots of *Cyathocline purpurea* were screened *in vitro* for antibacterial activity against eight human pathogenic bacteria. The essential oil of roots was analyzed by using GC–FID and GC–MS. The antibacterial activity of oil was tested against four Gram-positive and four Gram-negative bacteria and antibacterial activity was determined by the tube dilution method. The main constituents of the oil were thymohydroquinone dimethyl ether (57.4%) and β-selinene (14.0%), among twenty five identified compounds, which represented 90.1% of the total oil. The oil was found to be active against Gram-positive bacteria with minimal bactericidal concentration (MBC) values in the range of 0.26–0.57 mg/mL. The observation of MBC assay suggested that the Gram positive microorganisms were susceptible to essential oil, while oil was found to be resistant against Gram-negative bacteria, and the oil has bactericidal property.

The roots of *Cyathocline purpurea* are used to relieve stomach pains (Parrotta, 2001). This plant releases an essential oil that is reportedly owns antimicrobial, anthelmintic and hypotensive properties (Parrotta, 2001). *Cyathocline purpurea* is used to treat pulmonary tuberculosis (Qiang et al., 2006).

Therefore, based on the primary information available on this plant, further series of studies like isolation and identification of active constituents, pharmacological standardization of extracts and activities on isolated compounds as well as clinical and toxicological efficacy is still remained to explore so far. Sesquiterpene lactones isolated from other plants have been found to possess good anti-inflammatory activities (Hall et al., 1980). As the literature survey shows that *Cyathocline purpurea* may show anti-inflammatory activity due to presence of sesquiterpene lactones and be
utilized for treating pathological states like arthritis (Joshi et al., 2010) and up till now there were no studies reported on *in-vivo* activity of this plant as anti-inflammatory and antiarthritic; therefore the objective of the present study was to determine the efficacy of *Cyathocline purpurea* (whole plant) as analgesic, anti-inflammatory and antiarthritic in different animal models.