1. INTRODUCTION

Mother Nature has gifted natural products very long ago and it has been present in the ecosystems for over a billion years. Hundreds of plant metabolites are reported for their pharmacological activities. Although most of these reports are of academic interest, yet a very few find entry at clinical trials. Screening of natural products would help to endorse their acceptance and use of plant based drugs in mainstream of medicine.

Medicinal plants are renowned for their reputed medicinal properties. According to World Health Organization, about 80% of the world population still uses herbs and other traditional medicines for their primary health care needs. Correct identification and quality assurance are essential to ascertain the medicinal value of herbal drugs. Authentication is chiefly used in case of medicinal herbs commonly substituted or adulterated with other species or varieties which are morphologically and phytochemically indistinguishable. Like the same, several herbal drugs on the market still cannot be identified or authenticated based on their morphological or histological characteristics. Use of a wrong herb may be ineffective or it may worsen the condition (Khan et al. 2010).

Medicinal plants play a key role in world health. Even though there is an immense growth in modern medicine; plants still make an important contribution to health care. Each plant is like a factory competent of synthesizing infinite number of highly complex chemical substances. About 120 distinct chemical substances are in use throughout the world which are derived from plants and several other drugs are simple synthetic modifications of the natural products. All over the world herbal industries are growing tremendously and more number of
herbal products is emerging in the market. The safety and efficacy of herbal products depend on the standardization.

WHO also insists the importance of the qualitative and quantitative methods for characterizing the samples, quantification of the biomarkers, chemical markers and fingerprint profiles. Standardization as defined by American Herbal Product Association (AHPA), “The body of information and controls that serves to optimize the batch-to-batch consistency of a botanical product”. The traditional approach towards standardization is insufficient for current herbal product market and hence there is a need for more advanced techniques used in standardization. (Pravin 2012)

Rheumatoid Arthritis (RA) is a chronic autoimmune inflammatory disease that affects the joints and other tissues in the body. The disease prognosis starts from swelling of joints, synovial tissue inflammation and subsequent damage to the cartilage. About 1% of the population has been affected throughout the world. The epidemiology of RA in female to male is 3 to 1 and the crest age of RA is at the adult and the following phase. The etiology of RA is multifactorial. The disease can occur as a consequence of complex interactions between genetic, hormonal, immunological, infective, environmental and physiological factors.

Non-Steroidal Anti-Inflammatory Drugs (NSAIDs), steroidal agents, Disease Modifying Anti-Rheumatoid Drugs (DMARDs), corticosteroids and immuno-suppressants are usually used for RA treatment which will reduce the pain, joint inflammation, minimize loss of function and decrease the progression of joint damage. However, the side effects and toxicity was exclaimed for unconventional, secure and more effectual natural product based drugs (Zahidah et al. 2012).
Some clinical studies reveal that the diverse arthritic and inflammatory conditions can be managed efficiently through specific herbal agents, dietary and supplementation practices, in conjunction with other natural treatments including chiropractic, massage, exercise, etc. Unlike conventional anti-inflammatory/anti-arthritic drugs, natural compounds do not show damage to the gastro intestinal tract, liver, kidney and do not accelerate joint cartilage damage (Simon 1999).

In India many ayurvedic practitioners use various indigenous plants for treatment of different types of arthritic conditions. Although the application of these medicaments has a sound tradition and a rational background according to the Indian system of medicine, perhaps it is essential to investigate the rationality of their use in modern scientific terms.

1.1 STANDARDIZATION OF MEDICINAL PLANTS

As commercialization of the herbal medicine has happened, assurance of safety, quality and efficacy of medicinal plants and herbal products have become an important issue. The herbal raw material is prone to a lot of variation due to several factors, the important ones being the identity of the plants and seasonal variation, the ecotype, genotype and chemotypic variations, drying and storage conditions and the presence of xenobiotics (Dixit & Yadav 2008).
Methods of standardization should take into consideration all aspects that contribute to the quality of the herbal drugs, namely correct identity of the sample, organoleptic evaluation, pharmacognostic evaluation, volatile matter, quantitative evaluation (ash values, extractive values), phytochemical evaluation, test for the presence of xenobiotic, microbial load testing, toxicity testing and biological activity. Of these, the phytochemical profile is of special significance since it has a direct bearing on the activity of the herbal drugs. The fingerprint profile serves as the guideline to the phytochemical profile of the drug in ensuring the quality, while quantification of the marker compounds would serve as an additional parameter in assessing the quality of the sample (Calixto & Braz 2000).

Markers are mainly categorized into two classes: DNA markers and chemical markers. DNA markers are reliable for informative polymorphism as the genetic composition is unique for each species. Chemical markers generally refer to phytochemical constituents including primary and secondary metabolites. DNA markers are used in authentication of medicinal plants and their breeding etc. Chemical markers help in identification of adulterants, confirmation of collection site, quality evaluation and diagnosis of herbal intoxication. Chemical markers are pivotal in the current practice of quality control (Aanchal 2012).

1.1.1 DNA Fingerprinting

DNA analysis has been proved as an important tool in herbal drug standardization. This technique is useful for the identification of phytochemically indistinguishable genuine drug from substituted or adulterated drug. It has been reported that DNA fingerprint genome remained the same irrespective of the plant part used while the phytochemical content varied with the plant part used, physiology and environmental conditions (Shikha & Mishra 2009). This concept of
fingerprinting has been increasingly applied in the past few decades to determine the ancestry of plants, animals and other microorganisms.

Genotypic characterization of plant species and strains is useful as most plants, though belonging to the same genus and species, may show considerable variation between strains. Additional motivation for using DNA fingerprinting on commercial herbal drugs is the availability of intact genomic DNA from plant samples after they are processed. Adulterants can be distinguished even in processed samples, enabling the authentication of the drug (Mihalov et al. 2000). The other useful application of DNA fingerprinting is the availability of intact genomic DNA specificity in commercial herbal drugs which helps in distinguishing adulterants even in processed samples (Lazarowych & Pekos1998).

To ensure efficacy, selection of the correct chemotype of the plant is necessary even when there are many known chemotypes of a plant species, selection of the right chemotype to which clinical effects are attributed is difficult. DNA markers are reliable for informative polymorphism as the genetic composition is unique for each species and is not affected by age, physiological condition as well as environmental factors.

DNA can be extracted from fresh or dried organic tissue of the botanical material; hence the physical form of the sample for assessment does not restrict detection. Various types of DNA-based molecular techniques such as hybridization based methods, Polymerase Chain Reaction (PCR) based methods and sequencing-based methods are utilized to evaluate DNA polymorphism(Cai et al. 1999).

1.2 ROLE OF FREE RADICALS AND ITS EFFECT
Free radicals are chemical species which contains unpaired electrons, highly unstable and reactive in nature. They capture the electrons from the nearest stable molecules making them unstable or become free radical thereby cause damage to the body. Free radicals are generated as part of the body's normal metabolic process and play a dual role in our body as both harmful and useful agent. Excess production of Reactive Oxygen Species (ROS) may lead to the tissue damage and different diseases (Leong et al. 2008).

Figure 1.1 Formation of free radicals

Free radicals are incessantly produced by the body's normal use of oxygen. The cell produces energy by the use of oxygen, during which the free radicals are liberated by the mitochondria. These by-products are commonly called as ROS as well as Reactive Nitrogen Species (RNS).
ROS can be classified into oxygen-centered radicals and oxygen-centered non radicals. Oxygen-centered radicals are superoxide anion (O$_2^-$), hydroxyl radical (OH), alkoxy radical (RO·), and peroxo radical (ROO·). Other reactive nitrogen species are nitrous oxide (NO·), nitric dioxide (NO$_2^-$), and peroxynitrite (OONO$^-$). Oxygen centered non-radicals are hydrogen peroxide (H$_2$O$_2$) and singlet oxygen (O$_2$), Hypochlorous acid and Ozone(Simon et al. 2000) (Figure 1.1).

Both ROS and RNS are produced in a well regulated manner to maintain homeostasis at the cellular level in the normal healthy tissues and play an important role as signaling molecules. Low concentration of ROS is essential for normal physiological functions like gene expression, cellular growth and defense against infection. Sometimes they also act as the stimulating agents for biochemical processes within the cell. But the exceeding level of these ROS leads to cell damage (Droge 2002).

Cellular membranes are vulnerable to the oxidation by ROS due to the presence of high concentration of unsaturated fatty acids in their lipid components. ROS interaction with membrane lipids cause lipid peroxidation, resulting in formation of lipid hydroperoxide (LOOH) which can further decompose to an aldehyde such as malondialdehyde,4-hydroxy non-enal or cyclic endoperoxide, iso-protons and hydrocarbons. The consequences of lipid peroxidation are cross linking of membrane proteins, change in membrane fluidity and formation of lipid-protein, lipid-DNA adduct which may be detrimental to the functioning of the cell (Beckman & Ames 1997).

Free radicals caused by ROS leads to several damaging effects as they can attack lipids, protein/ enzymes, carbohydrates and DNA in cells and tissues. They induce undesirable
oxidation, causing membrane damage, protein modification, DNA damage and cell death induced by DNA fragmentation and lipid peroxidation.

In a normal cell, the oxidant: antioxidant balance is important. This balance gets affected, when production of free radicals is increased or levels of antioxidants are decreased. This is called as oxidative stress. Oxidative stress resulted in damage to nucleic acids, proteins, polyunsaturated fatty acids and carbohydrates. Oxidative stress causes serious cell damage leading to a diversity of diseases like Alzheimer’s disease, Parkinson’s disease, atherosclerosis, cancer, arthritis, immunological incompetence, neurodegenerative disorders, etc. (Peterhans 1997). Almost all organisms are well protected against free radical damage by oxidative enzymes such as superoxide dismutase (SOD) and catalase (CAT) or by chemicals such as α-tocopherol, ascorbic acid, carotenoids, polyphenols and glutathione. When the process of antioxidant protection becomes unbalanced, deterioration of physiological functions may occur, resulting in diseases and accelerated aging (Gulcin et al. 2002).

1.3 ANTIOXIDANTS FROM NATURAL SOURCE

An antioxidant is a chemical that prevents the oxidation of other chemicals. They protect the key cell components by neutralizing the damaging effects of free radicals. Antioxidants play a major role in protecting our body from disease by reducing the oxidative damage to cellular component caused by ROS (Huda et al. 2009).

Recent investigations proposed that the plant based antioxidants may have great therapeutic significance in free radical mediated diseases like diabetes, cancer, neurodegenerative disease, cardiovascular diseases, aging, gastrointestinal diseases, arthritis and aging process. Many synthetic antioxidant compounds have shown toxic and/or mutagenic
effects, while relatively plant-based medicines produce fewer side effects than the synthetic drug in some instances.

In recent years, there has been an increased interest in the development of "Natural Antioxidants". Various reports showed that plant derived products play a vital role in scavenging free radicals. Antioxidants protect the human body against free radicals that may cause pathological effects such as ischemia, asthma, anaemia, inflammation, neuro-degeneration and parkinson’s diseases (Narendhirakannan & Smeera 2010).

There are proven results that plant products such as flavonoids, polyphenols, terpenes exerted antioxidant activity (Asha et al. 2012). Phenolic compounds from medicinal plants possess strong antioxidant activity and may help to protect the cells against oxidative damage caused by free-radicals (Kahkonen1999). They are well known as radical scavengers, metal chelators, reducing agents, hydrogen donors and singlet oxygen quenchers (Preostos et al. 2006). Medicinal plant parts are commonly rich in phenolic compounds such as flavonoids, phenolic acids, stilbenes, tannins, coumarins, lignans and lignins. These compounds have multiple biological effects including antioxidant activity (Packer 1999).

Presently, much attention has been focused on the use of natural antioxidants to protect the human body especially brain tissues from the oxidative damage caused by free radicals. In the last two decades, several medicinal plants have shown such effectiveness through the traditional methods of psychoneuropharmacology (Dhawan 1995).

1.4 INFLAMMATION– PROCESS AND MEDIATORS
Inflammation is a part of the complex biological response of body tissues to harmful stimuli (Ferrero et al. 2007). It is a protective response that involves immune cells, blood vessels and molecular mediators. It can be classified as acute and chronic. Acute inflammation is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes from the blood into the injured tissues.

The process of acute inflammation is initiated by cells already present in the tissues. This is characterized by marked vascular changes, including vasodilatation and increased capillary permeability which are induced by the actions of the various inflammatory mediators (Okoli et al. 2006).

A series of biochemical events propagates and matures the inflammatory response, involving the local vascular system, the immune system and various cells within the injured tissue. Prolonged inflammation known as chronic inflammation, leads to a progressive shift in the type of cells present at the site of inflammation and is characterized by simultaneous destruction and healing of the tissue from the inflammatory process (Eming et al. 2007).
Figure 1.2 Steps involved in the inflammatory process
Figure 1.3 Mediators involved in inflammatory process

During inflammation, the cells undergo activation and release inflammatory mediators such as histamine, serotonin, Slow Reacting Substances of Anaphylaxis (SRS-A), prostaglandins and some plasma enzyme systems such as the complement system, the clotting system, the fibrinolytic system and the kinin system (Perianayagam et al. 2006). These mediators cause increased vasodilatation and permeability of blood vessels leading to blood flow, exudation of plasma proteins and fluids, migration of leukocytes, mainly neutrophils, outside the blood vessels into the injured tissues (Chaitanya et al. 2011).

The inflammatory response consists of an innate system of cellular and humoral responses following injury in which the body attempts to restore the tissue to its preinjury state. In the acute inflammatory response, there is a complex orchestration of events involving leakage of water, salt and proteins from the vascular compartment; activation of endothelial cells; adhesive interactions between leukocytes and the vascular endothelium; recruitment of leukocytes; activation of tissue macrophages; activation of platelets and their aggregation; activation of the complement; clotting and fibrinolytic systems; and release of proteases and oxidants from phagocytic cells, all of which may assist in coping with the state of injury (Figure 1.2). The earliest in vivo hallmark of the acute inflammatory response is the adhesion of neutrophils (Polymorphonuclear leukocytes) to the vascular endothelium.

Chronic inflammation may develop following acute inflammation and may last for weeks or months, and in some instances for years. During this phase of inflammation, cytokine interactions result in monocyte chemotaxis to the site of inflammation where Macrophage Activating Factors (MAF), such as Interferon-gamma (IFN-γ), Monocyte Chemo-attractant
Protein-1 (MCP-1), and other molecules activate the macrophages while Migration Inhibition Factors (MIF), such as Granulocyte-Macrophage -Colony Stimulating Factor (GM-CSF) and IFN-γ, retain them at the inflammatory site. The macrophages contribute to the inflammatory process by chronically elaborating low levels of Interleukin-1 (IL-1) and Tumour Necrosis Factor-α (TNF-α) (Figure 1.3), which are responsible for some of the resulting clinical symptoms such as anorexia, cachexia, fever, sleepiness and leukocytosis (Charles et al. 2010).

1.4.1 Search for Natural Anti-Inflammatory Agent

Unlike modern allopathic drugs which are single active components that target one specific pathway, herbal medicines work in a way that depends on an orchestral approach. Plant contains a multitude of different molecules that act synergistically on targeted elements of the complex cellular pathway (Kumar et al. 2013).

Chronic inflammation lies at the basis of many diseases of advanced age such as heart attacks, Alzheimer’s diseases, and cancer. The drugs used to reduce inflammation are Non-Steroidal Anti-Inflammatory Drugs (NSAIDs). These drugs block Cyclooxygenase-1 (COX-1) and Cyclooxygenase - 2 (COX-2) enzyme activity. COX enzymes assist with prostaglandin production.
NSAIDs and steroidal anti-inflammatory drugs are being used till now, long term uses of these drugs cause adverse side effects and damage human biological system such as liver, gastrointestinal tract, etc. Now there is a need for the new safe, potent, nontoxic or less toxic anti-inflammatory drug from plant source (Deepa & Renuka 2014).

The inherent problems associated with the current non-steroidal as well as steroidal anti-inflammatory agents leads to continuous search for alternative agents from natural source. Several herbal medicines constitute a potentially important avenue leading to novel therapeutic agents for inflammation and RA that may not only prevent structural damage of arthritic joints caused by tissue, which are inexpensive, highly tolerated and convenient for many patients (Agnihotri 2010).

1.5 ARTHRITIS

Arthritis is a common inflammatory joint disease characterized by inflammation of the synovial membrane, pain and restricted joint movement. It mainly affects people of age group between 40 and 50 years. There are about 100 types of arthritis reported. But most commonly occurring arthritis are as follows.

1. Osteo-arthritis

2. Rheumatoid arthritis

3. Gouty arthritis

4. Ankylosing spondylitis

5. Juvenile arthritis

Figure 1.4 Arthritis
The prevalence of osteo-arthritis is 22-39% and rheumatoid arthritis is 5% in India. The weight bearing joints such as feet, knees, hips and spine are generally affected (Figure 1.4) (Mujapara et al. 2009).

![Normal and Arthritic joints](image)

**Figure 1.5 Normal and Arthritic joints**

**Osteoarthritis:**

Osteoarthritis is a degenerative joint disease, resulting from the wear and tear from day-to-day life. It leads to pain, tenderness, swelling and decreased function of joints (Figure 1.5). The joints most often affected by osteoarthritis are knees, hips, hands and spine.

**Rheumatoid arthritis:**

Rheumatoid arthritis is an autoimmune disease that occurs when the body’s own immune system mistakenly attacks the synovium (cell lining inside the joint) causing joint pain, stiffness, swelling, and loss of joint function (Figure 1.5).
**Gouty arthritis:**

Gout is the clinical manifestation of disturbed purine metabolism. It is characterized by deposition of uric acid salts – especially sodium biurate in connective tissues such as cartilage, the walls of bursae and ligaments.

**Ankylosing spondylitis:**

It is primarily a disease of the spine, though in few cases, the arthritic changes involve also the proximal joints of the limbs, especially the hips. It is a chronic inflammatory affliction of the joints and ligaments of the spine, beginning in the sacro-iliac joints.

**Juvenile rheumatoid arthritis (JRA):**

It is an autoimmune, non-infective, inflammatory joint disease of more than 6 weeks duration in children less than 16 years of age. The disease commonly occurs in children from the ages of 7 to 12, but it may occur in adolescents as old as 15 years of age, as well as in infants(Alan et al. 1999).

1.5.1 Clinical Features of Rheumatoid Arthritis

It may affect any joint but the incidence is higher in the more peripheral joints such as the hand joints, wrists, feet, knees and elbows than in the lumbar or thoracic spine, shoulders or hips. The onset is gradual, with increasing pain and swelling of a joint. Soon a number of other joints are similarly affected. Pain and stiffness are often worse when activity is resumed after resting. Often there is constitutional disturbance, with tiredness, anemia and occasionally fever.
On examination, the affected joints are swollen from synovial thickening. The overlying skin is warmer than normal. The range of joint movements is restricted; movement causes pain, especially at the extremes.

### 1.5.2 Pathology of Rheumatoid Arthritis

The synovial membrane is thickened by chronic inflammatory changes; characteristically it is infiltrated with macrophage-like cells and T-cell lymphocytes, later the articular cartilage is gradually softened and eroded and the subchondral bone is also eroded, characteristically at the joint margins – probably from the action of lytic enzymes and inflammatory mediators produced in the thickened synovial membrane.

The eroded surfaces become covered by a soft membrane of inflammatory tissue known as ‘pannus’. The contained tendons may become softened, rupture and aggravate existing deformity. Inflammatory nodules may form in the soft tissues. After a month or year of activity the disease process tends to become less active, usually leaving a number of joints that are permanently damaged with consequent deformity, instability or ankylosis.

### 1.5.3 Treatment for Rheumatoid Arthritis

Method of treatment may be classified into the following categories:

1. Rest and constitutional treatment
2. Drugs
3. Intra-articular injections of hydrocortisone
4. Physiotherapy
5. Occupational therapy

6. Surgery

Drugs

1. Analgesics

Paracetamol, Opioids – Codeine, Trihydro codeine, Tramadol

2. Non-Steroidal Anti-Inflammatory Drugs (NSAIDs)

Diclofenc, Ibuprofen, Naproxen, Celecoxib, Etoricoxib

3. Disease-Modifying Anti-Rheumatic Drugs (DMARDs)

Monotherapies - Methotrexate, Sulfasalazine, Leflunomide

Combination Therapies - Hydroxychloroquine, Cyclosporin

In Systemic Disease - Azathioprine, Cyclophosphamide

Rarely Used - Injectable gold, Penicillamine, Auranofin

4. Biologics

Tumour Necrosis Factor inhibitors- Infliximab, Etanercept, Adalimumab, Certolizumab, Golimumab

B-cell inhibitor -Rituximab

T-cell inhibitor -Abatacept
Drugs used in rheumatoid arthritis fall mainly into the categories of NSAIDs and the potent anti-inflammatory agents are grouped under corticosteroids. A logical plan is to use aspirin since it has both analgesic and mild anti-inflammatory properties, but to be effective it may have to be given in fairly large doses. Alternative first-line drugs are from NSAIDs, which includes indomethacin, ibuprofen, naproxen, phenol profen and piroxicam. Due regard must be paid to the risk of side effects: gastric pain is a common complaint with most of these drugs and more serious complications, such as gastric bleeding are seen occasionally.

A different class of anti-rheumatic agents, to be regarded as second-line drugs, includes the potentially toxic group of compounds containing gold salts, usually given by intra-muscular injection. These must be used with care, but they are thought to be beneficial and therefore justified in severely afflicted patients who have failed to respond to the first-line drugs.

Sulfasalazine, a derivative of sulphapyridine, is now a second –line drug of choice. Its mode of action is uncertain, but it has the advantage over gold salts of oral administration and fewer side effects. Another drug in this category is penicillamine, the effect of which is comparable to that of gold. This is also potentially toxic for the kidneys and must be used with caution; also this category includes certain immuno-suppressive agents, such as azathioprine and methotrexate.

Newer developments in drug therapy have centered on biological molecule manufactured by genetic engineering that can block or reduce the production of destructive cytokines and enzymes from the cells of the rheumatoid synovium. The first of these, anti-TNF has given very
encouraging results in modifying the disease, but no long-term results are yet available. The place of corticosteroids in rheumatoid arthritis is still controversial. There is a wide agreement that because of their serious side effects they should be avoided altogether in the great majority of patients. There may be a small proportion of severely afflicted patients in whom the advantages outweigh the hazards.

**Intraarticular injections**

Injections of corticosteroids (usually hydrocortisone) into and affected joint can produce worthwhile relief, but the disadvantages have precluded their widespread use. The main disadvantages are: risk of infection, risk of accelerating a degenerative reaction, short duration to the relief obtained, repeated injections at several sites may become irksome to the patient as to be unacceptable (Raashid et al. 2008).

1.5.4 Cellular Mediators Involved in Inflammation and Arthritis

1.5.4.1 Inflammatory cytokines

Inflammation is mediated by a variety of soluble factors, including a group of secreted polypeptides known as cytokines. Inflammatory cytokines can be divided into two groups: those involved in acute inflammation and those responsible. Inflammation, the response of tissue to injury, is characterized in the acute phase by increased blood flow and vascular permeability along with the accumulation of fluid, leukocytes, and inflammatory mediators such as cytokines. In the sub-acute / chronic phase (hereafter referred to as the chronic phase), it is characterized by the development of specific humoral and cellular immune responses to the pathogens present at the site of tissue injury. During both acute and chronic inflammatory processes, a variety of
soluble factors are involved in leukocyte recruitment through increased expression of cellular adhesion molecules and chemo-attraction (Carol 1997).

**Fig. 1.6 Schematic representation of events occurring in arthritis**

Many of these soluble mediators regulate the activation of the resident cells (such as fibroblasts, endothelial cells, tissue macrophages and mast cells) and the newly recruited inflammatory cells (such as monocytes, lymphocytes, neutrophils and eosinophils), and some of these mediators result in the systemic responses to the inflammatory process. Several cytokines play a key role in mediating acute inflammatory reactions, namely IL-1, TNF-α, IL-6, IL-11, IL-8 and other chemokines, and GM-CSF. The cytokines known to mediate chronic inflammatory processes can be divided into those participating in humoral inflammation, such as IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-10, IL-13, and Transforming Growth Factor-β (TGF-β), and those contributing to cellular inflammation such as IL-1, IL-2, IL-3, IL-4, IL-7, IL-9, IL-10, IL-12,
Interferons (IFNs), IFN-\(\gamma\) inducing factor (IGIF), TGF-\(\beta\), and TNF-\(\alpha\) and TNF-\(\beta\) (Lin & Karwin 2007).

TNF-\(\alpha\) also triggers the production of other cytokines and endothelial adhesion molecules, stimulates collagenase and induces osteoclast differentiation leading to bone erosion. Therefore, IL-1, IL-2, and TNF-\(\alpha\) have been shown as the prevailing agents in the induction of inflammation and bone erosion (Yan et al. 2009).

**Tumor Necrosis Factor-\(\alpha\) (TNF-\(\alpha\))**

TNF-\(\alpha\) is a cell signaling protein involved in systemic inflammation and is one of the cytokines that make up the acute phase reaction. It is produced chiefly by activated macrophages, although it can be produced by many other cell types such as CD4(Cluster of Differentiation type 4 cells) lymphocytes, Natural Killer cells (NK cells), neutrophils, mast cells, eosinophils, and neurons. It plays an important role in inflammation. It stimulates neutrophils to transcribe and release cytokines and chemokines biosynthesis (Marucha et al. 1991)

TNF-\(\alpha\) is a pleiotropic cytokine, which plays a critical role in both acute and chronic inflammation. The formation of a number of small molecular mediators of inflammation is linked with TNF-\(\alpha\) and thus contributes to the range of mediators that critically control inflammation. It facilitates inflammatory cell infiltration by promoting the adhesion of neutrophils and lymphocytes to endothelial cells. When TNF-\(\alpha\) is specifically blocked, the severity of inflammation is reduced (Holtmann et al. 2002a) (Holtmann et al. 2002b).

Several lines of recent evidence have suggested that proinflammatory cytokines such as TNF-\(\alpha\) and IL-1 play a pivotal role in the pathogenesis of RA because they are increased in the synovial tissue, synovial fluid and serum of RA patients. IL-1 and TNF-\(\alpha\) contribute to
synoviocyte self-proliferation and increase the production of tissue enzymes such as matrix metalloproteinase via chondrocytes and synovial cells; resulting in cartilage degradation (Chu1991).

Figure. 1.7 Cellular Mediators involved in arthritis
In the process of bone erosion, TNF-α triggers the production of other cytokines and endothelial adhesion molecules, stimulates collagenase and induces osteoclast differentiation (Bazzoni 1996). Furthermore, TNF-α exerts its arthritogenic potency through the induction of IL-1. Therefore, IL-1, IL-2, and TNF-α have been shown as the dominant players in the induction of inflammation and bone erosion (Bonecchi 1998). In fact, anti-TNF-α antibodies and soluble TNF-α receptors have proven to be effective in ameliorating RA (Newton & Decicco 1999).

Interleukin – 2 (IL-2)

Interleukin 2 (IL-2) is a type of cytokine signaling molecule in the immune system. It is a protein that regulates the activities of white blood cells (leukocytes, often lymphocytes) that are responsible for immunity. IL-2 is also known as T Cell Growth Factor (TCGF). It is secreted by activated CD4 and CD8 T cells, neurons, microglia and hematopoietic stem cells in response to antigenic or mitogenic stimulation. IL-2 is required for T-cell proliferation, Natural Killer cells (NK) cytolytic activity, differentiation of regulatory T cells, modulation of T helper (Th) cell differentiation and activation-induced cell death. Increased expression of IL-2 has also been implicated in inflammatory conditions such as inflammatory bowel disease and chronic liver diseases. IL-2 is both a immune stimulator and immune suppressor cytokine which efficiently controls the immune system to deal with autoimmunity and adaptive immune response (Gaffen & Liu 2004)

Transforming Growth Factor-β (TGF-β)
TGF-β is an important regulator of cell proliferation, differentiation, and formulation of the extracellular matrix (Litterio et al. 1997). It plays a vital role in regulating the extracellular matrix by decreasing degradation of matrix proteins through a reduction in protease synthesis and an increase in the synthesis of protease inhibitors (Roberts et al. 1988). Like many cytokines, TGF-β has both pro- and anti-inflammatory effects. It functions as a biological switch, antagonizing or modifying the action of other cytokines or growth factors. The presence of other cytokines may modulate the cellular response to TGF-β, and the effect may differ depending on the activation state of the cell. TGF-β is capable of converting an active site of inflammation into one dominated by resolution and repair. TGF-β often exhibits disparate effects with immune-enhancing activity in local tissues and immune-suppressive activity in the systemic circulation. TGF-β₁ suppresses the proliferation and differentiation of T cells and B cells and limits IL-2, IFN-γ and TNF-α production. TGF-β₁ acts as a monocyte/macrophage deactivator in a manner similar to IL-10. However, TGF-β is less potent an inhibitor than IL-10 and has little or no effect on IL-1 production (Kingsley 1994).

1.5.4.2 Cluster of Differentiation 4 (CD4) cells

CD4 is a glycoprotein found on the surface of immune cells such as T helper cells, monocytes, macrophages and dendritic cells. CD4 T helper cells are white blood cells that are an essential part of the human immune system. They are often referred to as CD4 cells, T-helper cells or T4 cells. They are called helper cells because one of their main roles is to send signals to other types of immune cells, including CD8 killer cells, which then destroy the infectious particle.
CD4 T helper (Th) lymphocytes can differentiate into functionally dichotomous subsets of Th cells depending on the microenvironment of the cell. The cytokine-producing CD4 helper cells are classified into Th1- and Th2-type cells on the basis of the cytokines produced. A similar functional system has been recently described with CD8 cytotoxic T cells (CD8 T1 and CD8 T2 cells). Th1-type cells secrete high levels of IL-2, TNF-α, and IFN-γ. This activates macrophages and promotes cell-mediated immune responses against invasive intracellular pathogens. Th2-type cells produce a variety of anti-inflammatory cytokines, including IL-4, IL-5, IL-6, IL-10, and IL-13. Both Th1 and Th2 cells produce lesser amounts of TNF-α, GM-CSF, and IL-3. Th2-type cytokines promote humoral immune responses against extracellular pathogens (Steven et al. 2000).

1.5.5 Search for Anti-Arthritic Agent from Natural Source

Treatment of RA has moved from conventional strategies such as NSAIDs, corticosteroids, immuno-suppressants, DMARDs to newer biological agents such as TNF-α and monoclonal antibodies. Despite the progress made in the treatment of disease, these treatments fail to produce long term benefits and produce serious adverse effects such as gastrointestinal ulcer, renal morbidity, cardiovascular complications, hematological toxicity and nephrotoxicity, which limit their utility in the treatment of the disease. Besides their side effects the current treatment is also of high cost, so patients suffering from chronic musculoskeletal disorders are likely to seek alternative methods for symptomatic relief. Thus complementary and alternative medicines may meet the requirement of large number of patients suffering from this disease (Manjusha 2014).
Agents derived from plants such as flavonoids, terpenes, quinones, catechins, alkaloids, anthocyanins and anthoxanthins, can modulate the expression of pro-inflammatory signals and thus act against arthritis (Vikrant 2011).

Traditionally, herbal plants were used both externally as well as internally for treating inflammatory conditions like arthritis. The positive influence of herbal medicine in modifying pathophysiology of arthritis has resulted in a substantial increase in their use as a treatment for arthritis. While an abundant number of herbal medicines are available to effectively reduce chronic joint inflammation in case of arthritis, it is essential to prove their therapeutic effects through basic scientific research (Dhaval 2013).

1.6 REFERENCES


