Chapter I

Introduction
Arthritis represents one of the most prevalent chronic health problems and is a leading cause of disability (Ashburn, 2002), it has been an estimated 42.7 million Americans in 1998—nearly one of every six people. By 2020, 60 million Americans are projected to have arthritis (Hootman et al., 2012; Feldmann et al., 1996). The prevalence of RA globally is 1% while in the Indian population is 0.6 - 1% (Akhter et al., 2011; Chopra et al., 1988), this would give a total of about seven million patients in India. The prevalence of RA in India is quite similar to that reported from the developed countries. It is higher than that reported from China, Indonesia, Philippines and rural Africa. (Malaviya et. al. 1993) Arthritis is second only to heart disease as a major cause of missed work (National Arthritis Action Plan: A Public Health Strategy, 1999).

Rheumatoid arthritis (RA) is a systematic, inflammatory autoimmune disorder that is characterized by swelling and pain in multiple joints. Articular inflammation causes activation and proliferation of the synovial lining, expression of inflammatory cytokines, chemokine-mediated recruitment of additional inflammatory cells, as well as B cell activation with auto antibody production (Altenaha et al., 2010; Sweeney and Firestein, 2004). Chronic inflammation of synovial joints and infiltration by blood derived cells, chiefly memory T cell, macrophages and plasma cells (Feldmann et al., 1996). Progressive destruction of cartilage and bone is believed to be mediated by the cytokine induction of destructive enzymes chiefly the matrix metalloproteinases (Distler et. al, 2005). The small joints of feet and hand are preferentially affected, and synovial inflammation may ultimately result in bony and cartilaginous destruction and deformity.

Oxidative stress is an imbalance produced when an abundance of pro-oxidant forces (such as free radicals and ROS) are present in the cell. This stress could be the main cause for cellular damage and has been implicated in various pathological conditions including rheumatoid arthritis. However, the human body has an intrinsic ability to fight against oxidative stress. The redox imbalance thus may be related to progression of disease stimulation. Excessive oxidative stress is thought to have an important role in the pathogenesis of rheumatoid arthritis (Kalpakcioglu et al., 2008).

Reactive oxygen species (ROS) are transient chemical moieties with enormous potential to react with different proteins and cellular elements. This property makes these species dangerous as they can alter the normal biological functions of proteins. Macrophages, neutrophils, lymphocytes and endothelial cells have been demonstrated to generate ROS after stimulation (Knight JA, 2000). Because inflammation leads to activation and stimulation of the latter, it is imperative that ROS have pivotal role to play.
in pathogenesis of RA (Lunec J, 1990). While all the cells are equipped with machinery to neutralise these species but under persistent and chronic conditions it becomes an unavoidable task. ROS can initiate lipid peroxidation in membranes which in turn can lead to the activation of many signal transduction elements (Glenn et al., 2008). ROS deplete the antioxidant pool and depletion of other defence enzymes, protein functional alteration can lead to degradation of cartilages (Gay et al., 1993) in specific and other synovial tissues in general.

Increased ROS production leads to tissue damage associated with inflammation (Afonso et al., 2007). They might perpetuate inflammation by facilitating the production of chemotactic factors at the local site (Shivaprasad et al., 2011; Merry et., 1989). The hydroxyl radical, superoxide anion radical, and the peroxynitrite anion are the major ROS generated during the disease condition (Cuzzocrea S., 2006). Reactive species like nitric oxide (NO) serve as an important messenger molecule in inflammatory conditions. Decreased production of NO via suppressing or inhibiting iNOS may reduce arthritic symptoms and afford protection (Khanna et al., 2007).

Inflammation is one of the most prevalent conditions limiting productivity and diminishing quality of life. It involves a complex set of interaction among soluble factors and cells that can arise in any tissue in response to traumatic, infectious, post-ischemic, toxic or autoimmune injury (Nathan, 2002). The accumulation and subsequent activation of leukocytes is one of the central events in the pathogenesis of all forms of inflammation. Leukocytes migrate to and accumulate at the site of inflammation by locally produced chemoattractants, and are then activated to secrete granular contents and to release active oxygen metabolites during chemotaxis and phagocytosis (Campo et al., 2003). These active metabolites cause damage to the underlying tissue. Warmth and redness result from dilation of the small blood vessels as they become more permeable during inflammation. Protein rich exudates escape from blood plasma to the damaged tissue causing swelling. Pain is believed to result from tension of tissue over the inflamed area, and the release of chemical substances such as serotonin and eicosanoids (Simjee et al., 2007).

In RA, the balance between pro-inflammatory and anti-inflammatory cytokines determines the degree and extent of inflammation. Proinflammatory cytokines like interleukin (IL)-1β, tumor necrosis factor (TNF) α and IL-6 are highly expressed in the rheumatoid joint and play key role in the pathogenesis of RA (Vaillancourt et al., 2011). Fibroblast like synoviocytes (FLS), in response to these cytokines produce chemokines,
metalloproteinases (MMPs), prostaglandin E\textsubscript{2} (PGE\textsubscript{2}) and cyclooxygenase-2 (COX-2) which further promote inflammation, hyperplasia and cartilage destruction (Chabaud et al., 2000; Hammaker et al., 2007).

Prostaglandins (PG), derived from the cyclooxygenase (COX) pathway, are among eicosanoids that play important role in the initiation of inflammation and pain. PG and other eicosanoids such as thromboxanes are produced as a result of arachidonic acid metabolism involving prostaglandin synthase (Vane, 1971). Systemic inhibition of COX leads to decreased production of PG at the site of inflammation (De Witt, 1991). Two isoforms of COX, COX-1 and COX-2, have been identified. COX-1 is an inducible isoform of the enzyme, while COX-2 isoform is prominent at the sites of inflammation (Copeland et al., 1995). COX-2 is constitutively expressed in the macula densa of kidney and in brain. Currently available nonsteroidal anti-inflammatory drugs (NSAID) act by inhibiting the activity of both forms of COX enzymes (Copeland et al., 1995). Another pathway have been found to involved in arachidonic acid metabolism and that contributes to the production of eicosanoids is lipoxygenase pathway (Ding et al., 2003), which is inhibited by antioxidants (Carpenter, 1981). The inhibitors of cyclooxygenase pathway such as acetylsalicylic acid (aspirin), and the inhibitors of lipoxygenase pathway such as antioxidants have been used as pain relieving and anti-inflammatory agents.

Many specific drugs for the treatment of major inflammation and/or acute pain like steroidal anti-inflammatory agents or narcotic analgesics are available. Diclofenac, for example, is a widely used anti-inflammatory drug (Santos et al., 2004). However, these drugs have side effects like GI perforations, bleeding, nephrotoxicity and many more problems not listed here. United States Food and Drug Administration (FDA) have infact indicated for a black box warning to be carried on the drugs for chronic inflammatory disorders. (Salahuddin et. al., 2005) An ideal anti-inflammatory drug should affect only the aberrant, uncontrolled inflammation by modifying inflammatory response to disease but not interfere with the normal inflammatory process, which is the part of the body's defense mechanism to its major environmental insults or invading microorganisms.

Experimental models of rheumatoid arthritis have been developed to improve our understanding of the deleterious mechanisms involved in degeneration of cartilage and bone and to study the potential efficiency of therapeutic strategies. On the basis of
the known mechanism, the drug selected for the animals may be used for rheumatoid arthritis patients.

Aims and Objectives

There is no definite treatment or cure for rheumatoid arthritis, current treatment modalities for RA either produce symptomatic relief (NSAIDs) or modify the disease process (DMARDs). Though effective, their use is also limited by their side effects including gastrointestinal ulcers and perforation, cardiovascular complications and emergence of opportunistic infections due to immunosuppressant (Nair et al., 2010). In the US, 100,000 hospitalizations and 16,500 deaths per year are linked to NSAID-induced ulcers and gastrointestinal bleeding in arthritic patients (Tawwab et al., 2011).

Identification of common dietary substances capable of affording protection or modulating the onset and severity of arthritis may have important human health implications. Recently, some studies have reported the effects of the administration of synthetic and naturally occurring compounds on the progression of CIA in experimental animals (Haqqi et al, 1999). The aim of the present proposal is therefore to add more to the knowledge in modulation of the disease by screening the therapeutic efficacy of natural compounds/ plant extract (Thymoquinone, Bostellia serrata extract and Piperine) against collagen induced arthritis in experimental animals. The search of the current therapy would not only provide symptomatic relief to the RA but also delay/stop the progression of the disease.

The study has been done with the following objectives:

1. Development of rat model of rheumatoid arthritis induced by collagen type II.
2. Evaluation of inflammatory markers in model of rheumatoid arthritis.
4. Evaluation of therapeutic intervention by natural compounds against rheumatoid arthritis.