Chapter 1

Introduction
1.1. Introduction

Inflammation is one of the first and most important responses of the immune system after infection. Inflammation, a necessary evil, also called a two sided sword, is a protective attempt from the organism to remove the injurious stimuli and to initiate the healing process (Simmons, 2006). However, when the inflammation process goes awry and continues to simmer in our body for a prolonged period and starts to become detrimental, it is known as chronic inflammation. Thus, the progressive destruction of the tissue would compromise the survival of the organism. Chronic inflammation can also lead to diseases, such as hay fever, atherosclerosis, and rheumatoid arthritis, etc.

Rheumatoid arthritis (RA) is a chronic and debilitating autoimmune disorder mainly affecting joints and characterized by synovial hyperplasia, inflammatory cell recruitment, and progressive destruction of cartilage and bone (Choi and Kim, 2008). Aberrant glycosylation is one of the molecular pathogenesis which have been recognized and well studied in RA (Raghav et al., 2006c). The collagen-induced arthritis (CIA) rat model is commonly studied autoimmune model for RA. These are used to evaluate potential new therapeutic agents. CIA is a T-cell dependent animal model of RA in which rats develop experimental arthritis after immunization with heterologous type II collagen (Rioja et al., 2004). Both CIA and RA share many clinical, histological and immunological features. This model is widely used to identify and validate the potential therapeutic agents for RA (Choi, 2007). Activation of cell mediated immunity in CIA rats result in the secretion of Th1-related cytokines and free radicals that further contribute to articular degeneration. This local and systemic inflammatory response also induce and activate oxidant generating enzymes like NADPH oxidase, xanthine oxidase, myeloperoxidase, etc. Furthermore, these enzymes produce reactive oxygen (superoxide anion) and nitrogen species like nitric oxide which can exaggerate the pathogenesis through initiation of lipid peroxidation, alteration of antioxidant enzymes and depletion of glutathione (Fay et al., 2006). Thus, it follows that suppression of these
inflammatory mediators and oxidative stress can be used to treat RA. Currently prescribed anti-arthritic drug regimen mainly relies on non-steroidal anti-inflammatory drugs (NSAIDs) and disease modifying anti-rheumatic drugs (DMARDs), which can effectively reduce the symptoms of the disease but simultaneously pose potential side-effects (Yeom et al., 2006). Therefore, there is a strong interest in the development of better anti-inflammatory therapeutic agents for RA that can prevent the progression of disease and confer safe prolonged treatment. One solution to this problem is the use of herbal therapies, which have been considered safe and effective in alleviating chronic pain associated with arthritis (Soeken et al., 2003a). Plant kingdom is a rich source of active components that have been shown to promote the specific cellular and humoral immune response in different ways. This property has increased the interest of the scientific community to discover and develop various therapeutic interventions to be used as medicine against several ailments.

However, there are various other approaches to control the disease of RA. Such as using the small molecular inhibitors against the inflammatory mediators like cytokines, such as TNF-α, IL1β, etc. These molecules may be small chemical inhibitors or peptides. Including RA, TNF-α plays a very crucial role in the progression of many other inflammatory diseases. Approaches to inhibit TNF-α induced inflammatory response are of therapeutic values.

Suramin, a small chemical compound is known to bind and inhibit TNF-α (He et al., 2005). It specifically promotes dissociation of the biologically active trimeric form of TNF-α into inactive subunits, for multiple therapeutic effects including antineoplastic activity (Alzani et al., 1993; Alzani et al., 1995). It is known as an antagonist of ATP at P2X purinergic receptors.

Previous work of our laboratory showed that the plant *Ruta graveolens* showed anti-inflammatory activity *in vitro* (Raghav et al., 2006b). Such activity is owed to the presence of many active components present in the plant, such as rutin, quercetin, and
other polyphenolic and alkaloid compounds (Ratheesh et al., 2010). One such active compound, Rg1 was isolated by Raghav et al (Raghav et al., 2007) by bioassay guided extraction and found to be anti-inflammatory using cell culture. In the present study, we have isolated a compound 8-methoxy-chromen-2-one (Rg3) from the same plant using the method previously used with slight modifications. Rg3 was evaluated in the cell culture and carried forward to assess its anti-arthritic potency using the rat CIA model.

TNF-α was reported to be present at high concentrations in the blood and synovial fluid of RA patients (Raghav et al., 2006a). Also, we know that to combat the severe arthritic conditions, TNF-α has been a potential drug target, the suppression of which is used to reduce inflammation. Here, peptides were used for the inhibition of the TNF-α activity. The effects of which was evaluated using various parameters explained ahead. Suramin is also a small molecule inhibitor of TNF-α. The effect of which was evaluated in CIA model of RA.
1.2. Objectives

Primary objective of this thesis work was to evaluate potential therapeutic agents, which are anti-inflammatory in nature and may be used against RA or other inflammatory diseases as a drug of choice. To fulfill this objective, following studies were systematically carried out:

1) Screening of medicinal plants for anti-inflammatory activity.
2) Isolation and purification of active compounds.
3) Evaluation of the isolated natural active compound for its anti-inflammatory activity in cultured cells.
4) Evaluation of the isolated active compound in the CIA model.
5) Evaluation of suramin for its anti-arthritic potency in CIA rats.
6) Synthesis and evaluation of small peptides as anti-TNF-α agents in cultured cells.