Summary of Conclusion

Chapter VII
Rheumatoid arthritis (RA), the most common form of chronic inflammatory polyarthritis, represents a significant health burden in the developed world. It is the leading cause of disability, and increase mortality. Rheumatoid arthritis occurs worldwide with a variable incidence and severity. It affects approximately 1% of the population worldwide and 0.6 – 1% in Indian population. However, these figures may be underestimated since patients with mild disease may never seek a medical opinion. The damage and deformity of the synovial joints characteristic of RA most commonly develops in the sixth decade but can occur at any age and will usually require treatments and interventions for the rest of an individual’s life.

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. Due to the long-term use, side effects are associated with these agents, patients with arthritis rely on other substitute like use of complementary and alternative medicine (CAM) and according to reports CAM therapy is on rise as 60-90% dissatisfied patients are likely to seek option of CAM therapy.

In RA, the balance between pro-inflammatory and anti-inflammatory cytokines determines the degree and extent of inflammation. Proinflammatory cytokines are highly expressed in the rheumatoid joint and play key role in the pathogenesis of RA. Fibroblast like synoviocytes (FLS), in response to these cytokines produce chemokines, metalloproteinases (MMPs), prostaglandin E2 (PGE2) and cyclooxygenase-2 (COX-2) which further promote inflammation, hyperplasia and cartilage destruction. There has been growing evidence that the generation of reactive oxygen species (ROS) and other reactive nitrogen species (RNS) might have a role in the pathogenesis of rheumatoid arthritis. Increased levels of reactive oxygen species (ROS) are the major cause of tissue injury after rheumatoid arthritis, in which inactivation of antioxidant enzymes and consumption of antioxidants such that endogenous antioxidant defense mechanisms fail to protect cells from oxidative damage. Oxidative stress is the state of imbalance between the level of antioxidant defense mechanism and production of the free radicals that favor the latter leading to potential damage. Therefore, agents derived from plants that can modulate the expression of pro-inflammatory signals and boost antioxidant system is one of the most promising avenues for arthritis.
The cause and pathogenesis of many chronic diseases including rheumatoid arthritis (RA) remain a mystery. Nevertheless, the identification of an animal model representative of human RA is critical to furthering our understanding of the disease pathophysiology and identifying new strategies for its treatment. Numerous experimentally induced models, ranging from adjuvant arthritis and streptococcal cell wall arthritis in the rat to murine models of collagen-induced arthritis and antigen-induced arthritis, have been characterized with the hope of reproducing clinical RA as a means to study this disease from several different viewpoints. The use of appropriate animal models is essential to predict the value and effect of therapeutic approaches in human subjects. Collagen-induced arthritis (CIA) is most widely used animal model of inflammatory polyarthritis with clinical and pathological features similar to human rheumatoid arthritis (RA). This has prompted us to critically review the current animal models and discuss how these models may yield fresh insights into the pathogenesis associated to rheumatoid arthritis, as well as new therapeutic opportunities.

Based on current knowledge regarding the etiology, pathogenesis and mechanism of tissue injury in rheumatoid arthritis. Therapeutic intervention might be provided by agents that interfere with factors involved in pathogenesis. To date, most basic and clinical trials have focused on antioxidants and anti-inflammatory agents.

Chapter IV
The present study was undertaken to investigate antioxidant and antiarthritic activity of Thymoquinone (TQ) in Wistar rat by collagen induced arthritis (CIA). TQ was administered at a dose of 5 mg kg⁻¹ body weight once daily for 21 days. The effects of treatment in the rats were assessed by biochemical (articular elastase, MPO, LPO, GSH, Catalase, SOD and NO), inflammatory mediators (IL-1β, IL-6, TNF-α, IL-10, IFN-γ, and PGE₂), expression of Cox-2 and histological studies in joints. TQ was effective in bringing significant changes on all the parameters (articular elastase, MPO, LPO, GSH, Catalase, SOD and NO) studied. Oral administration of TQ resulted in significantly reduced the levels of pro-inflammatory mediators (IL-1β, IL-6, TNF-α, IFN-γ, and PGE₂) and increased level of IL-10. Our data revealed that COX-2 expression was gradually reduced by TQ as compared with RA rats. The protective effects of TQ against RA were also evident from the decrease in arthritis scoring and bone histology.

In conclusion, this study suggest that the antiarthritic effect of thymoquinone on joints cartilage in CIA rats is probably mediated by the controlling pro- and anti-inflammatory cytokines, nitric oxide and antioxidant enzymes followed by the inhibition of accumulation and activation of PMN cells. These observations suggest that thymoquinone may be a clinically viable protective agent against a variety of inflammatory conditions like RA. We
believe that our results will contribute to the clinical applications in the treatment of rheumatoid arthritis.

Chapter V

Gum resin extracts of Boswellia species have been traditionally used in folk medicine for centuries to treat various chronic inflammatory diseases. Our aim of this work was to evaluate the antioxidant and antiarthritic activity of *Boswellia serrata* gum resin extract (BSE) in Wistar rat induced by collagen induced arthritis (CIA). BSE was administered at doses of 100 and 200 mg kg$^{-1}$ body weight once daily for 21 days. The effects of treatment in the rats were assessed by biochemical (articular elastase, MPO, LPO, GSH, Catalase, SOD and NO), inflammatory mediators (IL-1β, IL-6, TNF-α, IL-10, IFN-γ, and PGE$_2$), expression of NF-κB and histological studies in joints. BSE was effective in bringing significant changes on all the parameters dose dependently (articular elastase, MPO, LPO, GSH, Catalase, SOD and NO) studied. Oral administration of BSE resulted in significantly reduced levels of pro-inflammatory mediators (IL-1β, IL-6, TNF-α, IFN-γ and PGE$_2$), increased level of IL-10 and suppressed NF-κB activity. The protective effects of BSE against RA were also evident from the decrease in arthritis scoring and bone histology.

We have demonstrated that *Boswellia serrata* resin is a major anti-inflammatory agent in herbal medicines as well as a common food supplement. Its anti-inflammatory activity has been attributed to boswellic acid and its derivatives. It is practically non-toxic in rats, with the high margin of safety exhibited by the extract in the present study. The findings also suggest that the administration of *Boswellia serrata* extract to RA rats markedly inhibited clinical sign of joint swelling, significantly decrease the free radical load, modulate inflammatory mediators and inhibits activation of the NF-κB in RA rats. Therefore, *Boswellia serrata* extract has significant potential as a phytomedicine in the treatment of rheumatoid arthritis. Inhibition of NF-κB activity by plant resins from species of the Boswellia family might represent an alternative for classical medicine treatments for chronic inflammatory diseases like rheumatoid arthritis.

Chapter VI

Piperine, a main component of *Piper longum* Linn. and *Piper nigrum* Linn., is a plant alkaloid with a long history of medical use. A few studies have shown that piperine exhibits anti-inflammatory activities in a variety of inflammatory disorders through mechanisms that are not fully understood. The aim of this work was to evaluate the anti-inflammatory and antiarthritic effects of piperine. We used a rat collagen-induced arthritis model of RA. Piperine was administered at a dose of 100 mg kg$^{-1}$ body weight once daily for 21 days.
Piperine was effective in bringing significant changes on all the parameters (articular elastase, MPO, LPO, GSH, Catalase, SOD and NO) studied. Oral administration of piperine resulted in significantly reduced the levels of pro-inflammatory mediators (IL-1β, TNF-α and PGF2α) and increased level of IL-10. The protective effects of piperine against RA were also evident from the decrease in arthritis scoring and bone histology.

In conclusion, the major findings of the present study were that piperine suppressed the accumulation of lipid peroxidation products, nitric oxide, enhanced the activity of antioxidant enzymes and eliminated the accumulation and activation of polymorphonuclear cell. We believe that our results will contribute to the clinical applications in the treatment of rheumatoid arthritis. The hypotheses about the mechanism of action in CIA model, reported above, need further investigations for confirmation. More work directed toward understanding molecular and immunological aspects of the disease is required.

In the end, summary and conclusion of the thesis work is presented which briefly describes the overall results and significance of the study of the respective chapters. These observations suggest that these compounds/extract may be a clinically viable therapeutic agent against a variety of conditions where cellular damage is a consequence of oxidative stress. Further understanding the mechanism underlying the antiarthritic potential of these will provide an avenue to disclose both the pathogenesis and therapeutic mechanisms underlying collagen induced arthritis. We believe that our results will contribute to the clinical applications in the treatment of rheumatoid arthritis. However, more studies elucidating the exact mechanism and pathways implicated in the action of these drugs should be carried out, before their clinical application can be recommended.