1. INTRODUCTION

Arthritis is a major human health problem affecting people of all ages, races, geographical areas, and as well as different socio-economic levels. In Greek, the name *arthritis* implies joint inflammation (*arth-* joint; *itis*-inflammation). The inflammation is not a single disease by itself, but rather a feature of many related diseases that can affect the different joints and parts of the body (Kushner, 1985).

In the course of more than 2000 years, when the tools of clinical observations were available, a series of well-defined disease entities could be distinguished and replaced the vague collective term of rheumatism. These entities comprise gout, acute rheumatism (rheumatic fever), rheumatoid arthritis which are solely connected by the symptoms of rheuma, during which 'mucous flux' leaves the brain for various foci in the entrails and also the joints, and there produce diseases (Fassbender, 1975). Rheumatoid disease, a generalised connective tissue disorder that may involve paraarticular structures such as bursae, tendon sheaths, and tendons as well as extra articular tissues such as cardiovascular system, lungs, spleen, lymph nodes, skeletal muscle, central and peripheral nervous system and eyes (Fassbender, 1975).

1.1 Biochemical Basis of Inflammation

Acute inflammation was characterised by Celsus in Roman times as consisting of redness, swelling, warmth, and pain. Biochemical basis of these phenomena have revolved around the study of three kinds of physiological
changes; (a) arteriolar dilation (which is responsible for erythema and warmth), (b) increased vascular permeability (which contributes to oedema), and (c) accumulation of phagocytic cells of polymorphonuclear leukocyte series (Davis and Kushner, 1983).

Chronic inflammation, on the other hand, is characterised by (a) the localisation and accumulation of mononuclear phagocytic cells and lymphocytes, (b) proliferation of local tissue cells, and (c) the abnormal formation and deposition of connective tissue elements. Occasionally, inflammation is also accompanied by destructive lesions, resulting in permanent loss of parenchymal and connective tissue elements. Hydrolytic enzymes released by phagocytic cells and proliferating tissues are responsible for this destruction. The mechanism mediating both the proliferative and destructive aspects of inflammation are of primary interest to the understanding of the pathogenesis of chronic inflammatory diseases (Davis and Kushner, 1983).

1.2 Types of Arthritis

The rheumatic diseases have some aspects in common. But each has its own pattern of symptoms and range of possible treatments. These diseases include:

1.2.1 Osteoarthritis

Osteoarthritis, the most common kind of arthritis, involves the breakdown of cartilage and bone. It is often considered to be the result of years
of normal wear and tear of joints, but can also develop after damage to a joint from injury or infection. Although the disease can affect any joint, the most commonly involved are those of the fingers, hip, knees, and spine (Kushner, 1985).

1.2.2 Rheumatoid arthritis (RA)

Rheumatoid arthritis is potentially a very serious form of arthritis and may lead to severe joint deformity. It involves a chronic inflammation that can attack not only joints but also the skin, muscles, blood vessels, lungs, and heart. It is a chronic disease that can cripple and even kill the person (Kushner, 1985).

1.2.3 Systemic lupus erythematosus (SLE)

SLE is an acute systemic disease related to rheumatoid arthritis. It causes inflammation and damage to joints and organs throughout the body, and sometimes even the kidneys; heart; lungs; brain; and blood vessels. A common feature of the disease is a skin rash on the face (Kushner, 1985).

1.2.4 Ankylosing spondylitis

Ankylosing spondylitis is a kind of arthritis that affects the spine. As a result of inflammation, the bones of the spine fuse or grow together. Spondylitis can also affect the shoulders, knees and ankles, and as well as the eyes, heart and lungs. Severe spondylitis restricts free movement of the back and neck (Kushner, 1985).
1.2.5 Gout

Gout is an acutely painful form of arthritis. It results from a chemical defect that allows too much of uric acid to build up in the body. Gout becomes severe when the uric acid forms crystals that become lodged in a joint, frequently the big toe - causing inflammation. Gout affects far more men than it does women (Kushner, 1985).

1.3 Joint Structure

In most rheumatic diseases, one or more joints are affected. A joint is any place in the body where two bones meet. The ends of the bones are covered by cartilage, which is a tough, elastic tissue that acts as a shock absorber and keeps the bones from rubbing against each other. The entire joint is enclosed in a capsule that is lined by an inner membrane known as the ‘synovial membrane’. The membrane forms a slippery fluid called ‘synovial fluid’, which fills the small space around and between the two bones. The synovial fluid nourishes the cartilage and keeps the joint lubricated, making movements smooth and easy (Kushner, 1985).

Just outside the joint are muscles, tendons, and ligaments. These structures provide support and help the bones to move in the right direction. Tendons are strong cord like structures that attach muscles to bones. Ligaments are similar to tendons except that they connect the bones to each other. Fluid-filled sacs called ‘bursae’ are spread among the muscles, bones, ligaments and tendons and help to keep these structures moving smoothly against each other (Kushner, 1985).
All of these tissues - the cartilage, muscles, ligaments, tendons, and synovial membranes - are made of different kinds of connective tissues. It is found throughout the body, and acts in varying capacity to form the body's support structures and keep the internal organs in place. Some rheumatic diseases affect many kinds of connective tissues in the body (Kushner, 1985).

1.4  Rheumatoid Arthritis

Rheumatoid arthritis is a multisystem, chronic relapsing inflammatory disease of unknown cause. Although the skin, eyes, heart, lungs and other organs may be affected rheumatoid arthritis is basically a severe form of chronic synovitis that sometimes lead to destruction and ankylosis of affected joints (Robins et al., 1984).

1.4.1  Incidence

It is estimated that about 1% of the world's population suffer from rheumatoid arthritis. Young adults are frequently affected, but with increasing age an increase in prevalence has been reported (Silberberg, 1966). Depending on the criteria used for diagnosis, women are 2.5 to 5 times more often affected than men (O'Sullivan, 1972). There is an increased frequency of rheumatoid arthritis among first degree relatives and an approximately 30% concordance rate in monozygous twins. Geographic distribution considered significant at one time, has recently been shown to be random. A slightly increased incidence is seen in men engaged in occupation requiring physical work as compared with those in professional or managerial position.
1.4.2 Pathologic anatomy

The basic tissue changes of rheumatoid disease are similar, regardless of site. In the joints the early changes involve the synovium, which becomes congested, edematous, and infiltrated by small and large lymphocytes, B-cells and T-cells, plasma cells, plasmoblasts, mast cells, and macrophages indicating the presence of both humoral and cellular immune response (Konttinen et al., 1981).

There are often small areas of superficial necrosis of synovial lining cells with formation of superficial erosions covered by fibrinoid deposits, composed of fibrin, small amount of gamma globulin and complement components. An exudate containing polymorphonuclear leukocytes, many with ingested immune complex accumulate in the joint cavity (Popert et al., 1982).

At later stages the synovitis is characterised by plasma cells, lymphoid centers, occasional multinucleated giant cells, and vasculitis. Granulation tissue composed of synovial fibroblasts and capillaries cause grossly recognizable villous thickening of the synovium, whose lining cells become hypertrophic and hyperplastic (Bonomo et al., 1970). The granulation tissue does not remain localized to the synovium but spread over the surface of the articular cartilage and produces adhesion between the opposing joint surfaces. This spread is apparently facilitated by the presence within the pannus of fibronectin, which is more abundant in rheumatoid pannus than in normal synovium (Matsubara et al., 1983). This pannus comes to be interposed between the cartilage and the lumen of the joint cavity and may interfere with the flow of synovial fluid into the cartilage. Thus, malnutrition of the cartilage
may contribute to its destruction, though most of the destruction is attributed to an extraordinarily complex interaction of degenerative processes including enzymatic collagenolysis, breakdown of proteoglycan, demineralization and enzymatic degradation of bones (Harris et al., 1975; Kogstad, 1965). As the pannus ages, vascularity decreases, fibrosis and collagenization lead to shrinkage of the capsule progressively narrowing the joint space, the displacement or increasing approximation of the ends of the opposing bones.

1.4.3 Possible causes of Rheumatoid arthritis

The causes of rheumatoid arthritis are not yet known, but suggestions center around a breakdown of the patients autoimmune system (Walker, 1977). Many researchers suspect that a virus triggers the disease in people who have genetic or inherited susceptibility to it and also suggested that this virus triggers the inflammatory process of rheumatoid arthritis. The genetic susceptibility to the disease is associated with the presence of the human leukocyte antigen known as \textbf{HLA-DR4} in the tissues of persons who have rheumatoid arthritis. Thus, while, the \textbf{HLA-DR4} antigen appears in about one-fourth of the general population, it is found in three-fourths of people with rheumatoid arthritis (Kushner, 1985).

1.5 Experimental Arthritis

The unravelling of the complex mechanisms which are concerned with the initiation and maintenance of rheumatoid arthritis necessitates the establishment of experimental models and other laboratory techniques.
Accordingly, attempts have been made for more than 40 years to discover an equivalent of rheumatoid arthritis in animal experimental models.

The first systematic experiments were made by Klinge in 1927. Rabbits were immunized with horse serum and antigen was then given intra-articularly. Within hours, a very severe arthritis developed with exudation of fibrin and partial necrosis of synovial villi. This model is still used by some investigators, but it cannot be regarded as an equivalent of rheumatoid arthritis.

The work on experimental arthritis was extended further by the introduction of the use of Freund's adjuvant. Stoerk et al (1954) observed generalised arthritis in rats which had been injected with complete adjuvant containing rat or bovine spleen cells. Further work by Pearson (1956) showed that adjuvant itself was responsible for the reaction. Subcutaneous sensitization induces a predominantly cellular reaction within 8-9 days and this can be transferred to other rats with the aid of lymphocytes.

1.5.1 Adjuvant induced arthritis

Adjuvant induced arthritis has been very widely adapted in pharmaceutical screening programmes, being generally considered over the past two decades as an appropriate model of rheumatoid arthritis (Newbould, 1963). This conclusion was reached from considerations of both the appearance and pathology of the disease and the fact that it is very easily inhibited by the aspirin-like drugs, which have been found only to inhibit the symptoms of the disease in man.
1.5.2 **Adjuvant mixture**

Many species of dead mycobacteria are capable of inducing adjuvant arthritis in the rat (*Mycobacterium tuberculosis*, *Mycobacterium butyricum* or *Mycobacterium phlei*) when suspended in vegetable or mineral oils. Earlier attempts to identify the arthritogenic substance in mycobacteria showed it to reside in the Wax D fraction and it was subsequently identified as a peptidoglycan (Jolles *et al.*, 1964; Migliore, 1968). Azuma *et al.* (1972) characterised the arthritogenic component of Mycobacterial Wax D as a mycolic acid - arabinogalactan mucopeptide. Key factors for the successful induction of adjuvant arthritis are the concentration of mycobacteria and its degree of dispersion. Newbould (1963) has emphasised that the fine suspension of bacilli in oil was needed for the induction of arthritis.

1.5.3 **Etiology**

The advantage of an animal model, in comparison with the human disease, is that the time of onset of the experimental condition is precisely known. For adjuvant arthritis, the first histopathological signs of a reaction are apparent in about days 5-7 after inoculation with *Mycobacterium tuberculosis*, with an accumulation of mononuclear cells in the loose connective tissues adjacent to periosteal surfaces. Newbould (1964) demonstrated a clear role for the lymph nodes, draining the site of inoculation in the early phase of development of the syndrome.

Key etiological information came from studies using rhodamine labeled *Mycobacterium tuberculosis*, to trace the dissemination of the bacillus
or its breakdown products in the early stages of the development of adjuvant arthritis. Dissemination of the tuberculous material to these sites occurred in either the free or within macrophages (Vernon-Roberts et al., 1976). Evidence of a key role of T-lymphocytes in the development of adjuvant arthritis came from studies of Kohashi et al. (1982) in which muramyl dipeptide was capable of inducing arthritis in euthymic rats, but not in athymic, nude mutants.

It was originally thought that immune mechanisms are involved in the development of adjuvant arthritis. Much of the published evidence favoured a delayed hypersensitivity (T-cell mediated) response to a disseminated antigen presumably derived from the *tubercle bacillus* (Waksman et al., 1960; Gery and Waksman, 1967).

The actual role of immunity to mycobacterial components is indicated by the experiments of Mackenzie et al. (1978) in which the humoral antibody response to *Mycobacterium tuberculosis* was suppressed by cyclophosphamide. Thus, immunity to the inducing agent, either humoral or cell-mediated, would appear to be an exacerbatory influence, rather than an initiating event in the development of these arthritic syndromes. Dissemination of the original injected material to various sites including synovial membranes, would dictate the actual sites of reactivity with specific antibody of T-cells. This should be regarded as a separate event to the periosteal proliferation and bone remodelling, which constitutes the major pathological feature of the arthritic syndromes seen with both immunogenic and non-immunogenic inducing agents (Smith et al., 1982).
Macrophage activating agents such as muramyl dipeptide can elicit the reaction which indicate a role for interleukin-1 in the activation of the periosteal cells leading on to the remodelling of bone. It is also possible that one role of the T-cells is to release the cytokine, osteoclast activating factor, which would influence the resorption and remodelling of bone (Wahl et al., 1979). Prostaglandins, such as PGE$_2$, are also known to have a role in bone remodelling and resorption. A role for prostaglandins is certainly inferred by the exquisite sensitivity of these arthritic lesions to inhibitors of the cyclooxygenase pathway of arachidonic acid metabolism (Mizel et al., 1981).

1.5.4 Assessment of adjuvant arthritis

Over the years individual laboratories investigating the effectiveness of therapies against adjuvant arthritis have developed their own in-house assessment techniques to detect an active compound or drug. More precise methods are required for the establishment of drug potencies and to help the medicinal chemist in establishing structure activity relationship. Billingham and Davies (1979) placed the various methods of measurements into two major categories (1) the gross physical changes which occur in the affected joints and limbs and (2) the biochemical changes referred by Glenn et al. (1968) as the systemic response to inflammation, which include the Erythrocyte Sedimentation Rate (ESR) and the acute phase proteins (APP). The size of the hind paws is another useful way of assessing the arthritis by measuring its volume, thickness between upper and lower surface, and the circumference of the tibiotarsal joint (Winter and Nuss, 1966; Perper et al., 1971; Graeme et al.,
1966). In addition, the body weight gain has been frequently used as an additional parameter for assessing drug effectiveness (Di Pasquale et al., 1975).

1.6 Treatment

Disease, decay, and death have always co-existed with life, and therefore study of diseases and their treatment must also have been contemporaneous with the dawn of the human intellect. The primitive man must have used therapeutical agents and remedial measures as those are things which he has been able to procure most easily.

1.6.1 Anti-inflammatory and anti-rheumatic drugs

Taking medication is a vital part of the treatment of most types of arthritis. The term ‘anti-rheumatic’ has been employed for substances that suppress the inflammation occurring in human rheumatoid arthritis and anti-inflammatory agents for drugs that inhibit any facet of inflammation of an experimentally induced or as part of a clinical syndrome (Smith, 1985). Screening procedures have been grouped arbitrarily into four categories to assess the anti-inflammatory and anti-rheumatic potential of drugs (Swingle, 1974) namely, (1) an interference with the manifestation of one of the cardinal signs of inflammation, (2) the modification of one of the events occurring during the inflammatory process, (3) biological or chemical properties of a class of known anti-inflammatory drugs and/or (4) the modification of those syndromes in laboratory animals which are believed to represent models for various rheumatoid disease states.
1.6.2 Non-steroidal anti-inflammatory drugs (NSAID)

The NSAIDs were given this name because they reduce inflammation. The NSAIDs have about the same ability as aspirin to reduce pain and inflammation by blocking the formation of prostaglandins (Wedmore and Williams, 1981). The side effects associated with NSAIDs include nausea, vomiting, stomach pain, diarrhoea or constipation, stomach bleeding, and ulcers. The side effects of oxyphenbulozone and phenylbutazone may be severe (Kushner, 1985).

1.6.3 Corticosteroids

The corticosteroids are man-made drugs that are related to cortisone and to hydrocortisone, the natural hormones made by the body's adrenal glands. Steroids are strong drugs which quickly reduce pain and inflammation. They may also cause serious side effects, such as thinning of the bones; depression, high blood pressure, cataract, muscle weakness, diabetes – an increased risk of infection and rarely, bleeding from the stomach. A single person seldom develops all these side effects and some occur only after high doses have been taken for months or years (Kushner, 1985).

1.6.4 Gold treatment

Injection of gold salts (gold sodium thiomalate and aurothioglucose) have been used to treat rheumatoid arthritis for more than fifty years. A person stays on gold treatment for as long as it works, or until it produces side
effects that require it to be stopped. These side effects may include skin rashes, damage to kidneys, bone marrow, liver and lungs (Kushner, 1985).

1.6.5 Cytotoxic drugs

Cytotoxic drugs are potent medications that are used mainly to treat people with cancer and those who receive organ transplants. More recently, they have been used to treat people with serious rheumatoid arthritis, psoriatic arthritis, systemic lupus erythematosus vasculitis. Their side effects are potentially dangerous and may outweigh their possible benefits. The most commonly used cytotoxic drugs are azathioprine, cyclophosphamide, and methotrexate (Kushner, 1985).

The modern drugs used for the amelioration of the symptoms of the disease offer only temporary relief and also produce severe side effects. In view of these, at present, a search has been made from the traditional remedies to find a drug that can effectively offer a permanent relief from arthritis with negligible side effects.

1.7 Indigenous Drugs

It is greatly to the credit of the people of India that they were acquainted with a far larger number of medicinal plants than the natives of any other country on the faces of the earth. The Indian indigenous drugs have great importance both from the professional and economic point of view. The Indian system of medicine in practice at present are Siddha, Ayurveda and
Unani. Although these three systems of medicine are based on humoral pathology, each has got certain characteristic and distinct features of its own.

1.7.1 Siddha medicine

The Siddha system of medicine is very old which is based on definite principles (Anandan and Veluchamy, 1987). Even then there are more parallelism between modern medicine and Siddha system of medicine. It can be easily broughtout and popularised by adopting accepted systematic screening procedures to evaluate their therapeutic potency. In the modern medicine, though much emphasis is not laid on the factors like edaphic, biotic, seasonal and so on, a greater emphasis on these factors is seen in Siddha system of medicine (Anandan and Veluchamy, 1987).

The word 'Siddha' comes from the word 'Siddhi' which means an object to be attained or perfection or heavenly bliss. Siddha also means one who has attained immortality. Ancient Tamils developed two ways by which man can achieve mastery over nature - the Yogie way and through medicines. Yogies were also known as "Siddhars". Hence, this system came to be known as Siddha medical system (Kannan, 1995).

According to the Siddha system, all diseases are caused by the mixture of the three cardinal humours - Vatham, Pitham, Kabam i.e. air, fire, and water; respectively and relative proportion of these are responsible for a person's well being or otherwise. The three humors are called 'Muppini' in Tamil and 'Tridosha' in Ayurveda. The external air corresponds to 'Vayu'. The
external heat to ‘pitta’ and the external water to kapa, man is, thus, linked
with the external world and change in the latter has its corresponding change
in the human body (Kannan, 1995).

The Siddhars have dealt with all subjects of medical sciences in an
efficient manner. Siddha medicine consists of herbs, minerals and animal
products. Inorganic substances were reduced to their atomic and ionic form for
easy absorption into the system. These medicines have the answer to a number
of diseases and are without much side effects.

1.7.2 Usefulness of crude drugs

By using crude drugs and preparations, the cost of treatment could
be considerably reduced. In many cases, the preparations comprise a few to
many constituents. While one constituent is considered to be a therapeutic
agent, the other constituents are supposed to suppress the side effects, if any
were to be caused due to the administration of the said constituent. In few
other cases, the presence of other constituents promote the effectiveness of this
constituent in treatment acting synergistically. Yet another therapy involves
preparations that are given orally or applied externally. In such cases, the
presence of other constituents facilitate the adsorption, uptake and transport
of this constituent in question thereby promoting the effectiveness and
efficiency of the constituent.

Eventhough indigenous system of treatment is superior to any other
system, it has not gained credit. The reason being:
i. The preparations and their purpose were told orally but the causes and the effects were neither written in scripts nor told explicitly.

ii. The course.

iii. The difficult impositions prescribed in diet and other conditions.

iv. The lack of interest and publicity.

v. The lack of intention to the scientific approach at the part of our own people.

vi. The socio-economic psychology of our people.

Siddha system of treatment is being increasingly recognised as an alternate approach to arthritic treatment. Among herbal drugs, certain drugs such as *Acalypha indica* Linn., *Solanum nigrum* Linn., *Hibiscus populnea*, *Allium sativum* Linn., *Asparagus officinalis* Linn., *Citrus bergamia*, *Moringa pterygosperma* have been shown to contain anti-arthritic properties (Nadkarani and Nadkarni, 1976). Anti-inflammatory activity of *Astercantha longifolia* has also been described (Balraj and Nagarajan, 1982). In the present study, attempt has been made to assess the anti-arthritic property of Siddha preparation of *Semecarpus anacardium* nut extract named as ‘Serankottai Nei’.

*Semecarpus anacardium* Linn. (Family: Anacardiaceae) is a moderate sized deciduous tree, reaching upto a height of 12-15 m and a girth of 1.25 m found in the outer Himalayan, from the Sulleg to Sikkim, and fairly common
Nut of *Semecarpus anacardium*
throughout the hotter part of India as far east as Assam. Bark - dark brown, rough; leaves - large, simple; flowers - small, dull greenish yellow (The Wealth of India).

The tree is not found under cultivation, but is common in forest. A large genus of trees are distributed in the Indo Malaysian region and extends to Australia. Six species are reported in India. In vernacular language, it is known as Bibba (Marathi), Bhilawa (Hindi) and Bhallataka (Sanskrit). The fruit is known as marking nut, since the juice of pericarp is used for marking cotton clothes (Kritikar and Basu, 1933).

Ayurveda describes *Semecarpus anacardium* to be a potent drug against variety of ailments and is popularly known as ‘Andha Vaidhya’ (Nadkarni and Nadkarni 1976). The fruits and oil have been claimed to be highly efficacious in the treatment of neuritis (Charaka, 1941), arthritis, (Satyavathi, *et al.*, 1969), leprosy, (Chopra *et al.*, 1956), helmintic infection (Selye, 1953) and venereal disorders (Sushruta Samhita, 1938). The fruit of *Semecarpus anacardium* is acrid, hot and anthelmintic, and is considered beneficial in tumours (Gothoskar *et al.*, 1971). The juice of the pericarp has antibacterial property and arsenic derivatives of bhiliwanol are non-vesicant (Chatopadhyaya and Chare 1969).

The chloroform soluble fraction of the whole nuts are reported to be very useful in the treatment of cancer of the oesophagus and leukemias (Vad and Kulkarni 1975). The fraction of petroleum ether extract, under high vacuum yielding an orange coloured oil (b.p. 200-20°/2-3 mm) has been found to possess good antitumour activity against P₃₈₈ tumour (Indap, 1978). It was
found to inhibit acute tuberculin reaction in sensitized rats and also the primary phase of adjuvant arthritis without having any significant effect on the development of the secondary lesions induced by the adjuvant (Satyavathi et al., 1969).

The crude extracts were found to be very toxic. The LD$_{50}$ of the chloroform extract of the *Semecarpus anacardium* nut was found to be 230 mg/kg body weight (Kesava Rao et al., 1979). In rheumatism and in the treatment of painful joints, it has been used along with garlic, dry kernals of coconut and jaggery.

On account of its wide therapeutical property, the chemistry of *Semecarpus anacardium* was studied extensively. Pillai (1931) reported the presence of phenolic compounds like semicarpol and bhilwanol in the nut. A number of biflavanones which have been characterised through spectral data were also extracted from the alcoholic extract of nut shells (Murthy, 1983). The phytochemical studies of the Siddha preparation of *Semecarpus anacardium* nut extract (*Serankottai Nei*) was found to contain phenols, flavonoids and carbohydrates.

1.8 Chemistry of Flavonoids

Flavonoids are benzo-$\gamma$-pyrone derivatives which resemble coumarin and are ubiquitous in photosynthesizing cells. Their occurrence is, therefore, widespread in the plant kingdom. For centuries, preparations which contain flavonoids as the principal physiologically active constituent have been used
by laymen and physicians in attempts to treat human diseases (Havsteen, 1983).

The high chemical reactivity of flavonoids is expressed in their binding affinity to biological polymers and heavy metal ions (Dick, 1981) as well as in their ability to catalyse electron transport (Heyman and Kinoshito, 1976) and to scavenge free radicals (Cavallini et al., 1978). The flavonoids have long been considered to possess anti-allergic and anti-inflammatory activities. They are also potent antioxidants (Yuting et al., 1990). They inhibit the release of lysosomal enzymes from rabbit polymorphonuclear leukocytes and the antigen induced secretion of histamine from human basophils (Berton, et al., 1980; Middleton and Drezewiecki, 1982).

Flavonoids are metabolised by animal cells especially those of liver. No residue of flavonoids is accumulated in the body. The toxicity of flavonoid is very low in animals. For rats, the LD$_{50}$ was found to be 2-10 g per animal for most flavonoids (Havsteen, 1983).

1.9 Biochemical Changes Associated with Rheumatoid Arthritis and Experimental Model

Chronic diseases such as rheumatoid arthritis in man and models of arthritis in animals, involves not only the obvious signs of inflammation, but also produce many other changes in a variety of hematological and biochemical systems (Billingham, 1983).
1.9.1 Lysosomal stability and glycohydrolases

Lysosomes are a group of cytoplasmic organelle present in numerous animal tissues characterised by their content of acid hydrolases (Novikoff, 1961).

In relation to the etiology of inflammation, some hypothesis have been proposed in which it is postulated that lysosomal contents such as hydrolytic enzymes or cationic proteins play an important role in the initiation of inflammation (Shen, 1967). In its simplest form, this hypothesis states that either due to direct injury or to excessive endocytosis, materials are released from lysosomes which provoke acute inflammation in the joint. Chronic inflammation with cartilage erosion is similarly due to the action of lysosomal enzymes upon the extracellular matrix of cartilage (Weissmann, 1968).

It is well known that lysosomes act as mediators of the inflammatory process (Dingle et al., 1961). Elevated level of lysosomal acid hydrolases has been detected in inflammed tissues including rheumatoid synovial membrane (Weissmann et al., 1969).

One of the characteristic features of adjuvant arthritis in rat is the correlation between development of inflammatory processes and the extra cellular release of lysosomal enzymes (Weissmann, 1972). A number of agents which impair the integrity of lysosomes provoke inflammation and induce experimental arthritis.
Endotoxin, which labilizes lysosomes in intact cell by promoting autophagy or endocytosis regularly induces an acute arthritis (Hollingswarth and Atkins, 1966). Weiss and Dingle (1964) have shown that antibodies directed against relatively crude preparation of liver lysosomes produced labilization of lysosomes within living fibroblast. Anderson (1976) has reported that the increase in oedema in both the hind paws after adjuvant injection into rat is paralleled by an increase in extracellular activities of lysosomal enzymes.

The discharge of hydrolytic enzymes from lysosomes may be responsible for a variety of disorders affecting the extracellular connective tissue components. It is well established that lysosomal enzymes possess the capacity to degrade the various components of connective tissue components such as collagen, proteoglycans, glycoproteins, and elastin (Peltonen, et al., 1981).

The usefulness of many anti-inflammatory drugs on the alleviation of swelling and pain associated with rheumatoid arthritis has been attributed to the capacity of such drugs to stabilize lysosomes and to inhibit lysosomal enzymes (Anderson, 1969).

1.9.2 Lipids and lipoproteins

The lipids are a heterogenous group of compounds. They are important dietary constituent not only because of their high energy value but also due to the fat-soluble vitamins and the essential fatty acid contained in the fat of natural foods.
One of the systems, that mediates lipid transport is the plasma free fatty acids. This system carries fatty acids from the adipocytes to other tissues, primarily for oxidation to provide energy. The other lipid transport system consists of a group of plasma lipoproteins that primarily carry cholesterol and triglycerides.

Chronic inflammatory diseases are fundamentally disorders of immunological regulation, but they affect the lipid and protein metabolism (London et al., 1963). Lipid metabolic disturbance characterised by a reduction of cholesterol and triglyceride levels in different lipoprotein moieties has been found in patients with rheumatoid arthritis (Svensson et al., 1987).

Several studies have shown variation in the lipids during disorders promoting the acute phase response. A Finnish (Heldenberg et al., 1980, Chomber et al., 1980) survey revealed that patients with rheumatoid arthritis had more cardiovascular diseases and atherosclerotic manifestation than the controls (Isomaki et al., 1975).

Adjuvant induced arthritis in rats, an animal model of rheumatoid arthritis, exhibits abnormal alteration in serum lipids during acute and chronic stages in the development (Yamaguchi, 1989). Both stages display pronounced changes in plasma acute phase proteins. Since some of these proteins interact with plasma lipoprotein which transport lipids, such associated changes in plasma lipids may be another form of biochemical derangement developing in the mammal responding to acute inflammation or tissue injury as well as to chronic inflammation (Bendilt and Eriksen, 1977).
Svenson *et al.* (1987) have reported that human chronic inflammatory diseases, particularly rheumatoid arthritis, are characterised by an altered lipoprotein composition and metabolism as well as an acute phase response in parallel with the inflammatory activity.

The ratio of high-density lipoprotein cholesterol to total cholesterol remained unaltered in rheumatoid arthritis (Lorber *et al.*, 1985). Increased VLDL and LDL concentrations are associated with increased risk of atherosclerosis, while low HDL may be equally important for the development of atherosclerosis (Miller and Miller, 1975).

**1.9.3 Free radical biology**

It is well established that free radicals and other reactive O$_2$ species are continuously produced *in vivo* (Halliwell and Gutteridge, 1989). The term reactive O$_2$ species is a collective one that includes not only O$_2$ centered radicals such as superoxide and hydroxyl but also some non-radical derivatives of O$_2$, such as H$_2$O$_2$, singlet O$_2$ and hypochlorous acid (HOCl). Hydroxyl radical is produced in living organisms by at least two mechanisms: reaction of transition metal ions with H$_2$O$_2$ and homolytic fission of water caused by exposure to ionizing radiation (Von Sonntag, 1987). Hydroxyl radical is a fearsome reactive species that can attack all biological molecules, usually setting off free radical chain reaction (Halliwell and Gutteridge, 1989). Superoxide radical is much less reactive than hydroxyl radical, but a number of biological targets are sensitive to it.
1.9.3.1 Reactive \( \mathrm{O}_2 \) species and human rheumatoid arthritis

Human diseases may be caused by oxidative stress. Interest in the role of free radicals and other \( \mathrm{O}_2 \) derived species in human rheumatoid disease, stems from the seminal work of McCord (1974) who pointed out to the decreased viscosity of synovial fluid from the joints of patients with rheumatoid arthritis and showed that a similar decrease in viscosity can be produced by exposing synovial fluid or hyaluronate to superoxide generating system \textit{in vitro}.

In rheumatoid arthritis activated neutrophils liberate \( \mathrm{O}_2^\cdot \), \( \mathrm{H}_2\mathrm{O}_2 \), elastase, \( \mathrm{HOCl} \) and eicosanoids to pathogens (Weiss, 1989). Neutrophil production of \( \mathrm{O}_2^\cdot \) and \( \mathrm{H}_2\mathrm{O}_2 \) may be effected by oxygen tension in the synovial fluid. (Edwards et al., 1984). In rheumatoid arthritis, the pannus overgrowing the cartilage contains many macrophages (Rainsford, 1986) presumably secreting \( \mathrm{O}_2^\cdot \), interleukin - 1 and nitric oxide. Halliwell et al. (1992) have hypothesized that cartilage erosion underneath the pannus is due to a combined attack by proteases secreted by the cartilage itself in response to interleukin-1 and by \( \mathrm{O}_2 \) derived species. It has been further suggested that reactive \( \mathrm{O}_2 \) species might accelerate bone resorption by osteoclasts.

1.9.3.2 Antioxidant defence mechanism

The production of free radicals is essential to normal metabolism but they can be destructive if their toxicity is not controlled by intra/extracellular defence mechanisms. A range of intracellular antioxidant defence system limit the toxic potential of intermediate formed during the four electron reduction
of $O_2$ to water. Of particular importance is the enzyme superoxide dismutase which, catalyses the dismutation of $O_2^-$ to $H_2O_2$. Once again, the cell is protected from the potential toxicity of $H_2O_2$ either by the haem enzyme catalase or by the seleno enzyme glutathione peroxidase. (Morris et al., 1995).

The non-enzymic antioxidants include $\alpha$-tocopherol, which delay lipid peroxidation by scavenging intermediate radicals such as lipid peroxyl. The tocopherol transfers a hydrogen atom to the peroxyl radical at a faster rate than which is required for the peroxyl radical to react with membrane proteins or with adjacent fatty acid side-chains. (Burton and Ingold, 1989).

Ascorbic acid has a multiplicity of antioxidant properties and has been claimed to be the most important antioxidant in human plasma because it usually disappears faster than other antioxidants when plasma is exposed to reactive $O_2$ species (Frei et al., 1989).

Glutathione is important as a substrate for glutathione peroxidase, transferases and other enzymes and as a general radical quencher in cells (Eaton, 1991). It might also help to recycle tocopherol radical in vivo (Sies and Murphy, 1991). However, loss of hydrogen from -SH groups produce thyl radicals, and they have been capable of reacting with $O_2$ to give potentially damaging oxysulphur radicals (Asmus, 1990). On the other hand, thyl and oxysulphur radicals generated in vivo might simply be removed by reaction with ascorbic acid available at their site of generation (Asmus, 1990).

Uric acid is an end product of purine metabolism. Ames et al. (1981) proposed that uric acid acts as a antioxidant in vivo. Hence, measuring the
products of attack of reactive oxygen species on uric acid might be a potential marker of oxidative damage. Uric acid is degraded on exposure to OH\(^-\), HOCl and mixture of hemoglobin with H\(_2\)O\(_2\). Ozone also oxidizes uric acid. (Cross \textit{et al.}, 1992).

The increase in lipid peroxidation can be attributed to alteration in the antioxidant defence system. Failure of antioxidant defence system has been reported in many pathological conditions including rheumatoid arthritis. (Barber and Harris, 1994) Hence, the study of antioxidant status during a free radical challenge can be used as an index of protection against the development of degenerative inflammatory processes in experimental condition for assessment purpose.

1.9.4 Adenosine triphosphatase

A number of membrane proteins, which are involved in solute transport, have been investigated in great detail, which includes-sodium potassium ATPase; calcium ATPase; magnesium ATPase. \(\text{Na}^+,\text{K}^-\)-ATPase or sodium potassium pump is essential for the transport of glucose and amino acids. It hydrolyses adenosine triphosphate (ATP) only, when sodium and potassium ions are present in addition to magnesium ions, which are tightly bound to the membrane. Translocation of sodium and potassium ions by this phosphatases is coupled with the hydrolysis of ATP. It maintains high concentration of potassium and low concentration of sodium inside the cell (Rao, 1980).
The concentration of calcium in the erythrocyte membrane is only $10^{-6}$M as compared to $10^{-3}$M in circulating plasma. This concentration gradient is maintained by the low permeability of calcium across the membrane and by calcium or calcium ATPase (Jain and Shonet, 1981).

Various factors are responsible for the altered levels of ATPase. Among these, lipid peroxidation, membrane fluidity and extracellular calcium are important determinants (Stekhoven and Bonting, 1981; Kimelberg, 1975). Besides these, oxidation of sulphydryl (-SH) groups by lipid peroxidation is considered to be an important factor in $O_2^-$ radical mediated inhibition of calcium ATPase activities in sarcoplasmic reticulum (Scherer, 1986). Loss of Ca$^{2+}$ and Mg$^{2+}$-ATPase activity in the membrane is accompanied by an increase in the formation of malondialdehyde, a thiobarbituric acid reactive product of lipid peroxidation (Moore et al., 1989).

1.9.5 Acute phase proteins

The host response to tissue injury involves, in addition to local inflammation, a wide range of systemic alterations known collectively as the acute phase response (Kushner, 1982). The acute phase proteins are a diverse group whose levels in plasma rise, as a part of this systemic response, during the first few hours to days following tissue injury. They are termed ‘acute’ because the prototype of this group (C-reactive protein) was first noted in sera from human patients with acute bacterial infection (Kushner, 1982).

Tissue damage associated with surgery, infarction, burns or trauma can elicit the response. Bacterial, viral, immune-mediated, and idiopathic
inflammatory conditions are all capable of inducing this response. Endotoxin is a particularly potent acute phase stimulus (La Montagne et al., 1984).

The primary site of production for most acute phase proteins is the liver and the observed increases are the result of accelerated hepatic synthesis (Koj, 1974). Numerous potential mediators have been studied to explain how this hepatic synthetic response is triggered by tissue damage at distant sites. Various hormones (cortisol, epinephrine, sex hormones), prostaglandins, fibrinogen degradation products have all been reported to play some role in controlling the synthesis of various acute phase proteins. However, leukocyte pyrogen (interleukin-1) appears to be emerging as a major mediator that is able to induce whether directly or indirectly, the characteristic protein changes as well as other systemic features of the acute phase responses (Bornstein, 1982).

Elevated interleukin-6 is detectable in sera of patients with rheumatoid arthritis and is correlated with C-reactive protein and other acute phase proteins (Houssiau et al., 1988). This is because interleukin-6, which is identical to hepatocyte stimulating factor (HSF) is a major inducer of the acute phase response in liver cells (Gauldie et al., 1987).

\[
\begin{align*}
\text{IL-1} & \quad \text{Liver} \\
\text{IL-6} & \rightarrow \\
\text{Tumor necrosis factor} & \quad \text{C-reactive protein} \\
& \quad \text{Serum amyloid A} \\
& \quad \text{Fibrinogen} \\
& \quad \text{Haptoglobin} \\
& \quad \text{Ceruloplasmin}
\end{align*}
\]
Acute phase proteins appear to play a significant role in host defence as well as maintaining homeostasis. Host defence protein include C-reactive protein (CRP) and fibrinogen. Acute phase protein with antioxidant capacity include ceruloplasmin and haptoglobin. Serum amyloid A may play a role in modulating the inflammatory processes (Blackburn 1994). Hirshcelmann et al. (1987) have reported that CRP level was elevated in rats with adjuvant arthritis.

1.9.6 Glycoproteins

Glycoproteins are proteins having oligosaccharide chain covalently attached to their polypeptide backbones. They usually contain 15 or fewer sugar units per covalently attached oligosaccharide chain and may have carbohydrate contents ranging from 1 to 85% by weight (Martin, 1981).

The physiological function of the glycoprotein in the connective tissue remains unclear. It has been suggested that the glycoproteins have some function in stabilizing the tissue and that may be involved in maintaining the structural stability of collagen fibrils (Jackson and Bentley, 1968). In addition, glycoprotein may be the components of connective tissue that are primarily responsible for its antigenic property in tissue transplants (Robert et al., 1968). It is well known that the secretion of glycoproteins by the liver occur after assembly of carbohydrates.

Exer and coworkers (1976) reported an increase in metabolic turnover of proteoglycan in the ligament and cartilage during the chronic inflammatory process of adjuvant arthritis. The contents of sialic acid, hexosamine, and
deoxy sugars have been found to be increased in the serum of patients with rheumatoid arthritis (Maiveikov, et al., 1977). In both acute and chronic phases of experimental arthritis, a significant increase in the level of carbohydrate moieties of tissue glycoproteins were observed (Kesava Reddy et al., 1986). The elevated level of glycohydrolases in inflammatory process of adjuvant arthritis may be responsible for the increased level of carbohydrate components of glycoproteins (Kesava Reddy and Dhar, 1987).

1.9.7 **Cartilage collagen**

Cells are surrounded by variable amounts of an extracellular matrix, the composition of which helps to determine the physical, mechanical, and functional properties of individual tissues and organs (Balazs, 1970). The matrix is made up of the fibrous elements - collagen, elastin, amorphous proteoglycans and structural glycoprotein. These molecules undergo physiological turnover, and their degradation is necessarily a part of the destruction of tissue in diseases.

The fibrous protein, collagen, is the primary structural element of cartilage. The collagen molecule, about 3000 Å and 15 Å wide, is composed of three intertwined polypeptide chains. Collagen fibres are formed by collagen molecules aligning themselves, principally on the basis of electrostatic interaction (Nimni, 1974).

The collagen molecule is not synthesised as such, but is derived from a precursor molecule, termed "procollagen" (Layman et al., 1971). Individual procollagen polypeptide chains are synthesised in endoplasmic reticulum. Prior
to release of procollagen from the ribosomes, certain peptide bound proline and lysine residues are hydroxylated by the specific enzyme proline hydroxylase or lysine hydroxylase (Miller and Udenfriend, 1970).

Articular cartilage is destroyed in many forms of arthritis and this process requires degradation of its two major constituents: collagen and protein polysaccharide (Weissmann and Spilberg, 1968). The precise mechanism of collagen fibre degradation is not clear. Collagenase, neutral proteinases and the lysosomal cathepsin have been demonstrated to degrade native collagen, although the collagenase remains as the only enzyme which will attack the triple helix of the native molecule (Gibson, 1978).

The collagen fibres are degraded to small fragments which can then be ingested by phagocytic cells. Fragmented and partially degraded collagen fibrils have been observed in phagolysosomes, where the intracellular phase of digestion is completed by the lysosomal system to free amino acids. (Brands and Anton, 1969; Perez, 1973). The degree of intramolecular cross-linking is important for freshly reconstituted collagen fibrils without cross linking, they being more easily degraded by the enzymes. (Harris and McCroskera, 1974).

1.10 Visceral Lesions Associated with Rheumatoid Arthritis

Patients suffering from rheumatoid arthritis frequently present much evidence that the disease is not confined to the joints. From a clinical standpoint, it is easy to detect profound disturbances in the functions of a number of organs among such patients. But curiously, only fragmentary data concerning the visceral pathologic changes of this disease have been reported.
Consequently, little is known about the morphologic changes which may be at the basis of the visceral or systemic manifestation of rheumatoid arthritis.

1.10.1 Liver abnormalities

Abnormal biochemical tests of liver function have been reported in a large proportion of patients with rheumatoid arthritis (Darby, 1956). Histological evidence of liver disease occurring in rheumatoid arthritis has been reported (Movitt and Davis, 1953). Fingerman and Andrus (1943) have suggested that amyloidosis was the only specific and significant hepatic lesion found is rheumatoid arthritis.

Apart from amyloidosis, occasional cases of cirrhosis have been reported in rheumatoid arthritis (Lovgren, 1953). Many studies revealed only minor changes including variable fatty change, kupffer cell hyperplasia, and mild mononuclear cell infiltration of the portal tracts. Another specific liver lesion which occurs in rheumatoid arthritis is necrotizing arthritis. But this has only been seen in the presence of widespread rheumatoid arthritis (Ball, 1954). A significant degree of hepatic central necrosis affecting parenchymal cells was reported. This central necrosis was always associated with rheumatic heart disease and with severe and extremely crippling rheumatoid arthritis (Baggenstoss and Rosenberg, 1943).

1.10.2 Cardiac abnormalities

Involvement of the heart in rheumatoid arthritis is well recognised (Khan and Spodick, 1972). Pericarditis is clinically apparent in 2-10% of such
patients (Wilkinson, 1962). Several postmortem autopsy and echocardiographic studies have demonstrated increased incidence of pericardial, myocardial, and valvular abnormalities in patients with rheumatoid arthritis. (Nomeir et al., 1979; MacDonald et al., 1977). Fibrinoid granuloma, a specific lesion of rheumatoid arthritis has been reported in all parts of the heart. The fibrinoid granuloma on one or more valves have led to intractable congestive heart failure (Bonfiglio, et al., 1969).

1.10.3 Kidney abnormalities

Studies of mortality in patients with rheumatoid arthritis shows an excess of death from renal failure due to glomerulonephritis (Laakso et al., 1986). It seems likely, therefore, that rheumatoid arthritis is itself associated with the development of glomerulonephritis.

Postmortem observation of the kidneys in rheumatoid arthritis include glomerulitis, chronic or subacute interstitial nephritis, amyloid degeneration. (Baggenstoss and Rosenberg, 1943).

Renal amyloidosis and membranous glomerulonephritis are the most common and renal abnormalities associated with rheumatoid arthritis. (Helin et al., 1986).

These morphological changes in the visceral organs led us to concentrate on the biochemical changes taking place in these organs in adjuvant induced arthritis and drug treated animals.
Scope of the Present Investigation

Siddha system of treatment is being increasingly recognised as an alternate approach for the treatment of rheumatoid arthritis. The present work is aimed to study the prophylactic and therapeutic anti-inflammatory effect of *Serankottai Nei*, the nut extract of *Semecarpus anacardium*.

Before exploring the anti-arthritic potential of the drug, its toxicity range (acute and subacute) was established in albino rats. The effective dose of the drug in adjuvant arthritis was also determined. Further, the biochemical alterations following treatment with *Serankottai Nei* in arthritic condition was studied.

The anti-arthritic/anti-inflammatory effect of the drug was confirmed by measuring the paw diameter of adjuvant arthritis and drug treated animals.

Haematological parameters such as Hb, RBC count, WBC count, PCV, and ESR were estimated to study the effect of drug on haematological changes in adjuvant arthritis. In addition the effect of drug on cellular constituents such as DNA, RNA, protein, lipids, and glycogen in tissues were also studied in arthritic rats.

Lipid peroxidation product, malondialdehyde in plasma, and tissue, such as liver, kidney and heart were studied. The enzymic antioxidants (SOD, Catalase, Glutathione peroxidase) and non enzymic antioxidants (GSH, Vitamin C, Vitamin E, NPSH and TSH) were studied in tissues and
hemolysate of control, arthritic and drug treated animals. In addition GSH metabolising enzymes were also estimated in control and experimental groups.

Changes in plasma and tissue lipids may be another form of biochemical derangement developed in the mammal responding to acute inflammation as well as to chronic inflammation. Hence, the anti-arthritic nature of the drug has been assessed through its impact on lipids, lipid metabolizing enzymes, and lipoproteins' changes in adjuvant arthritis.

Further to add strength to the inferences the anti-arthritic potential of the drug *Serankottai Nei*, adjuvant arthritis induced changes in acute phase proteins such as C-reactive protein, fibrinogen, haptoglobin and ceruloplasmin were also studied.

In order to bring out the modulating effect of the drug on membrane fluidity, changes associated with membrane bound adenosine triphoshatase, erythrocyte membrane lipid peroxides and osmotic fragility test were carried out in adjuvant induced arthritic condition and in drug treated animals.

X-ray analysis of arthritic joints was carried out to assess the curative and preventive effect of the drug on adjuvant arthritis.

Rheumatoid arthritis was reported to induce a number of pathological changes in the susceptible tissues. Hence, the histopathological examination of synovial membrane, cartilage, liver, kidney and heart tissue were done to understand the antiarthritic effect on adjuvant induced arthritis and in drug administered animals.