**INTRODUCTION**

Inflammatory diseases including different types of rheumatic diseases are a major cause of morbidity of the working force throughout world. This has been called the ‘King of Human Miseries’ (Chatterjee et al., 1984). The immune system is a well-organized and well-regulated system. The deregulation of the immune system may lead to the development of autoimmune diseases. Rheumatoid arthritis is a prototype of such groups of illness with chronic systemic disorders to be considered an autoimmune disease with destructive articular and periarticular structure. Persistent inflammation produces swollen joints with severe synovitis, decreased nociceptive threshold and massive sub-synovial infiltration of mononuclear cells, which along with angiogenesis leads to pannus formation. Expansion of the pannus induces bone erosion and cartilage thinning, leading to the loss of joint function (Feldmann et al., 1996; Koch, 1998).

In India, many Ayurvedic practitioners are using various indigenous plants for the treatment of different types of arthritic conditions (Shah et al., 2006). Although the applications of these medicaments have 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.

**Syzygium cumini** (Syn. Eugenia cumini, Eugenia jambolana, jambul, black plum) is a tree of the family Myrtaceae distributed in Asia. The barks, leaves and seed extracts of *Syzygium cumini* have been reported...
to possess anti-inflammatory (Chaudhurei et al., 1990), hypoglycemic (Chopra et al., 1958), antibacterial (Bhuiyan et al., 1996) and anti HIV activity (Kusumoto et al., 1995). In the indigenous system of medicine, the seed extract of *Syzygium cumini* is reported to be useful in the treatment of various inflammatory disorders. In recent years, the extract of seeds, leaves and bark of *Syzygium cumini* has been extensively studied for anti-inflammatory activity (Slowing et al., 1994; Muruganandan et al., 2001; Kumar et al., 2008).

In view of the importance of this herbal plant the present study aims to evaluate the comparative therapeutic effects of *Syzygium cumini* against Freund’s complete adjuvant induced arthritis in rat model, which is the best and most widely used experimental model for arthritis with clinical and laboratory features which closely mimic the clinical features of human rheumatoid disease (Pearson et al., 1963; Taurog et al., 1988).

**MATERIALS AND METHODS**

**Plant Material**

The fully mature *Syzygium cumini* seeds were collected locally during the month of June of 2007. The plant was botanically identified and authenticated by Prof. K. Srinivasa Rao (Department of Pharmacognosy, Roland Institute of Pharmaceutical Sciences, Berhampur, Orissa, India) and voucher specimen was deposited in the department herbarium.

**Preparation of Plant Extract**

The *Syzygium cumini* fruits were first washed well and pulp was removed from the seeds. Seeds were washed several times with distilled water to remove the traces of pulp from the seeds. The seeds were dried at room temperature and coarsely powdered. The powder was extracted with hexane to remove lipids. It was then filtered and the filtrate was discarded. The residue was successively extracted with methanol using cold percolation method (Kumar et al., 2008). The percentage yield was 10.38% in methanol. The extract was stored at -70°C until further use. Henceforth, the methanolic extract of *Syzygium cumini* seeds will be called as SME. The extract and standard drug were administered in the form of suspension in water with 1% sodium carboxy methyl cellulose (SCMC) as suspending agent.

**Preliminary Phytochemical Screening**

One gram of the methanolic extract of *Syzygium cumini* was dissolved in 100 ml of its own mother solvent to obtain a stock of concentration 1% (%w/v). The extract thus obtained was subjected to preliminary phytochemical screening (Kokate, 2001; Harborne, 1998).

**Animals**

Albino rats (175-200 g) procured from Mahaveer Enterprises, Hyderabad, India were used for the study. They were maintained under standard laboratory conditions at ambient temperature of 25±2°C and 50±15% relative humidity with a 12-h light/12-h dark cycle. Rats were fed with a commercial pellet diet (Rayans Biotechnologies Pvt Ltd., Hyderabad) and water *ad libitum*. The experiments were performed after prior approval of the study protocol by the institutional animal ethics committee of Roland Institute of Pharmaceutical Sciences, Berhampur, Orissa, India. The study was conducted in accordance with the guidelines provided by Committee for the Purpose of Control and Supervision of Experiments on Animals (Reg. No. 926/ab/06/ CPCSEA).

**Drugs/Chemicals**

Indomethacin was supplied by Recon, Bangalore, India. Sodium pentobarbitone was supplied by E-Merck, Mumbai, India. All other chemicals used for this study were of analytical grade.

**Pharmacological Experiment**

**Acute Toxicity Studies**

The acute oral toxicity study was carried out as per the guidelines of OECD (Ecobichon, 1997). One tenth of the medium lethal dose (LD$_{50}$) was taken as an effective dose (Anupama et al., 1990).

**Freund’s Complete Adjuvant (FCA)**

5 mg of heat killed mycobacterium tuberculosis cell (being killed at 60°C in 15-20 min in the autoclave) was finely ground using a mortar and pestle. Sufficient liquid paraffin was added and thoroughly triturated to make a 5 mg/ml suspension.

**Incomplete Freund’s Adjuvant**

The liquid paraffin is referred in the study as Incomplete Freund’s Adjuvant.

**Induction of Arthritis**

Albino rats of either sex were divided in to five groups of six animals each.

Group-1 : Vehicle control  
Group-2 : Arthritis control  
Group-3 : SME -250 mg/kg/day, p.o  
Group-4 : SME -500 mg/kg/day, p.o  
Group-5 : Indomethacin-10 mg/kg/day, p.o
The method described by Newbould (Newbould, 1963) was employed with some modifications. Adjuvant arthritis was induced by subcutaneous injection of FCA (0.1 ml) in to sub plantar tissue of the right hind paw of each rat. The test groups consisted of FCA-injected rats challenged with the respective doses of the test drugs administered orally 24 h before FCA injection while, the vehicle control rats were injected with 0.1 ml of liquid paraffin (incomplete Freund’s adjuvant) only. The drug treatments were continued daily on the same time after the challenge for 20 more days.

The swelling in the injected and contralateral hind paws of the rats were monitored daily using liquid displacement plethysmometer (Ugo Basile, Italy). Increase in the extent of erythema and edema of the tissues shows the severity of the inflammation. The difference in severity of arthritis between the experimental groups and arthritis control group were statistically analyzed. The changes in body weight were recorded daily.

**Biochemical Parameters**

On 20th day blood was withdrawn through retro orbital vein puncture of all groups and the biochemical parameters were analyzed. Hemoglobin (Hb) content was estimated by the method of Drabkin and Austin (Austin et al., 1935). Red blood cell (RBC) and white blood cell (WBC) counts were estimated according to the method of Chesbrough and Mc Arther (Chesbrough, 1972) in an improved Neubauer chamber. Estimation of erythrocyte sedimentation rate (ESR) was followed by the method of Westergren (Zlonis, 1993).

**Radiographic Analysis**

On 20th day animals were anesthetized with sodium pentobarbitone-60 mg/kg, i.p. (Baig et al., 2007) and placed in X-ray machine for the radiographic analysis of the knee joints. X-ray was taken at the knee joints for the confirmation and evaluation of the severity of arthritis in FCA induced rats.

**Statistical Analysis**

The experimental results were expressed as mean ± SEM. Statistical analysis was performed by one-way ANOVA followed by Dunnet’s ‘t’ test.

**RESULTS**

**Preliminary Phytochemical screening**

This investigation showed the presence of alkaloids, amino acids, flavonoids, glycosides, phytosterols, saponins, steroids, tannins and triterpenoids in the methanolic extract of *Syzygium cumini* seeds (SME).

**Acute Toxicity Studies**

From the acute toxicity study, the LD₉₀ cut-off dose for methanolic extract was found to be 5000 mg/kg body weight. Hence one tenth of LD₉₀ dose (500 mg/kg) of extract was selected as maximum therapeutic dose and 250 mg/kg was selected as lower dose for the study.

**Effect of SME on Primary Response of FCA Induced Arthritis Rat**

The paw volume of the right paw was measured and taken into consideration for evaluating the possible anti-inflammatory effect of SME on rheumatoid arthritis. After the onset of inflammation the peak incidence in swelling reached during 4th to 6th day with the increase in paw volume at the maximum of 1.18 ml for the arthritis control. The edema on the animals treated with SME (500 mg/kg) and Indomethacin (10 mg/kg) group began to subside gradually (p<0.001) when compared with arthritic control. The effect of SME -500 mg/kg on this primary reaction was found to be high at the earlier of 2nd day after FCA injection and was maintained until the termination of the experiment. Therefore SME-500 mg/kg observed for its significant effect in preventing the primary systemic response and it is capable of inhibiting the development phase of arthritis and this effect was compared with the standard indomethacin-10 mg/kg (Table 1 [Supplementary data]).

**Effect of SME on Secondary Response of FCA Induced Arthritis Rat**

The latent secondary response that occurs after few days and characterized by joint swelling and nodule formation in the contralateral paw was first evident on the 7th day. The administration of SME (250 mg/kg) significantly (p<0.05) protected against joint swelling in arthritis-induced paw when compared with arthritis control group. But the significant reduction was observed from day 11 to 13 in the SME (250 mg/kg) treated group. However the effect of SME-500 mg/kg treatment was found to be significant (p<0.001) from the initial stage of secondary response and maintained through out the experiment and shows p<0.001 level of significance during 15 to 19 days after FCA injection as that of the group treated with the reference standard Indomethacin-10 mg/kg (Table 2 [Supplementary data]).

**Biochemical Parameters**

Standard drug (Indomethacin) and SME have shown the increase in hemoglobin content and RBC count when compared to arthritic control group (Table 3 [Supplementary data]). The total WBC counts were remarkably increased in arthritic control group. However, indomethacin and SME treated group significantly decreased (p<0.01) the total WBC count. The drastic increase in ESR count in arthritic control
Anti-arthritic property of *Syzygium cumini* group has been remarkably counteracted by the standard and methanolic extract, restoring it back to normal thus justifying their significant roles in arthritic conditions.

**Effect on body weight**

The arthritic control animals exhibited a significant decrease in body weight when compared with vehicle control group. The result showed the indomethacin-10 mg/kg and SME-500 mg/kg could ameliorate the weight loss occurred during arthritis (Table 4 [Supplementary data]).

**Radiographic analysis**

The clinical analysis of rheumatoid arthritis allows therapeutic monitoring which remains the standard method for evaluating the disease progress. The loss of articular cartilage leads to diminished joint space, which may be brought about a variety of pathological mechanism. The degree of bone resorption, diminished joint space and tissue swelling were markedly reduced in SME (500 mg/kg) and the lower dose of SME also shows the similar result. The standard drug, indomethacin (10 mg/kg) also shows the fractional tibial emphysis and the femoral condol appearance normal with no soft tissue swelling. (Fig. 1)

**DISCUSSION**

Freund’s complete adjuvant (FCA) induced arthritis models are extensively used to study the pathogenesis of rheumatoid arthritis for testing therapeutics (Mizushima *et al.*, 1972). One of the reasons for the wide utilization of this model is due to the strong correlation between the efficacy of therapeutic agents in this model and in rheumatoid arthritis in humans and it is characterized by very rapid erosive disease (Hunneyball *et al.*, 1986). In adjuvant arthritis, bacterial peptidoglycan and muramyl dipeptide are responsible for arthritis induction (Crofford *et al.*, 1992; Rainsford,
Anti-arthritic property of Syzygium cumini

It occurs through cell mediated-autoimmunity by structural mimicry between mycobacteria and cartilage proteoglycans in rats (Vijayalakshmi et al., 1997). In the present study, rats were selected to induce arthritis because rats develop a chronic swelling in multiple joints with influence of inflammatory cells, erosion of joint cartilage and bone destruction (Singh et al., 1996).

Chronic inflammation involves the release of number of mediators like cytokines (IL-IB and TNF-α), granulocyte monocyte colony stimulating factor (GM-CSF), interferon’s and platelet derived growth factor (PGDF). These mediators are responsible for the pain, destruction of bone and cartilage that can lead to severe disability (Eric et al., 1996). Paw swelling is one of the major factors in assessing the degree of inflammation and therapeutic efficacy of the drugs (Begum et al., 1988).

After FCA injection on the rat hind paw, a pronounced swelling and hyperalgesia appeared with no involvement of contralateral paw. This response is usually considered as a primary reaction. There is also a delayed hypersensitive response which is considered as latent secondary systemic response known to induce arthritis occurs after few days on the contralateral paw and characterized by tibiotarsal joint swelling and nodule formation in the tail. The secondary response could be due to the liberation and over-production of bradykinin, prostaglandins and kinins in paw tissue, which accompanies leukocyte migration (Garcia et al., 1973). According to our result and investigation more pronounced and reliable anti-inflammatory activity was observed in SME-500 mg/kg, which significantly (p<0.001) inhibited the development phase of chronic joint swelling induced by FCA on both the paws. The activity exhibited by extract was in dose-dependent manner.

Changes in body weight have also been used to assess the course of the disease and the response to therapy of anti-inflammatory drugs (Winder et al., 1969). Adjuvant arthritis is characterized by reduced weight loss (Campos et al., 2003) and the body weight loss is associated with increased production of pro-inflammatory cytokines such as TNF-α and interleukin-1 (Roubenoff et al., 1997). Treatment with SME extract showed significant (p<0.05) increase in body weight as that of vehicle control group.

In the present study, the arthritic rats exhibited a reduced RBC count, reduced Hb level and an increased ESR. All these indicate the anemic condition, which is a common diagnostic feature in patients with chronic arthritis (Allar et al., 1977; Mowat, 1971). Erythrocyte sedimentation rate (ESR) is an estimate of the suspension stability of RBC’s in plasma. It is related to the number and size of the red blood cells and to the relative concentration of plasma proteins, especially fibrinogen, α and β globulins. Increase in the rate is an indication of active but obscure disease processes. The acute phase proteins in ESR and C-reactive protein (CRP) share the property of showing elevations in the concentration in response to stress or inflammation like injection, injury, surgery and tissue necrosis (William, 1996). The treatment with the Syzygium cumini extract improved the RBC count, Hb level and the ESR to a near normal level indicating the significant recovery from the anemic condition and arthritis progress thus justifying its significant role in arthritic conditions (William, 1996). White blood cells (WBC) are a major component of the body’s immune system. Indications for a WBC count include infectious and inflammatory diseases (Mowat, 1971). In arthritis condition there is a mild to moderate rise in WBC count due to release of IL-IB. IL-IB increases the production of both granulocyte and macrophages colony stimulating factor (Eric et al., 1996; William, 1996). WBC count was increased in arthritic group. The migration of leukocytes is significantly suppressed in extract treated groups as seen from the significant decrease in the WBC count. Apart from prostaglandins, other cyclooxygenase products and various cells involved in inflammatory changes, free radical activities have all been implicated in the development of rat adjuvant arthritis (Vijayalakshmi et al., 1997).

The radiographic analysis of the knee joint in arthritis and drug treated animals further supported and confirms the potent antiarthritic effect of SME in a dose dependent manner which suppress the pathological changes, such as pannus formation and bone destruction.

Our phytochemical investigation revealed the presence of saponins, triterpenoids, flavonoids, tannins, steroids and alkaloids in the methanolic extract. Presence of wide range of constituents indicates the good efficacy of this plant in various pathological disorders. Saponins, flavonoids, steroids, tannins and alkaloids are known to inhibit articular swelling, decrease arthritis index and regulate down the content of IL-IB and TNF-α in the inflammatory tissue of arthritis rats (Wang et al., 2006; Hirano et al., 2006; Shukla et al., 2008). Moreover, all the chemical constituents identified in SME are well known for their antioxidant and immunomodulatory activities (Liu, 2003; Manach et al., 1996; Latha et al., 1998; Akindele et al., 2007; Iikay Orhan et al., 2007; Badger et al., 1997; Ramprasath et al., 2006). As arthritis is a complex and multifactorial disease, the presence of wide range of constituents may contribute to the significant anti-arthritic activity of SME.

From the results observed in the current investigation, it may be concluded that the methanolic extract of Syzygium cumini seeds possess potentially useful anti-arthritic activity. This study warrants the investigation.
to isolate and identify the active principles and to elucidate the exact mechanism of action.

References


Anti-arthritic property of *Syzygium cumini*


