International Journal of Medical, Medicine and Health Sciences
ISSN: 2517-9969
Vol:10, No:9, 2016

Role of Inflammatory Markers in Arthritic Rats Treated with Ethanolic Bark Extract of *Albizia procera*

M. Sangeetha, D. Chamundeeswari, C. Saravanababu, C. Rose, V. Gopal

***Abstract*—**Rheumatoid arthritis (RA) is a chronic, progressive, systemic inflammatory disorder affecting the synovial joints and typically producing symmetrical arthritis that leads to joint destruction, which is responsible for the deformity and disability. Despite improvements in the treatment of RA over the past decade, there still is a need for new therapeutic agents that are efficacious, less expensive, and free of severe adverse reactions. The present study aimed to investigate role of inflammatory markers in arthritic rats treated with ethanolic bark extract of *Albizia procera*. The protective effect of ethanolic bark extract of *Albizia procera* against complete Freund's adjuvant (CFA) induced arthritis in rats. Arthritis was induced by an intradermal injection of 0.1 ml FCA in the foot pad of left hind limb of rats. ETBE (100 and 200 mg/kg b.wt./p.o) and the reference drug diclofenac (25 mg/kg b.wt./p.o) were administered to arthritic rats. Paw volume was measured for all the animals before inducing arthritis and thereafter once in seven days by using plethysmometer for 42 days. Gene expression of inflammatory markers such as IL-1β and IL-10 were investigated in paw tissues. Up regulation of IL-1β and Down regulation IL-10 were observed in CFA injected rats when compared to normal rats. ETBE attenuated these alterations dose dependently when compared to the vehicle treated rats. These results provide insights into the mechanism of anti-arthritic activity, and unravel potential therapeutic use of *Albizia procera* in arthritis.

***Keywords*—**CFA-Complete Freund's adjuvant, ETBE, Ethanolic Bark Extract, IL- Interleukins, RA-Rheumatoid Arthritis.

I. Introduction

RA is an autoimmune disease in which the body's immune system attacks joints and other tissues. The pattern of joints affected is usually symmetrical, involves the hands and other joints. Arthritis is a common inflammatory joint disease characterized by inflammation of the synovial membrane, pain and restricted joint movement [1]. In RA, an immunological trigger begins an inflammatory process that ultimately manifests clinically by typical signs and symptoms of disease, such as joint swelling and tenderness [2]. Inflammation is also responsible for stimulating destructive mechanisms in the joint, which lead to structural damage and subsequently to functional declines and disability. The rheumatoid joint contains numerous cell types that are involved in these inflammatory and destructive processes [3].

According to World Health Organization (WHO), 0.3-1% of the world population is affected from RA, and among them, females are three times more prone to the disease as compared to males [4]. RA patients need life-long treatment with a variety of drugs duly supported by non-pharmacological interventions such as, physical and occupational therapy. Prevention of tissue destruction is one of the main aims of modern antirheumatic therapy. Both non-steroidal and steroidal anti-inflammatory drugs and DMARDS are being used in the management of arthritis. The ability of NSAIDS to interfere with the production of prostaglandins during the inflammatory cascade is the reason behind their anti-inflammatory action.

In India, many Ayurvedic practitioners use various indigenous plants for the treatment of different types of arthritic conditions. Although the application of these medicaments has a sound tradition and a rational background according to the Indian system of medicine, natural herbal remedies can effectively inhibit the inflammatory process safely and offer alternative to synthetic anti arthritic drugs. [5] One such plant drug used by traditional healers is *Albizia procera*.

Albizia procera (Family: Mimosaceae) is Himalayan tracts from Yamuna eastwards to west Bengal. Bark of the tree is a smooth, yellowish-brown colour. The bark decoction of *Albizia procera* is traditionally used in the treatment of cancer, inflammation and arthritis [6]. The present study was undertaken to scientifically validate the role of inflammatory markers in arthritic rats treated with ethanolic bark extract of *Albizia procera*.

II. Materials and Methods

A. Plant Materials

The bark of *Albizia procera* was collected from Sengottai, Tirunelveli, Tamil Nadu, India in the month of November 2009. The plant material was identified and authenticated. The collected plant material was taxonomically identified by the plant Anatomy Research center Chennai (PARC/2011/ 2315).

Dr. M. Sangeetha is with the Department of Pharmacognosy, Sri Ramachandra University, Chennai, India (phone: +91 98654 52555; e-mail: sangeethamoorthy@yahoo.com).
Dr.D. Chamundeeswari is with the Department of Pharmacognosy, Sri Ramachandra University, Chennai (e-mail: dvchamu@yahoo.co.in).
Dr. C. Saravanababu is with the Department of Pharmacognosy, JSS college of Pharmacy, Mysore University, Mysore. (e-mail: csaravanababu@gmail.com).
Dr. C. Rose is with the Department of Biotechnology, Central Leather Research Institute, Chennai (e-mail: rose.clri@gmail.com).
Dr. V. Gopal is with the Department of Pharmacognosy, Mother Theresa Post Graduate Research Institute of Health Sciences, Pondicherry (e-mail: gopalveni@yahoo.com).

B. Preparation of Extract

The bark was cleaned, cut into small pieces, and shade dried. The plant material was pulverized into coarse powder. The bark of *Albizia procera* (2.5 kg) was subjected to ethanol solvent. The maceration period was 72h, 48h, and 24h. Solvents were filtered, distilled and dried in a vacuum desiccator and extractive yield was calculated (4.5 gm w/w).

C. Animal Ethical Approval Reference

The study was performed with female sprague dawley rats (125-150 g). Rats were acclimatised, fed commercial pelleted feed and water ad libitum. Experimental protocols were approved by the committee for the purpose of control and supervision of experiments on animals (CPCSEA), New Delhi, India (IAECXII/SRU/82/2008).

D. Acute Oral Toxicity Study

Acute oral toxicity study was performed according to the OECD test guideline 423-Acute toxic class method. Young healthy adult sprague dawley female rats weighing between 160-180g b.wt., were divided into seven groups of three animals/group. Animals were housed individually in a well-ventilated polypropylene cage. A 12-h light/12-h dark artificial photoperiod was maintained. Room temperature of 22°C (±3°) and relative humidity 50-70% were maintained in the room. Animals had free access to pelleted feed. (Nutrilab rodent, Tetragon Chemie Pvt Ltd., India) and Reverse osmosis (Rios, USA) purified water ad libitum. The test drug was administered once orally via gastric intubation at a dose level of 2000 mg/kg b.wt. Lethality and abnormal clinical signs were observed on the day of dosing and thereafter for 14 days [6]. Body weight was recorded before dosing and thereafter once in a week till the completion of the experiment. Gross pathological changes were also observed at the end of experiment. [7].

E. Anti-Arthritic Activity

Arthritis was induced by subcutaneous injection of 0.1 ml of CFA which contains 10 mg of heat killed mycobacterium tuberculosis per ml of paraffin oil into the plantar surface of the left hind paw [8]. Animals were divided into five groups of five animals each. Group I: control rats orally treated with vehicle. Test groups were injected with 0.1 ml of CFA intradermally. Group II: disease control. Group III: treated with standard drug, diclofenac sodium (0.3 mg/kg/day/p.o.) while Group IV and Group V with ETBE (100 and 200 mg/kg/day/p.o., respectively) from day 15th to 41st of induction. Inflammation was assessed by measuring the hind paw volume using mercury displacement plethysmometer. On the 42nd day, animals were sacrificed, blood was collected, and the paw tissue was collected for the investigation of inflammatory markers.

F. Gene Expression Study of Inflammatory Marker

Reverse transcription (RT) - PCR will be performed to determine the level of mRNA expression of pro and anti-inflammatory markers. Briefly, total RNA will be extracted from scrapped tissues of dissected paw using Trizol Reagent (Sigma, USA). After homogenization, the tubes will be incubated for 10 minutes and centrifuged at 1000 rpm for 5 min. 200µl of chloroform will be added to the supernatant and allowed to incubate for 5 min at room temperature and centrifuged at 12000 rcf for 20 min. The isolated RNA will be used for reverse transcription and polymerization reaction to get cDNA using PCR master cycler gradient. The formed cDNA will be load in agarose gel, allowed to run the electrophoresis at 80V for 30 min and the gene expression will be analyzed [9].

Primers sequence used were as follows, β-actin: sense, 5’-CCT CTA TGC CAA CAC AGT GC -3’; antisense, 5’- GTA CTC CTG CTT GCT GAT CC -3’, IL-1β: sense, 5’- CCC TGC AGC TGG AGA GTG TGG -3’; antisense 5’- TGT GCT CTG CTT GAG AGG TGC T -3’, IL-10: sense, 5’- TGC CTT CAG TCA AGT GAA GAC T -3’; antisense, 5’- AAA CTA TTC ATG GCC TTG TA -3’.

III. Result and Discussion

There were no deaths related to treatment, abnormal clinical signs, remarkable body weight changes or gross pathological changes observed in all the experimental animals. No mortality was observed up to a dose level of 2000 mg/kg b.wt. No changes in autonomic profiles (writhing, heart rate, defecation, light reflex, etc.), neurological response (abdominal tone, twitching, grip strength, limb tone, etc.). Behavioral response (alertness, irritability, fearfulness, touch response, etc.) was normal. The extracts do not cause any apparent acute toxicity and hence may be confirmed as non-toxic. Hence, the test drugs fall in the “category- 5” or “unclassified” in accordance to the Globally Harmonized System.

There were no significant changes in body weight between the experimental animals throughout the experimental periods [10]. Reported that loss in body weight in arthritic animals was due to alteration in metabolic activities. However, no such alteration was observed in the present experiments.

A significant ($p<0.01$) increase in paw oedema volume was observed in the vehicle treated arthritic rats from day 7 till the completion of experiment. Treatment with ETBE at 100 mg/kg significantly reduced the paw oedema on day 35 ($p<0.05$) and 42 ($p<0.01$). ETBE 200 mg/kg produced significant reduction in paw oedema volume on day 21 ($P<0.05$), 35 ($P<0.01$) and day 42 ($P<0.01$). CFA is a reagent frequently used for the induction of RA as a model of the disease [11]. The reduction in paw oedema with ETBE treatment may be related to its interactions with cell mediated immunity and also its capacity to suppress vascular permeability. This effect may be a systemic or peripheral one. The effect of ETBE was comparable with that of the standard anti-inflammatory agent diclofenac.

The ETBE inhibited the rat paw oedema by 61.36%, after 42 days. Gene expression of inflammatory markers such as IL-1β and IL-10 were also investigated in Paw tissues. In CFA injected rats up regulation of IL-1β and down regulation of IL-10 were observed when compared to normal rats. ETBE (100

& 200 mg/kg) showed significant changes in dose dependently when compared to positive Control.

Acid phosphatase seems to be an important index for the examination of the integrity of the lysosomal membrane and is responsible for the tissue damage and necrosis of hepatic tissue. Increased activities of plasma acid phosphates were observed in arthritic rats. This may be attributed towards persistent inflammation. These changes are due to decreased lysosomal stability in adjuvant induced arthritis. [12].

TABLE I
EFFECT OF ETBE OF *ALBIZIA PROCERA* ON BODY WEIGHT OF EXPERIMENTAL ANIMALS OF ACUTE ORAL TOXICITY

Treatment	Day 0	Day 7	Day 14
Control	160.67±0.88	176.00±1.00	180.33±0.88
ETBE (2000mg/kg)	161.33±4.81	173.33±3.38	180.00±3.61

TABLE II
EFFECT OF ETBE OF ALBIZIA PROCERA ON BODY WEIGHT IN ARTHRITIC RATS

Day	Body weight (g)				
	G1	G2	G3	G4	G5
0	237.00 ±5.10	239.00 ±7.92	235.00 ±5.78	235.00 ±8.30	239.00 ±8.20
7	246.00 ±6.16	245.00 ±10.31	239.00 ±5.21	233.00 ±6.85	218.00 ±7.49
14	243. 00 ±5.01	236.00 ±9.33	240.00 ±5.31	223.00 ±7.76	226.00 ±5.75
21	238.00 ±2.48	225.00 ±8.14	241.00 ±4.71	224.00 ±4.91	220.00 ±6.06
28	239.00 ±1.52	223.00 ±8.94	243.00 ±5.27	233.00 ±4.55	224.00 ±5.54
35	243.00 ±5.31	221.00 ±4.98	244.00 ±3.37	234.43 ±4.29	234.29 ±6.39
42	248.00 ±5.78	215.00 ±5.67	246.00 ±4.57	237.28 ±5.93	239.19 ±4.09

G1-Normal Control, G2-Positive Control, G3-Diclofenac (25 mg/kg), G4-ETBE (100 mg/kg), G5-ETBE (200 mg/kg).

TABLE III
EFFECT OF ETBE OF *ALBIZIA PROCERA* ON PAW OEDEMA IN ARTHRITIC RATS

Day	Paw oedema (% inhibition)				
	G1	G2	G3	G4	G5
7	0.00 ±0.00	0.79 ±0.04**	0.80 ±0.05	0.77 ±0.03	0.78 ±0.02
14	0.01 ±0.00	0.81 ±0.04**	0.82 ±0.06	0.78 ±0.05	0.79 ±0.03
21	0.00 ±0.00	0.94 ±0.07##	0.65 ±0.03** [30.58]	0.78 ±0.04 [17.02]	0.75 ±0.03* [20.21]
28	0.01 ±0.00	1.49 ±0.11##	1.01 ±0.09 [32.21]	1.22 ±0.16 [18.12]	1.08 ±0.17 [27.52]
35	0.01 ±0.01	1.78 ±0.12##	1.08 ±0.14** [39.33]	1.19 ±0.08* [33.15]	1.02 ±0.16** [42.70]
42	0.00 ±0.00	1.76 ±0.12##	0.63 ±0.16** [64.20]	0.88 ±0.10** [50.00]	0.68 ±0.11** [61.36]

G1-Normal Control, G2-Positive Control, G3-Diclofenac (25 mg/kg), G4-ETBE (100 mg/kg), G5-ETBE (200 mg/kg).

Values were expressed in mean±SEM; n=5 animals; Significance was analysed by using one-way Analysis of Variance (ANOVA) followed by Tukey's multiple comparison test; #, ## denotes P<0.05 and P<0.01 respectively when compared to normal control; *, ** denotes P<0.05 and P<0.01 respectively when compared.

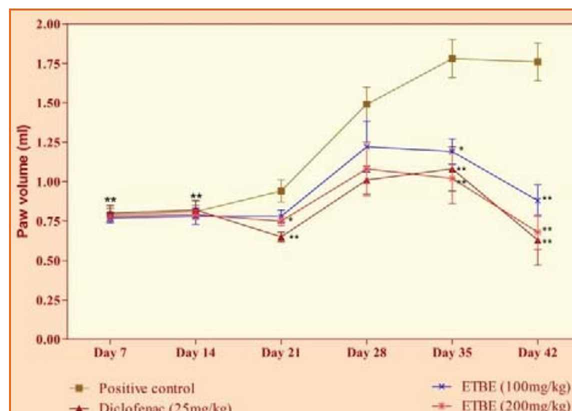

Fig. 1 Effect of ETBE of *Albizia procera* on paw oedema in arthritic rats

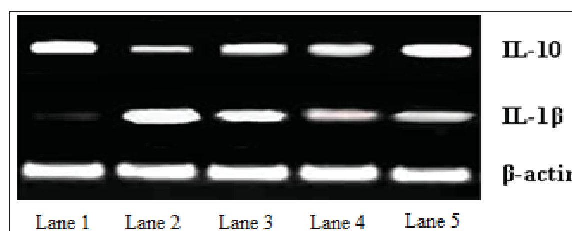

Fig. 2 Gene expression of inflammatory markers

Lane 1-Normal control, Lane 2-Positive control, Lane 3-Diclofenac (25 mg/kg), Lane 4-ETBE (100 mg/kg), Lane 5-ETBE (200 mg/kg).

IV. CONCLUSION

From the present experimental investigations, it is concluded that at the doses of 100 mg/kg and 200 mg/kg of ethanolic bark extract of *Albizia procera* possesses potentially useful anti-arthritic activity as it gives a positive result in controlling inflammation in adjuvant induced arthritic model in rats. The drug is a promising anti-arthritic agent of plant origin in the treatment of inflammatory disorders.

REFERENCES

[1] Pearson, C. M. Development of arthritis, periarthritis and periostitis in rats given adjuvants. Proc Soc Exp Biol Med, 1956, 91, 95-101.
[2] Choy, E. H. & Panayi, G. S. 2001. Cytokine pathways and joint inflammation in rheumatoid arthritis. N Engl J Med, 344, 907-16.
[3] Feldman, M., Brennan, F.M. & Maini, R.N. 'Role of cytokines in rheumatoid arthritis', Annu Rev Immunol, 1996, 14, 397-440.
[4] Tripathy S, Pradhan D, Anjana M. Anti-inflammatory and antiarthritic potential of *Ammania baccifera* Linn. Int J Pharm Bio Sci. 2010,1:1–7.
[5] Shah BN, Nayak BS, Seth AK, Jalalpure SS, Patel KN, Patel MA, Search for medicinal plants as a source of anti-inflammatory and anti-arthritic agents - A review. Pharmacog Mag. 2006;2:77–86.
[6] Kritikar K.R. and B.D. Basu "International book distribution", Dehradun, India, Indian medicinal plants, 1987,943.
[7] OECD, "Guidelines for the Testing of Chemicals / Section 4: Health Effects Test No. 423: Acute Oral toxicity - Acute Toxic Class Method", Organization for Economic Cooperation and Development, Paris, France, 2002.

[8] Paran, D., Kidron, D., Mayo, A., Ziv,O., Chowers, Y., Caspi, D., Yaron,M. & Paran, H, "Somatostatin analogue treatment attenuates histological findings of inflammation and increases mRNA expression of interleukin-1 beta in the articular tissues of rats with ongoing adjuvant induced arthritis", Rheumatol Int, 2005,25(5),350-356.
[9] Hall, L.R., Mehlotra, R.K., Higgins, A.W., Haxhiu, M.A & Pearlman, E, "An essential role for interleukin-5 and eosinophils in helminth-induced airway hyper responsiveness", Infection and Immunity, 1998, 66, 4425–4430.
[10] Olsen, I., Bon-Gharios, S. & Abraham, D. The activation of nresting lymphocytes is accompanied by the biogenesis of lysosomal organnels. Eur J Immunol, 1990, 20, 2161-2170.
[11] Choi, S. S., Lee, J. K. & Suh, H. W. Anti nociceptive profiles of aspirin and acetaminophen in formalin, substance P and glutamate pain models. Brain Res, 2002,921, 233-9.
[12] Walz, D.T., Dimartino, M.J. & Misher, A. Adjuvant induced arthritis in rats. II. Drug effects on physiologic, biochemical and immunologic parameters. J Pharmacol Exp Ther, 1971, 178(1), 223-231.

M. Sangeetha is from Coimbatore district of Tamilnadu and born on 14/09/1976. Working in Sri Ramachandra University, Chennai. She has M.Pharm in Pharmacognosy (Trichengodu, Tamilnadu, 2000), M.B.A in Human Resources (Delhi, 2008) and PhD (Studies on the Effects of *Albizia procera* Benth.on Inflammatory Markers in Rheumatoid Arthritis, 2014).

Dr. M. Sangeetha has over 14 years of teaching experience in the field of Pharmacognosy. She has held various designations in teaching such as a LECTURER for 5 years, an ASSISTANT PROFESSOR for over 7 years and an ASSOCIATE PROFSSOR for 1 year 4 months. She has presented several presentations in international and national conferences. She has also published papers in journals. She has guided several PG and UG projects for the students of Faculty of Pharmacy, Sri Ramachandra University. Currently she is working on a funded project in Sri Ramachandra University. She is member in Tamil Nadu Pharmacy Council, No.3804/A1, Indian Pharmacist Association and Association of Pharmacy Teachers of India- LM-555.

Dr. M. Sangeetha has been appreciated with a cash award of INR 5000 as a special award for the Oral Presentation in the conference organized by ICPG 2012 held at Chettinad Health City, Chennai in October 2012.