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Curative Effect of *Albizia lebbeck* Methanolic Extract against Adjuvant Arthritis-With Special Reference to Bone Erosion

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ABSTRACT
Localized bone loss in the form of bone erosions and peri-articular osteopenia constitutes important signs for the diagnosis of rheumatoid arthritis. In the present study, the effect of *Albizia lebbeck* Methanolic extract (AL) on the bone erosion turn over was studied by analyzing various markers of bone erosion like histological and radiological analysis of the joints in Freund' complete adjuvant induced- arthritis in rats. The anti-arthritic activity of methanol extract of the bark of *Albizia lebbeck* (Mimosaceae) was evaluated against Freund's complete adjuvant induced- arthritis induce arthritis model in rats. Arthritis was induced in rats by injecting 0.1ml of Freund's complete adjuvant containing 6 mg of heat killed mycobacterium tuberculosis in 1ml paraffin oil into the left hind paw of the rat intradermally. AL (200 mg/kg, 400 mg/kg, 600 mg/kg body weight/day) was administered orally for 12 days. On 21st day of experiment, the histopathological and radiological observation was carried out along with rheumatoid factor and arthritic index. It can be concluded that *Albizia lebbeck* Methanolic extract (AL) possesses strong anti-arthritic property by modulating bone erosion.

Keywords: *Albizia lebbeck,* freund's complete adjuvant induced arthritis, bone erosion.

INTRODUCTION

Bone loss is a common feature of various inflammatory arthritis Bone erosions results from the activation of an inflammatory response that increases the number and activity of osteoblasts. Bone erosions and peri-articular osteopenia constitutes an important signs for the diagnosis of rheumatoid arthritis. [1-2] The complete freund"s adjuvant induced arthritis model represents a systemic inflammatory disease with bone and cartilage changes similar to those observed in RA. The common pathological features of adjuvant arthritis in rats and RA inhuman are joint swelling associated with cellular and pannus invasion of the joint space and bone resorption. [3-4] Strong bone loss after intense arthritis is induced when adjuvant is injected into the foot pad. [5-6] The major site of irreversible tissue damage originates from synovium lining the joint capsule with cartilage and bone, which is often termed the "pannus" and this is a characteristic feature of RA.

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Albizia lebbeck Benth. (Family: *Mimosaceae*) is an unarmed deciduous which grows about 12-21 m high, bark pale with glabrous young shoot. The bark is bitter, cooling, alexiteric, anthelmintic, cures "vata", diseases of the blood, leucoderma, itching, skin disease, piles excessive perspiration, inflammation, bronchitis, good in rat bite. The bark is good for opthalmia. The flowers are given for asthma and for snakebite. All parts of the plant are recommended for the treatment of snake-bite. [7] It is reported to possess nootropic [8-9], anxiolytic [9], anticonvulsant [10-11], antifertility [12] and antidiarrhoeal. [13] Different phytochemicals have been Isolated from beans which include albigenin - a triterpene [14] and albigenic acid -a triterpenoid sapogenin. [15] Albiziahexoside– a bioactive saponin isolated from bark. [16]

MATERIALS AND METHODS

The *Albizia lebbeck* was collected in the month of February from Balwa chokadi (Chiloda) gandhinagar Gujarat, India. & aunthetification was done by pharmacognosy department and voucher specement was deposited in hurbenuium museum of SKPCPER. Freshly colleted plant parts were washed. The bark was pulverized to coarse powder. The powder was extracted with methanol in a Soxhlet apparatus. The extract was evaporated under reduced pressure by a

rotary vacuum evaporator until all the solvent had been removed to give an extract. Preliminary qualitative analysis of methanol extract showed the presence of Tannins, saponins, reducing sugars and triperpenoids. Methanolic extract was administered orally to animals after suspending it in 2 % v/v Tween 80 aqueous solution. Freund's complete adjuvant was procured from Sigma chemicals, St. Louis, USA. All other chemicals used were of analytical grade. The drugs were prepared as described in the Formulary of Siddha medicine.

Experimental design

Adult female wistar rat (prevalence of rheumatoid arthritis is more in female) with an initial body weight of 200 to 300 g were taken, and divided into six groups each containing six animals. On day zero, all rats were injected into the sub plantar region of the left hind paw with 0.1 ml of Freund's complete adjuvant. This consist of Mycobacterium butyricum suspended in heavy paraffin oil by thorough grinding with motor and pestle to give a concentration of 6 mg/ml. Dosing with the test and standard compounds was started on the first day and continued for 12 days according to the following schedule:

Group I: Normal control (Distilled water),
Group II: Disease control (suspension of 1% CMC),
Group III: Dexamethasone (5 mg/kg, p.o., standard),
Group IV: Methanolic extract of *Albizia lebbeck* (200 mg/kg, p.o.),
Group V: Methanolic extract of *Albizia lebbeck* (400 mg/kg, p.o.),
Group VI: Methanolic extract of *Albizia lebbeck* (600 mg/kg, p.o.).

From day 13^{th} to 21^{st}, the animals were not dosed with the test compound or the standard. The following parameters were measured. [17]

Paw edema

Paw volumes of both hind limbs were recorded on the day of CFA injection, and again measured on day 1^{st}, 3^{rd}, 6^{th}, 9^{th}, 13^{th}, 21^{st} using mercury column plethysmometer. The 6^{th} day measurement is indicative of primary lesions and13^{th} day measurement will aid in estimating secondary lesions. On the day 21^{st}, the secondary phase of rheumatoid arthritis becomes more evident and inflammatory changes spreads systemically and becomes observable in the limb not injected with Freund's adjuvant.

Arthritic index

All the animals were closely observed for organs like ears, nose, tail, fore paws and hind paw and arthritic index (Pearson CM, 1959) was calculated. [14]

Rheumatoid factor

The latex turbidimetry method was used in the present study using RF turbilatex kit of SPINREACT Company. Calibration was carried out for linear range up to 100 IU/ml. The reading of RF factor of all the groups obtained was compared with the control animals and was expressed as IU/ml RF. [19]

Radiography

Female wistar rats were sacrificed on 21^{st} day of Freund's complete adjuvant administration and legs are removed and placed on formalin containing plastic bag. This plastic bag was kept at a distance of 90 cm from the X-ray source was and Radiographic analysis of arthritic and treated animal hind paw were performed by X-ray machine (International journal Electron Company) with a 300-mA exposition for 0.01 s. An investigator blinded for the treatment regime performed radiograph score. The following radiograph criteria were considered: These scores (destroyed or intact joint) were used as a quantal test for bone necrosis. Radiographs were carefully examined using a stereo microscope and abnormalities were graded as follows:

(i) Periosteaic reaction, 0 - 3 (none, slight, moderate, marked);
(ii) Erosions, 0 - 3 (none, few, many small, many large);
(iii) Joint space narrowing, 0 - 3 (none, minimal, moderate, marked);
(iv) Joint space destruction, 0 - 3 (none, minimal, extensive, ankylosis).

Bone destruction was scored on the patella as described previously. [20]

Histological processing and assessment of arthritis damage

Rats were killed by ether anesthesia. Knee joints were removed and fixed for 4 days in 4 % formaldehyde. After decalcification in 5 % formic acid, the specimens were processed for paraffin embedding tissue sections (7 µm thick) and were stained with haematoxylin and eosin, or safranin. An experienced pathologist, unaware of the different drug treatments scored the condition of tibiotarsal joints. Histopathological changes were scored using the following parameters. Infiltration of cells was scored on a scale from 0 to 3, depending on the amount of inflammatory cells in the synovial tissues. Inflammatory cells in the joint cavity were graded on a scale from 0 to 3 and expressed as exudate. A characteristic parameter in Freund's complete adjuant is the progressive loss of articular cartilage. This destruction was separately graded on a scale from 0 to 3, ranging from the appearance of dead chondrocytes (empty lacunae) to complete loss of the articular cartilage. Bone erosion was scored on a scale ranging from 0 to 3, ranging from no abnormalities to complete loss of cortical and trabecular bone of the femoral head. Cartilage and bone destruction by pannus formation was scored ranging from 0, no change; 1, mild change (pannus invasion within cartilage); 2, moderate change (pannus invasion into cartilage/subchondral bone); 3, severe change (pannus invasion into the subchondral bone); and vascularity (0, almost no blood vessels; 1, a few blood vessels; 2, some blood vessels; 3, many blood vessels). Histopathological changes in the knee joints were scored in the femur region on 5 semiserial sections of the joint, spaced 70 µm apart. Scoring was performed on decoded slides by two observers, as described earlier. [20-23]

RESULTS

The hind paw injected with complete Freund's adjuant became gradually swollen and reached its peak at 21st day. Table 1 showed the results obtained for the different formulation of AL and the standard drug (Dexamethasone 5mg/kg) in the complete freund's adjuant-induced (CFA) paw edema test at specific time intervals. It was obvious that during 21st day treatment paw edema in disease control inflamed paw is increase in time dependent manner and all administration groups significantly inhibited the development of joint swelling induced by complete Freund's adjuant.

Arthritic index and rheumatoid factor were significantly decreased in treatment with AL (200 mg/kg, 400 mg/kg and 600mg/kg) and dexamethasone (5 mg/kg) treated animal as compare to disease control treatment.

Bone destruction, which is a common feature of adjuvant arthritis, was examined by radiological analysis. Freund's Complete Adjuvant treated rats had developed definite joint space narrowing of the intertarsal joints, diffuse soft tissue swelling that included the digits, diffuse demineralization of bone, marked periosteal thickening, and cystic enlargement of bone and extensive erosions produced narrowing or pseudowidening of all joint spaces. In contrast, in rats treated with AL attenuate abnormalities consisted of asymmetric soft tissue swelling and small erosions, periosteal thickening, and minimal joint space narrowing, predominantly localized to the proximal areas of the paws.

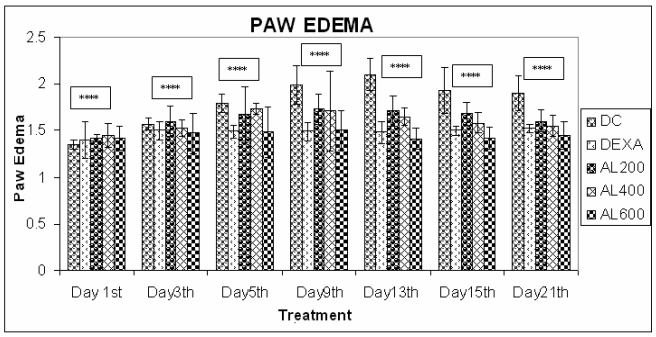
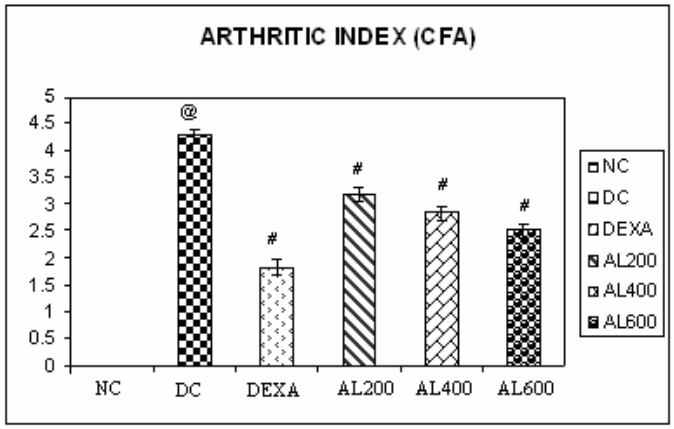


Fig. 1: Effect of *Albizia lebeeck* methanolic extract (AL) on paw edema in Freund's complete adjuvant induced arthritis in rat. Data are presented as Mean ± SEM (n=6), * P < 0.001, when compared with Disease control. (One Way ANOVA).



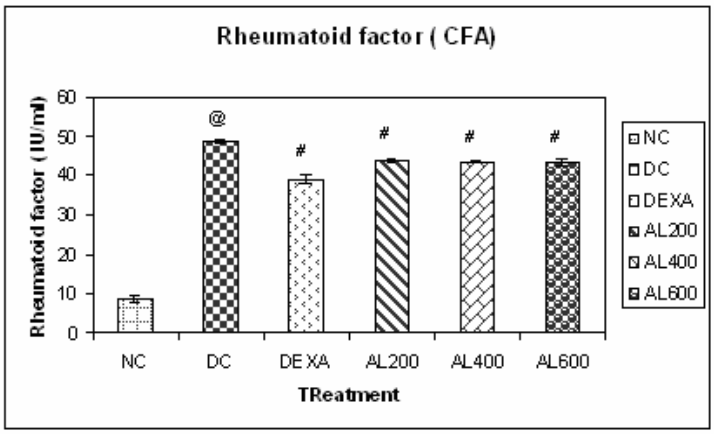


Fig. 2: Effect of *Albizia lebeeck* methanolic extract (AL) on arthritic index and rheumatoid factor in Freund's complete adjuvant induced arthritis in rat. Data are presented as Mean ± SEM (n=6), @ P < 0.001, when compared with normal control, # P < 0.001, when compared with disease control. (One Way ANOVA)

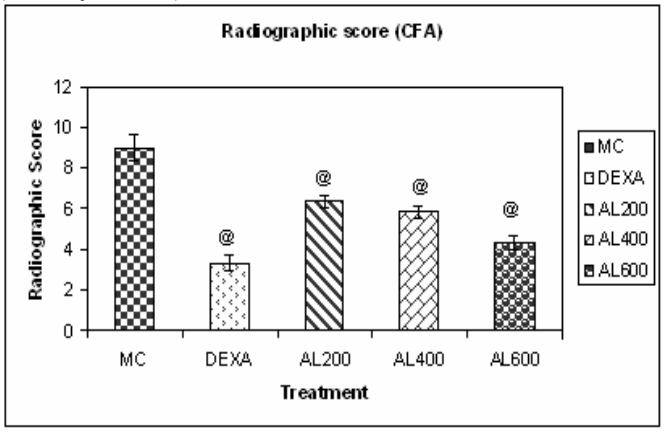


Fig. 3: Effect of *Albizia lebeeck* methanolic extract (AL) on radiographic score in Freund's complete adjuvant induced arthritis in rat. Data are presented as Mean ± SEM (n=6), @ P < 0.001, when compared with disease control, (One Way ANOVA)

As shown in Fig 5 a [NC]: Histology of synovial joint of normal control rat with intact morphology of synovium and synovial lining Fig 5 b [DC]: FA induced disease control rat showed plenty of lymphocytic infiltration [↓]in synovial lining with severe inflammation and marked angiogenesis [↕] studied with proliferation of synovial cells [↓↓]Fig 5 c [DEXA]: Dexamethasone [5 mg/kg] treated rats showed significant protection with mild lymphocytic infiltration [↓] with no evidence of thickening of synovial lining and angiogenesis Fig 5 d AL200: AL 200 treated rats showed milder angiogenesis [↕], lymphocytic infiltration [↓] and synovial lining thickening[↓↓]. Fig 5 e AL400: AL400 treated rats showed milder angiogenesis [↕], synovial lining thickening [↓↓] with no evidence of lymphocytic infiltration. Fig 5 f AL600: AL600 treated rats showed milder lymphocytic infiltration [↓] and synovial-lining thickening [↓↓] with no evidence of angiogenesis.

DISCUSSION

Rheumatoid Arthritis is an autoimmune disorder, the immunologically mediated complete Freund's adjuvant induced arthritic model of chronic inflammation is considered as the best available experimental model of RA. [19] Complete Freund's adjuvant-induced arthritis is a model of chronic polyarthritis with features that resemble RA. [24] Therapeutic efficiency of herbal drug like *Glycerhhiza glabra* & *moschus moschipus* were mainly investigated in the rat adjuvant arthritis model. [25] Evaluation of the inflammatory stratus in RA is reflected inflammation in the hind paw. Progression of disease in AL treated group shows reduction in edema in dose dependent manner as compare to tissues control animals. Symmetric involvement of small hand joints (especially proximal interphalangeal and metacarpophalangeal), foot joints (metatarsophalangeal), wrists, elbows, and ankles is typical, but initial manifestations may occur in any joint. Inflammation and / or nodules are observed on ears, nose, and tail, fore paws and hind paws. Arthritic index is the average of the score given to severity of the lesions in these places. This gives full picture of the disease. [17] AL treated animal showed significant lesser arthritic index as compared with disease control animals. Prominent immunological abnormalities that may be important in pathogenesis of RA include immune complexes are found in joint fluid cells and in vasculitis. Plasma cells produce antibodies e.g., rheumatoid factor (RF) that

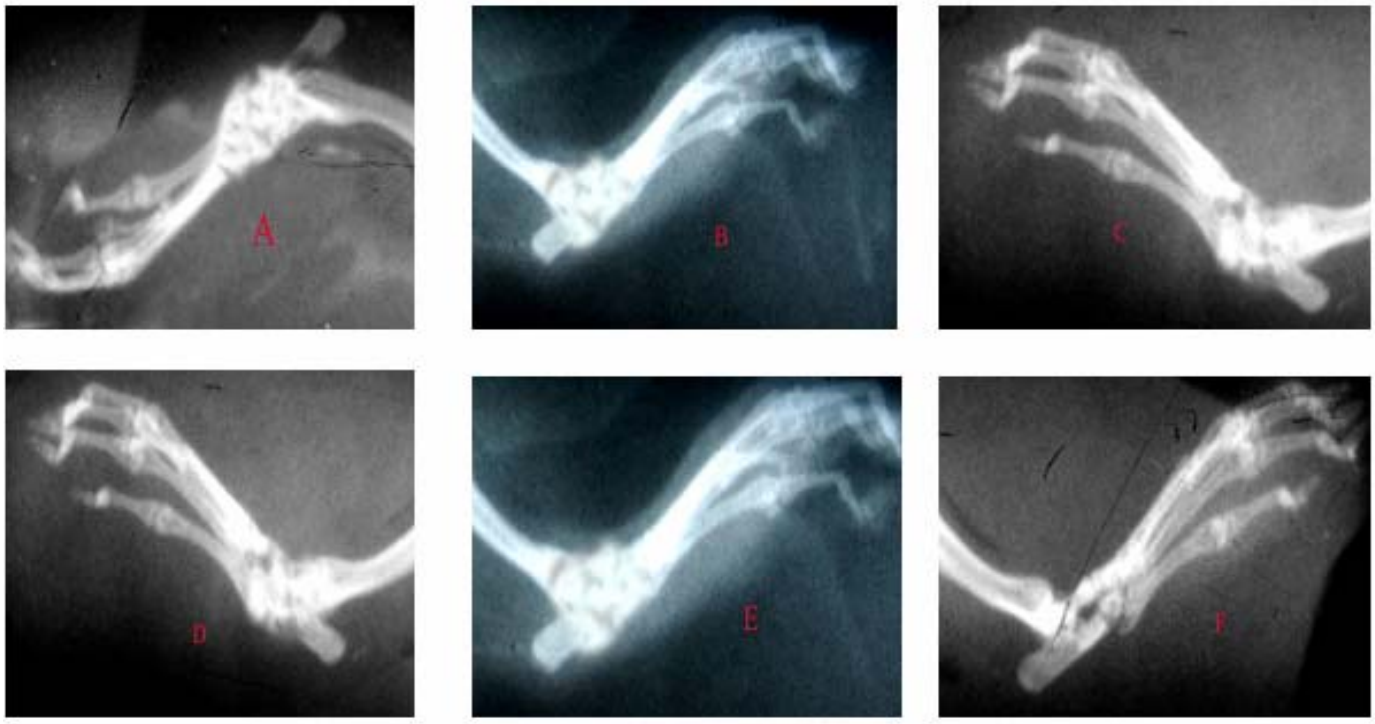


Fig. 4: Effect of *Albizia lebeeck* methanolic extract (AL) on Radiographs of tibiotarsal joints

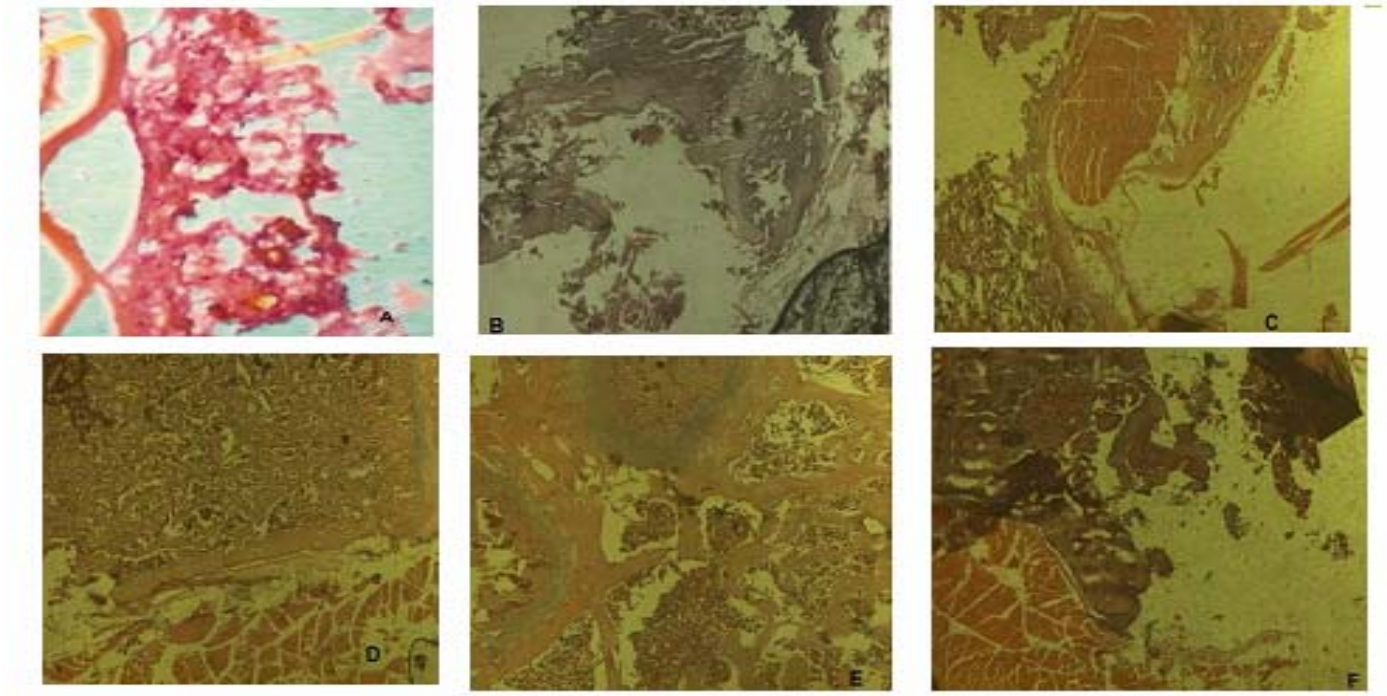


Fig. 5: Describe histopathology of joints indicate treatment with *Albizia lebbeck* methanolic extract (AL) prevent bone erosion

contribute to these complexes. Serum rheumatoid factor (RF) is the immunological expression of an individual's immune system reaction to the presence of an immunoglobulin molecule that is recognized as "non-self." This response to the "non-self" immunoglobulin results in the presence of immune complexes. These, in turn, bind complement and may eventually lead to synovium, cartilage, and bone destruction. Higher the levels of serum rheumatoid factor, higher are the development of inflammation. [26] Serum rheumatoid factor (RF) measures the amount of antibody IgM titer present in the serum. [27] AL treated animal showed significantly lesser serum RF when compared to disease control animals.

Bone destruction, which is a common feature of adjuvant arthritis, was examined by radiological analysis. Adjuvant treated rats had developed definite joint space narrowing of the intertarsal joints, diffuse soft tissue swelling that included the digits, diffuse demineralization of bone, marked periosteal thickening, and cystic enlargement of bone and extensive erosions produced narrowing or pseudowidening of all joint spaces. Despite a similar clinical course of arthritis, disease control rats suffered from more pronounced bone destruction than AL treated group.

RA is characterized by synovial tissue leukocyte ingress and angiogenesis. [28] The disease is thought to occur as an immunological response to as yet unidentified antigen. Even

in early RA, some of the earliest histological observations are blood vessels. [29] A mononuclear infiltrate characterizes the synovial tissue along with a luxuriant vasculature. Angiogenesis is integral to formation of the inflammatory pannus and without angiogenesis, leukocyte ingress could not occur. Changes in the density of blood vessels in the synovium and alterations in endothelial proliferative responses in RA have been shown in a range of studies. The number of synovial blood vessels has been found to correlate with hyperplasia of synovial cells, infiltration of mononuclear cells, and indices of joint tenderness. [30] Histopathology study of synovial joint showed that treatment with *Albizia lebbeck* Group decreased vascularity, lymphocytic infiltration with less rheumatoid inflammation and angiogenesis, with no thickening of synovial membrane and absence of lymphoid follicles. As compared to disease control and orally treated animals.

Our data suggested that *Albizia lebbeck* possesses significant antiarthritic activity. The possible mode of anti- arthritic activity of Methanolic extract of *Albizia lebbeck* appears to be, Possessing anti–inflammatory activity showed in arthritic parameters like Paw edema, Arthritic index, Rheumatoid factor, improving bone erosion.

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REFERENCES

1. Rehman Q, Lane NE. Bone loss therapeutic approaches for preventing bone loss in inflammatory arthritis, Arth. Res. 2001; 3:221–227.
2. Goldring SR, Gravallese EM. Mechanisms of bone loss inflammatory arthritis: diagnosis and therapeutic implications,Arth. Res. 2000; 2: 33–37.
3. Osterman T, Virtamo T, Lauren L, Kippo K, Pasanen I, Hannuniemi R, Vaananen K, Sellam R. Slow-release clodronate inprevention of inflammation and bone loss associated with adjuvantarthritis, J. Pharmacol. Exp. Ther. 1997; 280:1001–1007.
4. Allen MJ. Biochemical markers of bone metabolism in animals:uses and limitations, Vet. Clin. Pathol. 2003; 32 (3):101–113.
5. Yamamura K, Yonekawa T, Nakamura T, Yano S, Ueno K, The histamine H2-receptor antagonist, cimetedine, inhibits the articular osteopenia in rats with adjuvant—induced arthritis bysuppressing the osteoclast differentiation induced by histamine, J. Pharmacol. Sci. 2003; 92: 43–49.
6. Iwamura H, Moore AR, Willough DA. by, Interaction between neutrophil derived elastase and reactive oxygen species in cartilagedegradation, Biochim. Biophys. Acta 1993; 1156: 295–301.
7. Kirtikar KR, Basu BD Indian Medicinal Plants.2nd ed, Vol. 2 International Book Distributor, Dehra Dun, India 1999, pp. 936-938.
8. Chintawar SD, Somani RS, Kasture VS, Kasture SB. Nootropic activity of Albizia lebbeck in mice. Journal of Ethnopharmacology. 2002; 81:299-305.
9. Une HD, Sarveiya VP, Pal SC, Kasture VS, Kasture SB. Nootropic and anxiolytic activity of saponins of Albizia lebbeck leaves. Pharmacology Biochemistry and Behavior. 2001; 69: 439-444.
10. Kasture VS, Chopade CT, Deshmukh VK. Anticonvulsive activity of Albizia lebbeck, Hibiscus rosa sinesis and Butea monosperma in experimental animals. Journal of Ethnopharmacology. 2000; 71: 65-75.
11. Kasture VS, Kasture SB, Pal SC. Anticonvulsant activity of Albizia lebbeck leaves. Indian J of Exp Biol. 1996; 34:78-80.
12. Gupta RS, Kachhawa JB, Chaudhary R. Antifertility effects of methanol pod extract of Albizia lebbeck (L.) Benth in male rats. Asian J Androl. 2004; 6: 155-159.
13. Besra SE, Gomes A, Chaudhury L, Vedasiromoni JR, Ganguly DK. Antidiarrhoeal activity of seed extract of Albizia lebbeck Benth. Phytotherapy Research. 2002; 16: 529-533.
14. Barua AK, Raman Pattabi S. Triterpenoids—X: The constitutionof albigenic acid—a new triterpenoid sapogenin fromAlbizia lebbeck benth. Tetrahedron 1959; 1: 19.
15. Barua AK, Raman SP. Triterpenoids-XII: The constitutionof albigenin—a new triterpene from Albizia lebbeckbenth. Tetrahedron 1962; 18: 155-159.
16. Ueda M, Tokunaga T, Okazaki M, Satan NU, Ueda K, YamamuraS. Albiziahexoside: a potential source of bioactivesaponin from the leaves of Albizia lebbeck. Nat. Prod. Res. 2003; 17: 329-335.
17. Colpert KM. Evidence that adjuvant arthritis in the rat is associated with chronic pain: Pain. 1987; 28:201-222
18. Pearson CM, Wood FD. Studies on polyarthritis and other lesions induced in rats by injection of mycobacterium adjuvant. I. General clinic and pathological characteristics and some modifying factors. Arth rheum. 1959; 2: 440-459.
19. Van de Berg WB, Joosten LAB, Helsen MMA, van de Loo FAJ. Amelioration of established murine collagen-induced arthritis with anti-IL-1 treatment. Clin Exp Immunol. 1994; 95:237–243.
20. Joosten LAB, Helsen MMA, Van de Loo FAJ, van den Berg WB. Anticytokine treatment of established collagen type II arthritis in DBA/1 mice: a comparative study using anti-TNF alpha, anti-IL-1 alpha, beta and IL-1Ra. Arthritis Rheum. 1996; 39:797–809.
21. Joosten LAB, Lubberts E, Helsen MMA, van den Berg WB. Dual role of IL-12 in early and late stages of murine collagen type II arthritis. J Immunol. 1997; 159:4094–4102.
22. Taniguchi K, Kohsaka H, Inoue N, Terada Y, Ito H, Hirokawa K, Miyasaka N: Induction of the p16INK4a senescence gene as a new therapeutic strategy for the treatment of rheumatoid arthritis.Nat Med. 1999, 5:760-767.
23. Robert H, Shmerling, et al. The Americn Journal of Medicine. 1991; 91: 528-534.
24. Corvo, M.L., Jorge, J.C.S., Hof, R.V., Curz, M.E.M., Crommelin, D.J.A.,Storm, G., 2002. Superoxide dismutase entrapped in long-circulating liposomes: formulation design and therapeutic activity in rat adjuvant arthritis. Biochimica et Biophysica Acta. 1564; 227-236.
25. Siddiqui H. Effect of Glycerrhiza glabra and Moschus moschiferus on arthritis induced in rats by mycobacterium adjuvant. The Indian Journal of Pharmacy. 1965; 27:80-81
26. Koopman W, et al. Rheumatoid factor and human disease. Clin Immunol News.1990; 10:137-141.
27. Pagano MP. Rheumatoid arthritis: An update, 1996.Szekanecz Z, Szegedi G, Koch A. Angiogenesis in rheumatoid arthritis: pathogenic and clinical significance. J Invest Med. 1998; 46:27-41.
28. Walsh D. Angiogenesis and arthritis. Rheumatology. 1999; 38:103-112.
29. Rooney M, Condell D, Quinlan W, Daly L, Whelan A, Feighery C, Bresnihan B. Analysis of the histologic variation of synovitis in rheumatoid arthritis. Arthritis Rheum. 1988; 31:956-963.
30. G. Simon, Alterations in joint space (arthritis) and associated bonechange, in: Principles of Bone X-ray Diagnosis, 2nd ed., Butlerworth & Co. Ltd., Great Britain, 1965, pp. 157–163.