



Biological Evaluation of Free Radical Scavenging Activity of *Albizia Lebbeck* Methanolic Extract in Arthritic Rats

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Abstract

Rheumatoid arthritis (RA) is a prevalent and debilitating disease that affects the joints. Infiltration of blood-derived cells in the affected joints upon activation generates reactive oxygen/nitrogen species, resulting in an oxidative stress. One approach to counteract this oxidative stress is the use of antioxidants as therapeutic agents. The methanolic extract of *Albizia lebbeck* (AL) which exhibited significant anti-inflammatory activity, was evaluated for the possible mode of action by studying its antioxidant potential in adjuvant-induced arthritic rats. The biological defense system constituting the superoxide dismutase, catalase level showed a significant increase while the lipid peroxide content was found to decrease to a large extent on AL treatment thereby indicating the extracts has free radical scavenging property. 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. *Albizia lebbeck* Methanolic extract (200 mg/kg, 400 mg/kg, and 600 mg/kg body weight/day) was administered orally for 12 days. On 21st day of experiment; the biological estimation and radiological observation were carried out along with rheumatoid factor and arthritic index. It can be conclude that *Albizia lebbeck* methanolic extract possesses strong anti-arthritic and anti-oxidant property.

Key-word: *Albizia lebbeck*, friend's adjuvant, arthritis, antioxidant activity, anti arthritic activity.

Introduction

The adjuvant arthritic model represents a systemic inflammatory disease with bone and cartilage changes similar to those observed in RA. The common patho-logical 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 [1, 2]. *Albizia lebbeck* Benth. (Family: Mimosaceae) is an unarmed deciduous which grows about 12-21 m high, bark pale with glabrous young shoot The root is used in hemicrania. 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 ophthalmia. The flowers are given for asthma and for snakebite. All parts of the plant are recommended for the treatment of snake-

bite [3]. It is reported to possess nootropic [4, 5], anxiolytic [6], anticonvulsant [6, 7], antifertility [8] and antidiarrhoeal [9]. Different phytochemicals have been isolated from beans which include albigenin- a triterpene [10] and albigenic acid- a triterpenoid saponin [11]. Albiziahexoside- a bioactive saponin isolated from bark [12].

Materials and Methods

Preparation of extract

The *Albizia lebbbeck* was collected in the month of February from Gandhinagar, Gujarat, India. & authentication was done by pharmacognosy department and voucher specimen was deposited in herbarium museum of SKPCPER. Freshly collected 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 triterpenoids. 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.

Animal

Wistar rats 200-300gm and of either sex were housed under standard laboratory conditions of light and dark cycles of 7.00 a.m. to 7.00 p.m. temperature of 25 ± 2 °C and 55% relative humidity. The animals were given standard rat pellets and tap water and libitum. The study protocol was approved by institutional animal ethical committee, SKPCPER, Kherva, India.

Complete Freund's adjuvant arthritis

Adult wistar female rat with an initial body weight of 200 to 300g 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 0.1ml of Freund's complete adjuvant. This consists of *Mycobacterium butyricum* suspended in heavy paraffin oil by thorough grinding with mortar and pestle to give a concentration of 6mg/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 lebbbeck* (200 mg/kg, p.o.),
- Group V: Methanolic extract of *Albizia lebbbeck* (400 mg/kg, p.o.),
- Group VI: Methanolic extract of *Albizia lebbbeck* (600 mg/kg, p.o.).

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

Paw edema

Paw volumes of both hind limbs were recorded on the day of CFA injection, and again measured on day 1st, 3rd, 6th, 9th, 13th, 21st using mercury column plethysmometer. The 6th day measurement is indicative of primary lesions and 13th day measurement will aid in estimating secondary lesions. On the day 21st, 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 [13].

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 [15].

Radiography

Female wistar rats was sacrificed on 21st 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 kept at radiographic analysis of normal and arthritic rat hind paws was 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:

1. Periosteal reaction, 0 - 3 (none, slight, moderate, marked);
2. Erosions, 0 - 3 (none, few, many small, many large);
3. Joint space narrowing, 0 - 3 (none, minimal, moderate, marked);
4. Joint space destruction, 0 - 3 (none, minimal, extensive, ankylosis).

Bone destruction was scored on the patella as described previously [16].

Biochemical estimation

The animals were sacrificed by cervical dislocation on the 21st day and the blood was collected by cardiac puncture prior to the sacrifice. The spleen were rapidly removed and washed with ice-cold saline. The tissues were cut into small pieces and homogenised using Tris buffer (0.01 M, pH 7.4) at 4 °C to give 10% homogenate. The haemolysate was extracted [17]. The collected blood with anti-coagulant was centrifuged to remove the plasma. The packed cells were washed first with isotonic saline to remove the buffy coat and then thrice with isotonic Tris-HCl buffer (0.3 M, pH 7.4). The haemolysate was prepared by suspending washed red blood cell with hypotonic buffer (Tris-HCl buffer, 0.01 M, pH 7.2). The levels of protein [18], lipid proxide [19], and enzyme SOD superoxide dismatase [20], were estimated.

Result

The hind paw injected with complete Freund's adjuvant 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 Freud's adjuvant-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 adjuvant.

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

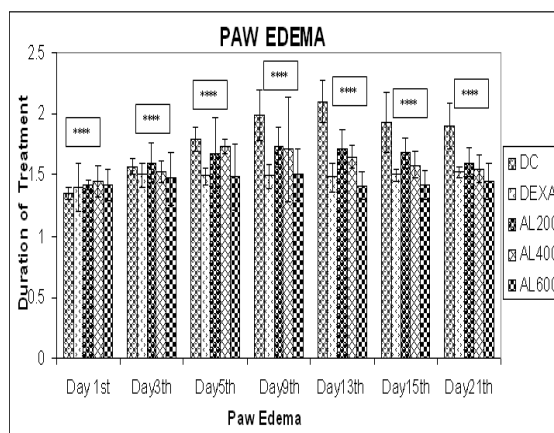


Fig 1 Effect of *Albizia lebeek* methanolic extract (AL) on paw edema in complete Freund's adjuvant induced arthritis in rat. Data are presented as Mean \pm SEM (n=6), * P < 0.001, when compared with Disease control

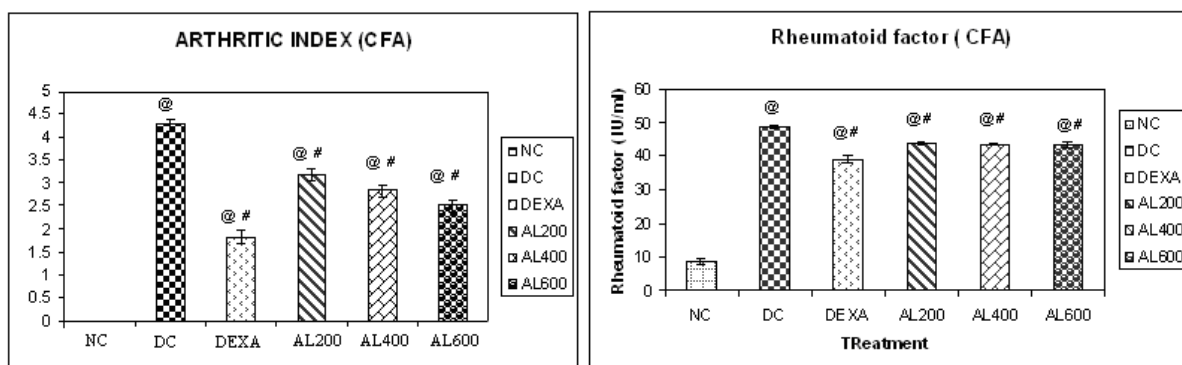


Fig 2 Effect of *Albizia lebeek* methanolic extract (AL) on arthritic index and rheumatoid factor in complete Freund's adjuvant induced arthritis in rat. Data are presented as Mean \pm SEM (n=6), @ P < 0.001, when compared with normal control, # P < 0.001, when compared with disease control

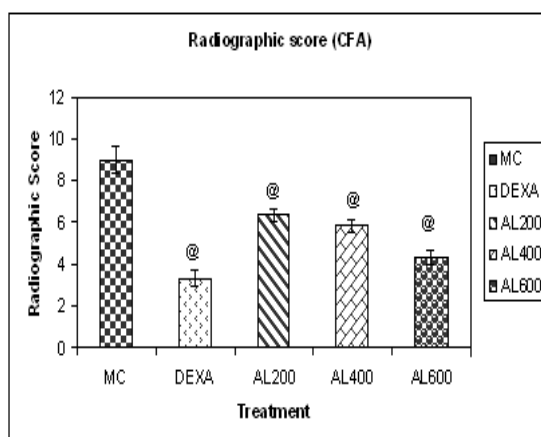


Fig 3 Effect of *Albizia lebeek* methanolic extract (AL) on radiographic score in complete Freund's adjuvant induced arthritis in rat. Data are presented as Mean \pm SEM (n=6), @ P < 0.001, when compared with disease control



Fig 4 Effect of *Albizia lebeek* methanolic extract (AL) on Radiographs of tibiotarsal joints

Table 1: Effect of *Albizia lebeek* methanolic extract (AL) on spleen level of SOD, catalase and MDA content in complete Freund's adjuvant induced arthritis in rat. Data are presented as Mean \pm SEM (n=6), @ P < 0.001, when compared with disease control, (One Way ANOVA)

Treatment	MDA (nmoles/mg tissue protein)	CATALASE lvel (units/mg tissu protein)	SOD level (U /mg protein or/ml RBC)
NC	51.72 \pm 3.23	85.57 \pm 0.87	1.18 \pm 0.12
DC	91.17 \pm 3.06@	35.83 \pm 0.64@	0.5 \pm 0.008@
DEXA	60.33 \pm 2.14#	61.57 \pm 0.83#	1 \pm 0.07#
AL200	69.3 \pm 3.68#	39 \pm 0.43#	0.8 \pm 0.04#
AL400	69.3 \pm 3.68#	58.56 \pm 3.134#	0.8 \pm 0.05#
AL600	66 \pm 2.48#	43.64 \pm 0.44#	0.9 \pm 0.08#

Bone destruction, which is a common feature of adjuvant arthritis, was examined by radiological analysis. Complete Freund's 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 pseudo widening 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.

The lipid peroxide activity significantly increased in the tissues and plasma of the arthritis-induced group animals when compared to control. AL treated animals showed remarkably reduced lipid peroxide activity, as compare to disease control group.

A reduced activity of SOD and catalase were observed in the disease-induced group, whereas a substantial increase in the same was found in AL and standard treated groups.

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 [21]. Complete Freund's adjuvant-induced arthritis is a model of chronic polyarthritis with features that resemble RA [22]. Therapeutic efficiency of herbal drug like *glycyrrhiza glebra* & *moschus moschopus* were mainly investigated in the rat adjuvant arthritis model [23]. Evaluation of the inflammatory status 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. Which 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 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 [24]. Serum rheumatoid factor (RF) measures the amount of antibody IgM titer present in the serum [25]. 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. In arthritic condition, the granulocytes and macrophages accumulate in the effected area and produce large amount of super oxide and H₂O₂ radical [26]. Estimation of these active species in disease induced and drug treated animals' helps in assessing the free radical (FR) scavenger property and indirectly anti-arthritic potential of the plant drug. High levels of free radicals were formed during inflammation causes decline in antioxidant enzyme levels leading to cell damage, inactivation of various enzymes and increase in lipid peroxidation. Oxygen radicals are recognized mediators of inflammatory disease like RA [27]. Therefore, oxidative stress, antioxidant defense, cellular redox status are regarded as the central players in chronic inflammatory disease like RA. Increased oxidative stress is defined as a persistent imbalance between the production of highly reactive oxygen and nitrogen species and antioxidant defenses. However, biological systems have evolved an array of enzymatic and non-enzymatic antioxidant defense mechanism to combat the deleterious effects of oxidative free radicals (OFRs). Superoxide dismutase (SOD) and catalase play an important role in the detoxification of superoxide anion and H₂O₂, respectively, thereby protecting the cells against OFRs induced damage [28]. H₂O₂ may be reduced by enzymes glutathione peroxide, but alternatively may react

again with superoxide anion to form free hydroxyl radicals, which have a greater toxicity and a longer half-life than superoxide anion [29]. There occurs marked increase in oxidative stress in RA as indicated by elevated concentrations of malendehyde (lipid peroxidation indicator), ceruloplasmin, and decreased levels of Catalase, as observed in serum of patients with RA [30]. In present study, complete Freund's adjuvant induced arthritic rats showed significant increase in Malendehyde level and decrease in SOD & catalase levels in the rat spleen homogenates compared to normal control animals indicating dysfunction in antioxidant system in RA. Treatment with Methanolic extract of *Albizia lebbek* significantly reduced RA induced increase in Malendehyde level, and there will little increased in SOD and Catalase levels in arthritic rats.

Conclusion

Our data suggested that *Albizia lebbek* possesses significant antiarthritic activity. The possible mode of anti-arthritic activity of Methanolic extract of *Albizia lebbek* appears to be, Possessing anti-inflammatory activity showed in arthritic parameters like Paw edema, Arthritic index, Rheumatoid factor, improving bone erosion and By normalization of pro-oxidant and improving anti-oxidant parameters indicating its anti-oxidant potency.

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