

Histological findings of *Lasia spinosa* extract in Arthritic Model

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ABSTRACT

Background: Arthritis is a phrase that encompasses more than 100 different medical disorders. Arthritis is a condition in which one or more joints become inflamed. Arthritis causes cartilage to break down. A joint's cartilage protects it and allows it to move freely. When pressure is applied to the joint, such as when walking, cartilage absorbs the stress. The bones rub together when there isn't enough cartilage, producing discomfort, swelling (inflammation), and stiffness. Lymphadenopathy, Oedema, ocular inflammation, glaucoma Bursitis, urethritis, tenosynovitis (tendon sheath effusions) (swollen bursa), Diarrhea, Ulcers in the orogenital area. **Materials and Methods:** *Lasia spinosa* is a herbal medication with roots in many different traditional medical systems all over the world. **Results:** In this investigation, histopathology of the synovial joint revealed that *Lasia spinosa* group therapy reduced vascularity, lymphocytic infiltration, there was no thickening of the synovial membrane and no lymphoid follicles, indicating angiogenesis. **Conclusion:** This study reveals the histology of synovial joints, treated with the *Lasia spinosa* that reduced the vascularity, lymphocytic infiltration, and angiogenesis without causing the synovial membrane to thicken. Our research findings indicated that *Lasia spinosa* has potent anti-arthritic properties.

Keywords: Arthritis, Freund's adjuvant, Histopathology, *Lasia spinosa*.

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INTRODUCTION

Arthritis is a type of joint condition characterised by inflammation of one or more joints (from Greek Arthro: joint + -itis: inflammation; plural: arthritides).^[1-2] Arthritis is a word that encompasses over 100 medical diseases and is not a single disease. Arthritis is a condition in which one or more joints become inflamed. Arthritis is characterized by cartilage degradation. A joint's cartilage generally protects it and allows it to move freely. While pressure is applied to the joint, such as when walking, cartilage absorbs the stress. The bones rub together when there isn't enough cartilage,

producing discomfort, and swelling (inflammation). Arthritis can afflict people of any age; the average age of onset is between 25 and 50, with a peak between 40 sec and 50 sec.^[3]

Because herbal products are "natural," they are frequently thought to be safe. Traditional ayurvedic herbal remedies have been the subject of significant investigation in recent years, owing to their proven efficacy in the treatment of illnesses for which they have historically been used.^[4] In India, arthritis affects more than 20% of the population. Arthritis most commonly affects weight-bearing joints, such as the feet, knees, hips, and spine, as well as other joints, such as finger and thumb joints. Reduced joint mobility, stiffness, especially in the morning, trouble completing everyday tasks, impairment, and long-term (chronic) pain are all symptoms of arthritis. Age, gender, excess weight, injuries, dietary pattern, excessive alcohol use, lifestyle, inheritance, hormonal factors, environmental factors,

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and lack of physical activity are all important risk factors for arthritis.^[5]

Alkaloids may be found in all edible sections of the *Lasia spinosa* plant, as well as phenolic and tannins compounds in the leaves. Because the tender, stem, and leaves, along with rhizomes have economic value, they are commonly farmed and eaten. Anti-helminthic, anti-tumour, antibacterial, anti-hyperlipidemic, anti-diabetic, anti-inflammatory, antioxidant and other disease-preventive properties are all present in the plant.^[6] The present study was carried out to authenticate the traditional claim of *Lasia spinosa* scientifically, with the alkaloids, tannins, and polyphenols enriched methanolic aerial extract to investigate the anti-arthritis potential of *L. spinosa*.

MATERIALS AND METHODS

Chemicals

Piroxicam (East West Pharmaceuticals Ltd., Roorkee, India); complete Freund's adjuvant (Sigma, USA); and other chemicals were purchased from local vendors of Dibrugarh (Assam, India). All of the chemicals utilized were of the analytical grade.

Animals

The study's protocol was approved by the Institutional Ethics Committee of the Department of Pharmaceutical Sciences, Dibrugarh University (Regd. No. 1576/GO/a/11/CPCSEA Dated: 17/2/2012). The study was carried out with adult male Wistar rats weighing 80-120 g. Animals were acclimated to experimental circumstances in cages and kept in a controlled setting ($22 \pm 3^\circ\text{C}$; 12/12 h light/dark cycle). The rats were given food and water.

Plant Material

The whole plant of *Lasia spinosa* was gathered in August and September 2012 from Dibrugarh University campus, Assam and nearby areas. Botanical Survey of India, Eastern Regional Centre, Shillong's Dr N. Odyo identified and authenticated the plant. For future references, a voucher specimen is stored in the Department of Pharmaceutical Sciences, Dibrugarh University, Assam (Specimen no. Du/MTJ/2012/07, Referenceno.BSI/ERC/2013/Tech/Plantidentification/638). After 15 days of drying the leaves in the shade for further investigation, coarsely powdered and stored in an airtight container.

Preparation of Methanolic extracts

200gm of air-dried leaves of *Lasia spinosa* were ground into a coarse powder using a mechanical grinder. The

extraction is done with 1 litre ($40^\circ\text{-}60^\circ\text{C}$) petroleum ether in a soxhlet apparatus until the powder is entirely drained. In a soxhlet device, the defatted material was extracted with 1 lit. of methanol. Filtered, concentrated, dried, and stored in desiccators, the resulting methanolic extracts were used in experiments.

Administration of the Extract

The rats were separated into five groups ($n = 5$) with an equal number of male Wistar rats in each group. With 5% methanol in mineral water, a stock solution of the 100 mg/mL concentration extract was made. Tween 20 was given to Group I, the standard control group. Group 2 disease control received Freund's adjuvant (CFA) with 0.1 mL (6mg/mL). Group 3 standard treatment received Piroxicam as the standard of dose 10mg/kg. Group 4 and for 14 days, group 5 were given a methanolic extract of *Lasia spinosa* in doses of 250 mg/kg and 500 mg/kg, respectively.

Throughout this time, all of the animals were monitored on a daily basis for indicators of toxicity and mortality. According to the technique given in OECD, changes in body weight, food and drink intake, and clinical symptoms were documented.

Histopathological Analysis

The hind paws of rats were removed from the femur bone after the injection of adjuvant on day 14 of the experiment, after the animals had been sacrificed and the adjuvant had been injected then the following process was done:

- a. **Fixation:** For 5 days, the tissue was fixed in 5% buffered formalin.
- b. **Decalcification:** 15 % formic acid was used to decalcify the bones for three to fourteen days. Every day, a new formic acid solution was created and changed. A direct mechanical method was used to determine the termination point. The bone was pierced with a pointed pin that easily penetrated the bone if it was adequately decalcified.
- c. **Processing:** At first the bones were washed with distilled water upon decalcification then acetone was used to dehydrate the pieces in a series of solvent treatments. (50 %, 70 %, 90 %) keeping them each for 5 changes. Then they were kept in benzene for 2 hr for only one change. This is called the method of rapid dehydration.
- d. **Preparation of moulds:** After dehydration, they were kept in a paraffin bath at 60°C for three hours. Moulds out of paraffin wax were prepared and kept cold for them to get solidified properly.

- e. **Preparation of slides:** Before microtome sectioning, the slides were spread with egg albumin fixative (albumin: glycerin= 1:1 using preservative Thymol/ Menthol/ Sodium chloride). Serial sagittal sections of the tibiotarsal and metatarsal joints of the paw were cut (5 μ m thick) with the help of a microtome. The slides with the sectioning of the joint were incubated for some hr.
- f. **Staining of the slides:** After incubation, the slides were first dipped in xylene for 10 min for two changes then with absolute alcohol, 90%, 70 % and 50% alcohol, for a period of 5 min. Then it was run under tap water for 5-10 min and then kept in hematoxylin for 5- 10 min and again for 5 min, it ran under tap water. After this, the slides were decolourized with 1% acid- alcohol and then again ran under tap water for 5 min then kept in 1% eosin for 2 min and again ran under tap water. These slides were now washed with descending order of alcohol (50%, 70%, 90% and absolute respectively) for 1 min then they were air dried and mounted with DPX solution.
- g. **Examination of the slides:** The pathologist assessed the stained slides in a blinded way for the degree of synovitis and bone damage.

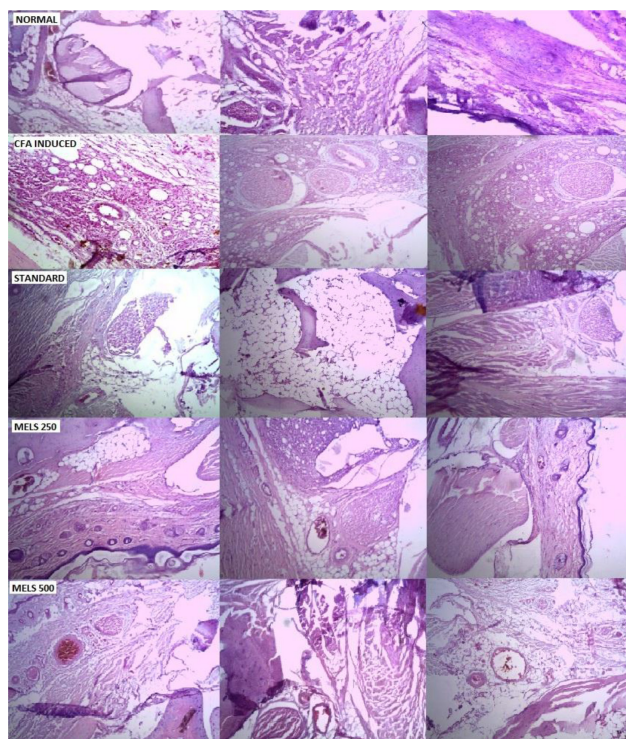


Figure 1: Histopathological representation of tibiotarsal joints stained with H&E of Normal rat, CFA induced rat, Piroxicam (10 mg/kg) treated rat, MELS (250 mg/kg and 500 mg/kg) treated rat. Images (10 X magnification) are typical and representative of each study group.

RESULTS

Histopathological Analysis

Figure 1 depicts the histological alterations in the experimental groups' proximal interphalangeal joints. The histology of a normal tibiotarsal joint was shown in Group I. Edoema formation, degeneration with partial erosion of the cartilage, destruction of bone marrow, pannus formation, fibrosis, joint space narrowing, vascular proliferation, and extensive infiltration of inflammatory exudates in the articular surface were all observed in the Group II Disease Control arthritic rat joint. The bone marrow in the usual drug-treated rat joint was normal, with fewer cellular infiltrates. Treatment with *Lasia spinosa* (250 and 500 mg/kg) for 14 days resulted in reduced inflammatory symptoms such as a sparse cellular infiltrate, no edoema formation, and normal bone marrow. In the 14-day drug-treated group, the overall avoidance of inflammatory symptoms of the rat tibiotarsal joints was considerable. When compared to the disease control, any of the drug-treated groups showed moderate tibiotarsal joint degeneration. The summary of this study includes the histology of synovial joints, treatment with the *Lasia spinosa* group decreased vascularity, lymphocytic infiltration, and angiogenesis without causing the synovial membrane

to thicken or the development of lymphoid follicles compared to animals used to control disease and animals that had received an oral medication. Our research indicated that *Lasia spinosa* has potent anti-arthritic properties. As shown by arthritic measurements such as paw edoema, arthritic index, and rheumatoid factor, as well as by improving bone erosion, the anti-arthritic effect of methanolic extract of *Lasia spinosa* appears to be anti-inflammatory. Histological studies confirmed *Lasia spinosa* having anti-arthritic activity in CFA-induced arthritis.

DISCUSSION

Rheumatoid Arthritis (RA) is an autoimmune disease in which the body's immune system attacks itself. The best accessible experimental model of RA is Freund's adjuvant-induced arthritic model of persistent inflammation.^[7] Freund's Complete Adjuvant-induced arthritis is a well-established rat model and has been widely used for many years for the evaluation of the anti-inflammatory and anti-arthritic potential of various agents.^[8-9]

Synovial joint histopathology research revealed that therapy with the *Lasia spinosa* group reduced vascularity,

lymphocytic infiltration, and angiogenesis, with no thickening of the synovial membrane and no lymphoid follicles. In comparison to disease-control animals and animals that had been given an oral treatment. Our data suggested that *Lasia spinosa* possesses significant anti-arthritis activity. The anti-arthritis effect of Methanolic extract of *Lasia spinosa* appears to be anti-inflammatory, as evidenced by arthritic measures such as paw edema, arthritic index, and rheumatoid factor, as well as improving bone erosion. The methanolic extract mixture of *Lasia spinosa* is a highly polar solvent, and several fractions of alkaloids are present in this medium may in form of alkaloidal salts. Bone joints are the original locations of the inflammatory progression of Rheumatoid Arthritis. The histological modifications of disease control of ankle joints revealed congestion of blood vessels, with hyperplasia, presence of inflammatory cells, and accretion of profuse mononuclear and polymorphonuclear cells in the joint region that produced inflammatory cytokines. Further studies are required to illuminate the antiarthritic plant metabolites and their mechanisms of action, and their significant molecular-based system.

Our bodies have a strong defensive system against harm caused by free radicals. It is made up of a collection of natural antioxidant enzymes, one of which is catalase (CAT). CFA depletes CAT levels, according to the findings of this study. Reduced glutathione (GSH), a non-enzymic antioxidant, is a crucial predictor of tissue sensitivity to oxidative damage. It's a type of intracellular reductant that's plentiful in cells. It protects cells from xenobiotics that are electrophilic, such as free radicals and peroxides. CFA depletes GSH concentration in rat livers, according to the findings of this study. This effect is reversed by *Lasia spinosa* (250 and 500mg/kg) and Standard (Piroxicam) treatment, which could be attributed to de novo GSH synthesis or GSH regeneration.

CONCLUSION

Natural products study vestiges one of the main mediums of discovering bioactive compounds. The Standard drug-treated group revealed a lower edema response with common connective tissue at the region of tibiotarsal joint, with the further absence of necrosis. In our research, histopathological tests validated *Lasia spinosa*'s anti-arthritis effectiveness in CFA-induced arthritis at the prescribed dose levels of 250 mg/kg and 500 mg/kg. With this research, the true mechanism of action of *Lasia spinosa* on adjuvant-caused arthritis is not obvious. This study establishes the role of

ethnopharmacological plants, where *Lasia spinosa*, is one such potential medicinal plant. The effect of *Lasia spinosa* on pro-inflammatory mediators such as TNF- α , Interleukins, and other important mediators will be investigated in the future. Further, it is obligatory to discover the prospective lead molecules and develop a suitable antiarthritic herbal formulation.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

CFA: Complete Freund's adjuvant; **° C:** Degree Celsius; **Sec:** Second; **%:** Percentage; **gm:** gram; **lit.:** Litre; **n:** sample size; **mg/mL:** milligram per milliliter; **mg/kg:** milligram per kilogram; **OECD:** Organisation for Economic Co-operation and Development; **hr:** Hour; **µm:** Micrometre; **DPX:** Digital Picture Exchange; **MELS:** Methanolic Extract of *Lasia spinosa*; **H&E:** Haematoxylin and Eosin; **RA:** Rheumatoid Arthritis; **CAT:** Catalase; **GSH:** Reduced glutathione; **TNF α :** tumor necrosis factor-alpha.

SUMMARY

The tibiotarsal joints of normal rats revealed the occurrence of normal connective tissues with a lack of necrosis. Besides, no impact of lymphocytic infiltration could be observed. In the disease control rats, the tibiotarsal joint revealed immense foray of inflammatory cells, and accretion of polymorphonuclear cells in the joint synovial hyperplasia accompanied by edema. In addition, a higher degree of necrosis could be observed. In Complete Freund's Adjuvant-induced arthritis rat model, the methanolic extract of *Lasia spinosa* emerges to be a potential anti-inflammatory herbal drug therapy, as substantiated by arthritic measures such as paw edema,

arthritic index, rheumatoid factor, and improvement in bone erosion that reduces the joint inflammation, reduced vascularity, lymphocytic infiltration, with no thickening of the synovial membrane, as evidenced in synovial joint histopathology research study.

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