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Radiological Analysis of Hind Paw Joint in Murine Rheumatoid Arthritis Model Treated Prophylactically Or Therapeutically with *Cassia fistula* versus Naproxen

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ABSTRACT

Introduction: *Cassia fistula* has anti-inflammatory activity. Rheumatoid arthritis (RA) is an autoimmune disease, causing inflammation and disability of the affected joints. It is mostly treated symptomatically with NSAIDs, which themselves have a lot of adverse effects. In this study the anti-arthritic effect of *Cassia fistula* versus naproxen has been observed radiologically. **Aims & Objectives:** The present study was radiological analysis of anti-arthritic effect of *Cassia fistula* compared to naproxen in Complete Freund's Adjuvant (CFA) induced rat model of rheumatoid arthritis. **Place and duration of study:** This study was conducted at the Animal House of University of Veterinary and Animal Sciences, Lahore for a period of three months. **Material & Methods:** The study was carried out on 96 male rats divided into 12 groups of 8 rats each. Single 0.2ml dose of Complete Freund's Adjuvant (CFA) was injected in the right hind paw of each rat in all groups except Group 1 (negative control). Group 2 was positive control. The prophylactic (3-7) and therapeutic (8-12) groups were given naproxen (25mg/kg), anthraquinone extract (250 &500mg/kg) or methanolic extract (250 & 500mg/kg) of *Cassia fistula* orally BD on days 1,2 &3 (first dose preceding CFA injection by 30 minutes) and on days 9,10 &11 for therapeutic groups respectively. On day 15, x-rays of right ankle joints of all groups were taken as a confirmatory investigation on the final status of the arthritis.
Results: Our results showed both anthraquinone and methanolic extracts of Cassia fistula have dose dependent (500>250mg/dl) prophylactic and therapeutic anti-arthritic potential albeit lesser than naproxen 25mg/kg, to reduce the swelling of hind paw and deformity of interphalangeal joints on radiological examination. **Conclusion:** The efficacy of Cassia fistula makes it a potential weaker candidate to naproxen in prevention and treatment of rheumatoid arthritis

Key words: *Cassia fistula,* anthraquinone, RA, CFA, NSAID, anti-arthritic effect.

INTRODUCTION

Cassia fistula known as Golden shower and Amaltas, has great variety of uses ranging from constipation to glandular tumors in traditional medicine.[1,2,3] Each part of this plant has therapeutic potential but specifically its fruit pulp has anti-inflammatory property and has demonstrated useful activity in various joint diseases.[4] The common active principle found in all parts of the plant is a phenolic antioxidant: anthraquinone.[5]

Rheumatoid arthritis is an autoimmune long lasting disease characterized by pain, swelling and stiffness of small joints which ultimately results in their deformity and disability.[6,7] It has a better prognosis if treated early and militantly.

The aim of treatment is to reduce pain and swelling, prevent bone deformity and improve quality of life. NSAIDs and disease modifying anti-rheumatic drugs (DMARDs) are usually used. NSAIDs are the most commonly prescribed drugs to subside pain and swelling throughout the world.[1]

Naproxen (NSAID) inhibits tissue cyclo-oxygenase and decreases pro-inflammatory prostaglandins, the important mediators of pain and inflammation.[8,9] Thus, alleviates symptoms of rheumatoid arthritis but has some adverse effects.[10,11] Fruit pulp of Cassia fistula has been used traditionally in many herbal medicines and shown to possess anti-inflammatory activity.[12]

Our previous experiments showed reduction in RA factor levels and ankle caliper measurement with Cassia fistula given prophylactically and

therapeutically.[13] The present study was carried out to further verify those effects radiologically.

This research was unique, as comparison of anti-arthritic activity of the standard drug naproxen with Cassia fistula methanolic fruit pulp extract and anthraquinone extract has not been done radiologically before to the best of our knowledge.

MATERIAL AND METHODS

After approval from the Ethical Review Board committees of Shaikh Zayed Federal Postgraduate Medical Institute, Lahore, this comparative study was completed over a period of six months at UVAS, Lahore.

Collection of *Cassia fistula* bark and fruit pulp was done from Botany Department of University of Punjab, Lahore.

***Cassia fistula* extract preparation:**

The extraction process of *Cassia fistula* bark and fruit pulp was performed in the labs of PCSIR, Lahore by following method.[14] The extract was utilized after confirmatory anthraquinone test.

Anthraquinone extraction:

Powdered 30gm fruit pulp of *Cassia fistula* + Ethanol 150ml (1:5) in Soxhlet apparatus

↓

Heated for 24hours at boiling point of solvent

↓

Obtained 9% anthraquinone extract stored with desiccant after being concentrated and dried

Methanolic extract preparation:

Cassia fistula powdered bark

↓

Extraction in Soxhlet apparatus with double distilled water & methanol

↓

9% Concentrated Extract was produced in Rotary evaporator and refrigerated till further use

Confirmatory Test for Anthraquinones:

Cassia fistula bark and fruit pulp extract was boiled for 5 minutes after addition of 10ml of 1% HCl. Sample was filtered and allow`ed to cool at room temperature. Then using equal volumes of 10% ammonia and chloroform partition of the cooled filtrate was done twice and the layer was allowed to separate. The presence of combined anthraquinones was indicated by rose pink colour.[13, 14]

Experimental Setup:

Ninety six male wistar albino rats weighing 170-200gm, acclimatized for a week, fed with standard laboratory diet, maintained at 25±2°C with relative humidity of 45-55% under 12 hours light and dark cycles were kept at UVAS Animal House, Lahore.

Preparation of rat model of rheumatoid arthritis:

To induce arthritis a single 0.2ml dose of CFA was injected in the right hind paw of each rat on Day 1.[15] A gradual increase in the swelling around injection site was noticed within few hours. The clinical evidence of arthritis was observed with gradual increase in the swelling around injection site and decreasing mobility of rats during 9th to 15th days of post CFA injection.

1% carboxymethyl cellulose was used as suspending agent for making water suspension of test extract and standard drug.

Extracts were given orally BD on days 1, 2 and 3 to the prophylactic groups (3-7) preceding CFA injection, and on days 9, 10 and 11 to the therapeutic groups (8-12).[16]

At the end of study on Day 15 X-rays of right ankle joint were taken to evaluate bone deformity and disease progression.

After careful numbering of rats, division into twelve groups was made with eight rats each.

Group 1: Healthy male rats, not treated. Given normal saline in equal amount.

Group 2: Diseased control rats left for natural recovery after induction of rheumatoid arthritis with single 0.2ml CFA injection in right hind paw.

Prophylactic Groups:

Group 3: Tab. naproxen 25mg/kg

Group 4: Anthraquinone extract at250mg/kg

Group 5: Anthraquinone extract at 500mg/kg

Group 6: Methanolic extract at 250mg/kg

Group 7: Methanolic extract at 500mg/kg

Therapeutic Groups:

Group 8: Tab. naproxen 25mg/ kg

Group 9: Anthraquinone extract at 250mg/kg

Group 10: Anthraquinone extract at 500mg/kg

Group 11: Methanolic extract at 250mg/kg

Group 12: Methanolic extract at 500mg/kg

Radiological analysis:

X-ray apparatus (Siemens-60MA, Germany) and industrial X-ray film (Fuji Photo Film, Japan) were used to take radioghraphs with operating conditions of 220V with peak of 40V, exposure time of 0.2 seconds and a 60cm tube to film distance for anterior-posterior projection.

Grading of Radiographic images was done as follows:

MILD: Diminished swelling of hind paw, No deformity of interphalangeal joints.

MODERATE: Minimal swelling of hind paw, Low deformity of interphalangeal joints.

INTENSE: Increased swelling of hind paw, Highly deformed interphalangeal joints.[17, 18]

RESULTS

X-rays showed no swelling (NS) of hind paw and no deformity (ND) of interphalangeal joints of group 1, 3 and 8 (Fig-1,3&8). Swelling (S) of hind paw and deformity (D) of interphalangeal joints of group 2, 6 and 11 (Fig-2,6&11). Low swelling (LS) of hind paw and low deformity (LD) of interphalangeal joints of group 4, 7, 9 and 12 (Fig-4,7,9&12). Low swelling of hind paw and no deformity of interphalangeal joints of group 5 and 10 (Fig-5&10).

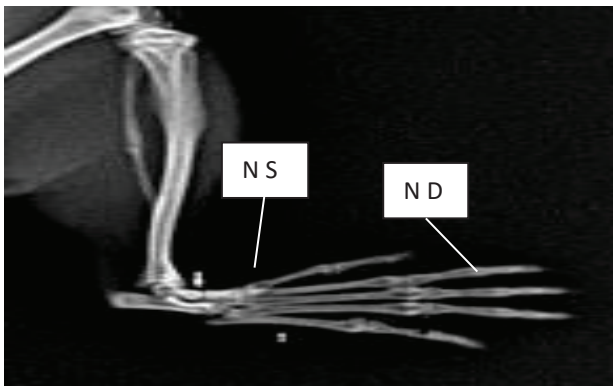

Fig-1: Group 1 Healthy control shows no swelling (NS) of hind paw and no deformity (ND) of interphalangeal joints.

Fig-2: Group 2 Disease control shows swelling(S) of hind paw and deformity(D) of interphalangeal joints.

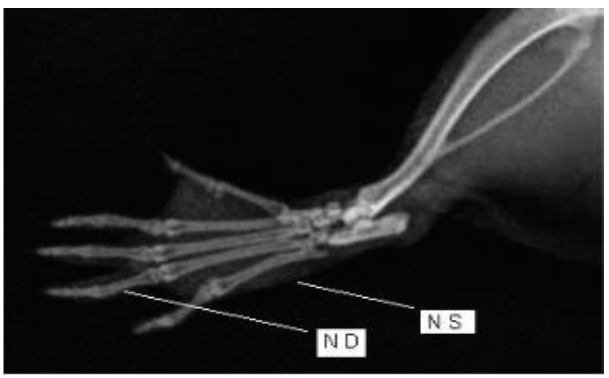

Fig-3: Prophylactic Group 3 Naproxen treated rats shows no swelling (NS) of hind paw and no deformity (ND) of interphalangeal joints.

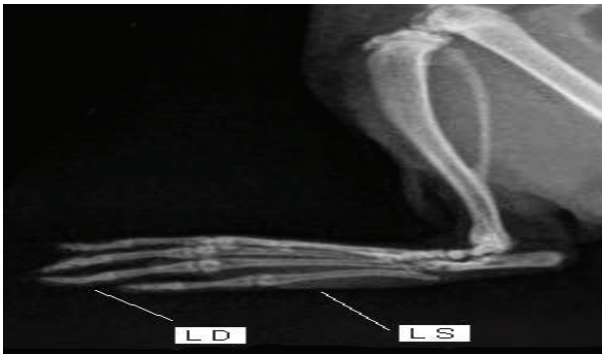

Fig-4: Prophylactic Group 4 Anthraquinone 250mg/kg treated rats shows low swelling (LS) of hind paw and low deformity (LD) of interphalamgeal joints.

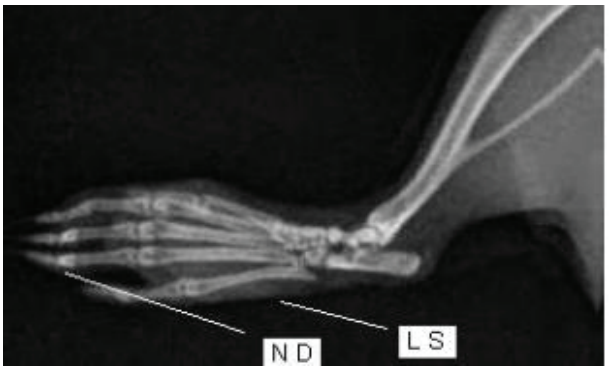

Fig-5: Prophylactic Group 5 Anthraquinone 500mg/kg treated rats shows low swelling (LS) of hind paw and no deformity (ND) of interphalamgeal joints.

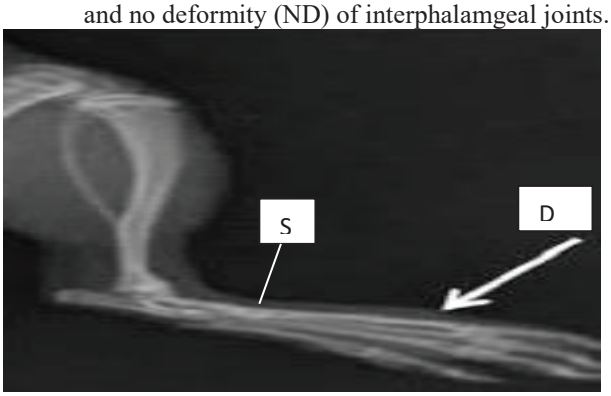

Fig-6: Prophylactic Group 6 Methalonic extract of Cassia fistula 250mg/kg treated rats showing mild swelling with less deformity of interphalangeal joints.

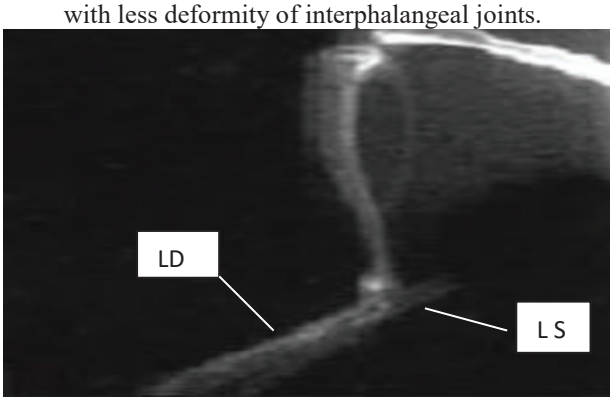

Fig-7: Prophylactic Group 7 Methalonic extract of Cassia fistula 500mg/kg showing low swelling of hind paw and low deformity of interphalangeal joints. Improvement in joint space and cartilage deformity.

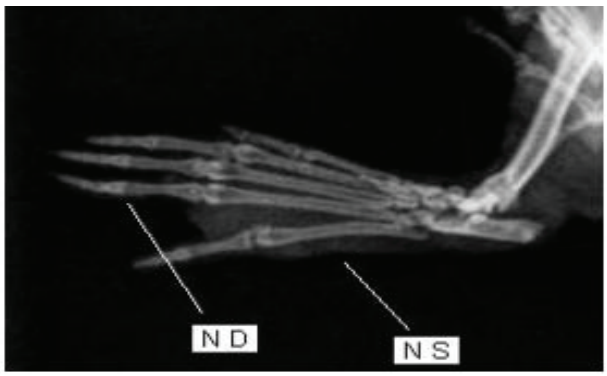

Fig-8: Therapeutic Group 8 Naproxen 25mg/kg treated rats showing no bony destruction and swelling of joint with reduced edema and swelling of soft tissues.

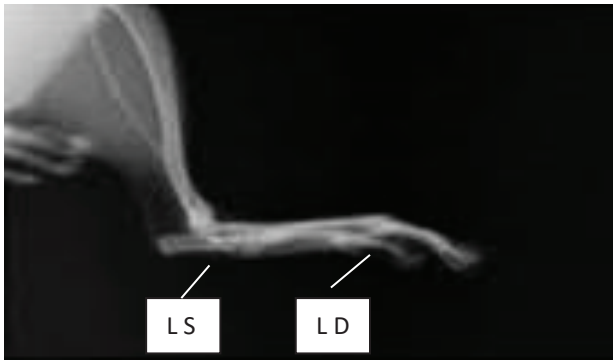

Fig-9: Therapeutic Group 9 Anthraquinone 250mg/kg treated rats showing reduced progression of RA with low joint swelling and less narrowing of joint space.

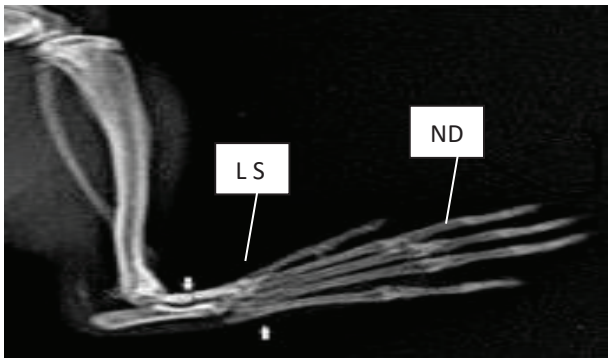

Fig-10: Therapeutic Group 10 Anthraquinone 500mg/kg treated rats showing significant improvement of joint swelling by less soft tissue swelling.

Fig-11: Therapeutic Group 11 Methanolic extract of Cassia fistula 250mg/kg treated rats shows swelling (S) of hind paw and deformity (D) of interphalangeal joints.

Fig-12: Therapeutic Group 12 Methanolic extract of Cassia fistula 500mg/kg treated rats shows low swelling (LS) of hind paw and low deformity (LD) of interphalangeal joints.

DISCUSSION

Rheumatoid arthritis, which is an autoimmune disease of progressive bones and cartilage damage, is associated with severe disability and has systemic effects as well.[6] Naproxen, a nonsteroidal anti-inflammatory drug (NSAID) is used to treat rheumatoid arthritis but itself has many side effects.[11, 12] Plants have been used to treat different ailments including arthritis since ages.[2] Remarkable effects were produced by *Cassia fistula* in earlier researches in murine model of arthritis.[13,14] The current research was conducted to further verify this effect radiologically.

Radiological studies of hind paw joints:

The radiological studies further verified the dominant anti-arthritic effect of methanolic and anthraquinone extracts of *Cassia fistula* proven in our earlier experiments. Clinically the diagnosis of rheumatoid arthritis, which requires therapeutic monitoring, is undeniable and it remains the standard method in assessing the disease progression. The reduced joint space, apparent in X-rays, is a hallmark of arthritis.

Radiological studies of hind paw joints of disease control groups showed swelling of hind paw and deformity of interphalangeal joints as shown in Fig 2. When treated by naproxen (both prophylactic and therapeutic groups; 3 and 8) there was almost complete reversal of radiological changes as compared to disease control group 2, with no deformity of joints and no swelling of hind paw as seen in Fig-3 & 8. Anthraquinone at the dose of 500mg/kg in group 5 and 10 had similar but lesser effects to that of naproxen group 3 & 8 with no joint deformity and low swelling of hind paw as shown in Fig 5 & 10. Anthraquinone has anti-inflammatory activity, inhibits production of superoxide anions from neutrophils and has antioxidant effect as well

due to inhibition of lipid peroxidation.[19,20,21] Lowest or negligible improvement on rat hind paw joints radiological features was with that of 250mg/kg dose of methanolic extract of *Cassia fistula* in group 6 & 11 as seen in Fig-6 & 11.

These radiological findings were novel and provided insight into the development of rheumatoid arthritis in the murine ankle joints and its resolution with *Cassia fistula* extracts.

CONCLUSION

In this innovative study, joint X-rays of both the prophylactic and therapeutic models were taken as a confirmatory investigation on the final status of arthritis which verified our findings regarding *Cassia fistula* anthraquinone and methanolic extracts anti-arthritic potential in comparison to naproxen. Therefore, the efficacy of *Cassia fistula* make it a potential weaker candidate in prevention and treatment of rheumatoid arthritis. Furthermore, anthraquinone extract in the dose of 500mg/kg was found to have more potent anti-arthritic effect.

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