

INVIVO EVALUATION OF ANTIARTHRITIC ACTIVITY OF SEED COAT OF *TAMARINDUS INDICA* LINN

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ABSTRACT

Introduction: The Alcoholic and Aqueous extract of seed coat of *Tamarindus Indica* Linn. of family Ceasalpiniaceae was studied for anti-arthritic activity against Freund's Complete Adjuvant induced arthritis in Wister Albino rats of either sex., the active phenolic component in *Tamarindus Indica* Linn extract.

Methods: The antiarthritic activities of *Tamarindus Indica* Linn extract were investigated on rat models of arthritis by Freund's Complete Adjuvant in rats induced acute paw pain and arthritis, evaluated by measuring the squeaking score, paw volume, and weight distribution ratio. The alcoholic and aqueous extract of seed coat of *Tamarindus Indica* Linn was administrated orally to rats for 19 days.

Results: *Tamarindus Indica* Linn inhibited the expression of IL and reduced the production of PGE₂. Both extracts (aqueous and alcoholic) were found to significantly inhibit paw edema induced arthritis by Freund's Complete Adjuvant in rats. The result indicates that alcoholic extract of seed coat of *Tamarindus Indica* Linn. had significant (P<0.01) anti-arthritic activity when compared with standard and untreated control.

Conclusions: These results suggest that *Tamarindus Indica* Linn has anti-inflammatory, antinociceptive, and antiarthritic effects in an arthritis animal model. Thus, *Tamarindus Indica* Linn should be further studied with regard to use either as a pharmaceutical or as a dietary supplement for the treatment of arthritis.

Keywords: *Tamarindus Indica* Linn., Adjuvant-induced arthritis, Immunoglobulins, IL- 4, Anti-inflammatory, Rat.

INTRODUCTION

The plant *Tamarindus indica* Linn popularly known as imli, chincha, belong to the family caesalpiniaceae, found throughout India. The plant used in traditional medicine for the treatment of cold, fever, stomach disorder, diarrhea and jaundice and as skin leanser, antimicrobial agents, (5). Seeds are useful in diarrhoea, dysentery, burning sensation, haematuria, inflammations, hepatic disorders, chronic ulcers. (6).

Adjuvant-induced arthritis (AIA) is an erosive autoimmune polyarthritis involving both humoral and cell mediated immune responses that resemble human rheumatoid arthritis (RA) (4). The immune system is a well-organized and well-regulated system. The deregulation of the immune system may lead to the development of autoimmune diseases. Rheumatoid arthritis (RA) is proto-type of such groups of illness with chronic systemic disorders to be considered an autoimmune disease with destructive inflammatory polyarticular joint potentially resulting in progressive destruction of articular and periarticular structure. Persistent inflammation produces swollen joints with severe synovitis, decreased nociceptive threshold, and massive subsynovial infiltration of mononuclear cells, which along with angiogenesis leads to pannus formation. Expansion of the pannus induces bone erosion and cartilage thinning, leading to the loss of joint function (3). Hence there is an urgent need to find safer compound for the management of rheumatoid arthritis.

MATERIALS AND METHODS

The seeds were obtained from the Local area Pune., heated in a hot air oven at 140 °C, for 45 min, cooled and cracked to separate their outside brown layer. Only brown-red seed coats were collected and these were then ground into fine powder. Authenticated by Dr. A.M. Mujumdar, Head, Plant Science Division, Agarkar Research Institute, Pune. (Authentication no: 09-06)

Preparation of extracts

The dried, coarse powder of seed coat of *Tamarindus indica* Linn were extracted with soxhlet extraction apparatus using ethanol and distilled water. The resultant extract was concentrated using rotary

vacuum evaporator. The yield of ethanolic and aqueous extract was found to be 23 and 30 gm respectively.

Animals

Wistar rats (150 – 200 g) of eithersex were used in this study. They were maintained under controlled temperature (23 ± 2oC) and relative humidity (40 – 60%) with standard environmental conditions of 12/12 light/dark cycle in the Departmental animal house. They were housed in polypro-pylene cages with free access of food and water ad libitum. The cages were cleaned daily by changing the bedding. The experimental protocol was approved by Institute's animal ethical committee (Proposal no.941/C/06/CPCSEA/08-09/06); care and use of laboratory animals were confirmed to national guidelines.

Pharmacological experiment

All the experiments were conducted in the pharmacological research laboratory between 9.00 am - 9.00 pm at a standard environmental condition (24 ± 2°C).

Freund's adjuvant Induced Arthritis

This model (1) useful to assess the anti-arthritic activity in Albino Rats. Animals were randomly divided into five groups of six animals. They were received Group 1 - Vehicle control (0.5ml normal saline), Group 2 - Arthritis control Group 3 , diclofenac sodium and group 4 - alcoholic and group 5 -aqueous extract. Adjuvant arthritis was induced by subcutaneous injection of 0.1ml suspension of killed Mycobacterium Tuberculosis homogenized in liquid paraffin into right hind paw. Drug treatment started from initial day i.e. from the day of adjuvant injection (0 day), 30min before adjuvant injection and continued 19 days. Swelling in injected and normal hind paw of rats were monitored daily using plethysmometer. The percentage inhibition of paw volume of injected paw over vehicle control at 21st day was evaluated by using formula

$$i = [1 - (\Delta V_{\text{treated}} / \Delta V_{\text{untreated}})] \times 100$$

Where, I = %inhibition of paw edema

$\Delta V_{\text{treated}}$ = Mean Change in paw volume of treated rat

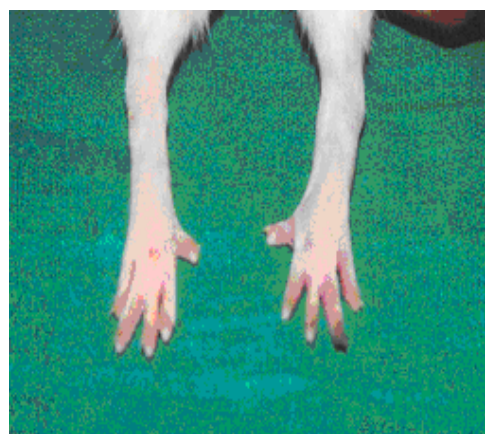
Arthritic Rat (FCA) on 21st dayAlcoholic Extract of seed coat of TI on 21st dayAqueous Extract of seed coat Of TI on 21st dayDiclofenac Control on 21st day

Fig. 3: Effect of different drugs on Rat paw on 21st day of treatment

Statistical Analysis

The result were expressed as mean \pm SEM. Difference among the data were determined using one way ANOVA followed by Tukey-kramer multiple comparison test for multiple comparison. Difference between the data were considered significant at $P < 0.001$

RESULT

Preliminary phytochemical studies revealed the presence of Tannins, Alkaloids, Flavanoids. The Aqueous and Ethanolic extract of seed coat of *Tamarindus Indica Linn.* was found to be non toxic up to 1000 mg/kg.(2)

Table 1: Effect of Various Extracts of *Tamarindus indica* Linn. in Arthritis Induced Rat Paw Edema

Groups	Mean Changes In Paw Oedema (Mean \pm S.E.M.)				%Inhibition of paw oedema on 21 st day
	4 th day	8 th day	14 th day	21 st day	
Normal control	0.61 \pm 0.13	0.65 \pm 0.12	0.73 \pm 0.14	0.8 \pm 0.16	83.33%
Arthritic control	4.0 \pm 0.08**	4.2 \pm 0.08**	4.4 \pm 0.14**	4.8 \pm 0.12**	0
DiclofenacSodium	1.33 \pm 0.21*	1.5 \pm 0.20**	1.73 \pm 0.23**	1.93 \pm 0.22**	58.53%
AlcoholicExtract	2.36 \pm 0.21**	2.53 \pm 0.18**	2.91 \pm 0.19**	3.08 \pm 0.20**	74.02%
Aq. Extract	2.0 \pm 0.21**	2.23 \pm 0.19**	2.41 \pm 0.21**	2.63 \pm 0.20**	69.58%

Results are mean \pm SEM, n = 6, Significance relative to control ($p < 0.01$)

**= $P < 0.01$ = Very significant;

*= $P < 0.05$ = Less significant.

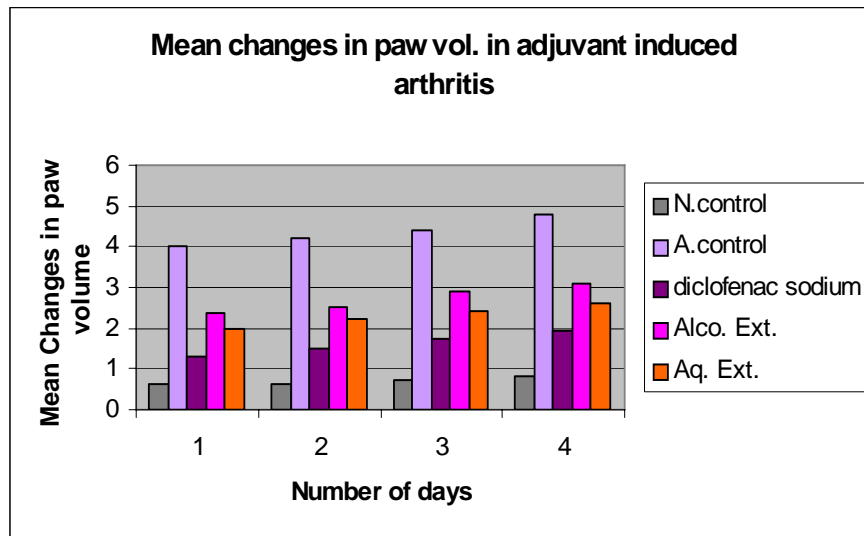
Aqueous and Alcoholic extract of seed coat of *Tamarindus indica* linn significantly inhibited the inflammatory edema, through the expression of IL6 and reduced the production of PGE₂ (10). The inhibition was highest in aqueous extracts (Table 1). FCA induced Arthritis in rats is probably the best and most widely used model since it has close similarity to human rheumatoid disease. Shortly

after administration of adjuvant into left hind paw of rat, a pronounced swelling appears in injected paw, which persists for several weeks. The determination of swelling appears in injected paw is most objective measurement that can be made to assess the activity (1). The inflammatory mediators are responsible for development of clinical symptoms of inflammation. They cause

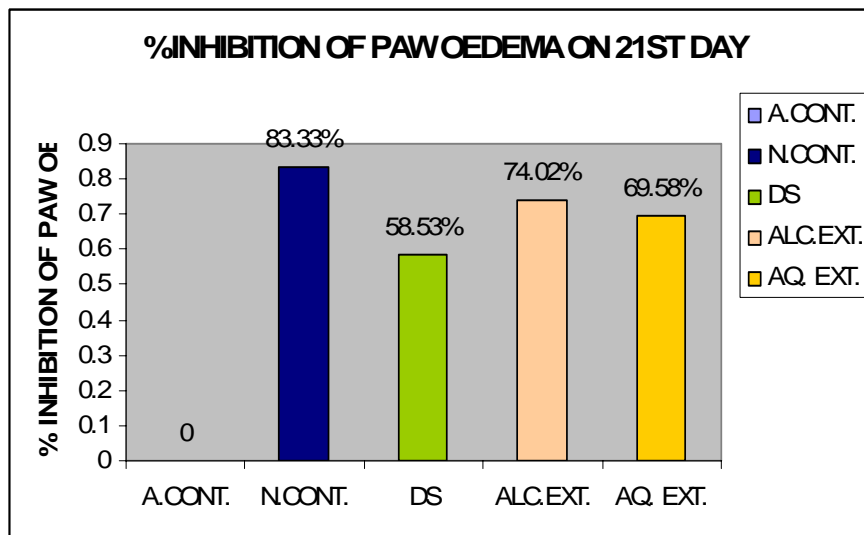
vasodilation, increased permeability of blood vessels and migration of leukocytes to the site of inflammation. Cytokines are locally acting protein mediators that are involved in almost all the biological processes including cell growth and activation, inflammation, immunity and differentiation. (7).

Analysis of the expression of cytokines at mRNA levels in patients with arthritis has revealed that many pro-inflammatory cytokines are abundant in synovial tissues and. A preferential activation of type 1 cells in the target tissue suggests that Th1 cytokines are involved in the pathogenesis. The increased expression of inflammatory mediators in arthritic joints is counteracted to some degree by the production of anti-inflammatory Th2 cytokines. Since the balance of Th1/Th2 cytokines is thought to influence the autoimmune diseases like arthritis and that the study allows better understanding of the main mechanisms involved in such diseases.

We tested the ability of aqueous extract and alcoholic extract to control Th1/Th2 cytokine balance in arthritic rats. Aqueous extract and alcoholic extract have been found to show fruitful immunomodulatory activity with respect to arthritis. They induced inhibition of IL-2 production by CD4+ T-cells. IL-2, being a central regulator of immune response, stimulates the synthesis of IFN- γ in T-cells and also induces the secretion of pro-inflammatory cytokines such as TNF- α by activated macrophages (8). The inhibition of IL-2 by aqueous and alcoholic extract of seed coat of *Tamarindus indica* linn is possibly responsible for reduced IFN- γ secretion by CD8+ T cells and TNF- α by macrophages. Since IL-2, IFN- γ and TNF- α are Th1 type (pro-inflammatory) cytokines (9), their inhibition shows a strong correlation with the antiarthritic activity of aqueous extract and alcoholic extract. The findings, therefore, show that aqueous extract and alcoholic extract have potent anti-arthritic activity.



Graph 1: The Mean Changes In Paw Volume against no. of days Indicate Significant Antiarthritic Activity of Aqueous And Alcoholic Extracts of *Tamarindus Indica* Linn.



Graph 2: Shows % Inhibition of Paw Edema On 21 St Day In Which Significant Inhibition Observed In Standard Group. Diclofenac Sodium and Aqueous Extract of *Tamarindus Indica* Linn Shows Significant Inhibition Of Paw Volume (Graph 2)

DISCUSSION

Anti-inflammatory drugs used for treating chronic inflammatory diseases such as rheumatoid arthritis are typically prescribed long term to properly control the disordered immune system. Thus, there is a strong need to develop safe and effective drugs for the long-term use. Many groups have studied non-steroidal anti-inflammatory small molecules that were derived from natural sources with the aim of developing new treatments for clinical use [11]. For example, curcumin is a polyphenolic compound derived from the dietary spice, turmeric. Recently, curcumin has been shown to possess diverse pharmacological properties, including anti-inflammation, antiproliferation, and antiangiogenesis. [12]

Rheumatoid arthritis is a chronic inflammatory and systemic autoimmune disease characterized by a number of inflammatory and destructive events such as joint pain and swelling, synovial hyperplasia, pannus formation, cartilage and bone destruction, and joint malformation. Many cell populations, various cytokines, and different inflammatory mediators are involved in the generation of the pathological events characteristic of Rheumatoid arthritis. Combination therapy strategies for Rheumatoid arthritis may be more appropriate to target on the complexity and redundancy of the pathological mechanisms of Rheumatoid arthritis.

The plant *Tamarindus indica* Linn popularly known as imli, chinch, belong to the family caesalpinaceae, found throughout India. The plant used in traditional medicine for the treatment of cold, fever, stomach disorder, diarrhea and jaundice and as skin leanser, antimicrobial agents, (5). Seeds are useful in diarrhoea, dysentery, burning sensation, haematuria, inflammations, hepatic disorders, chronic ulcers. (6).

We found that *Tamarindus indica* linn significantly inhibited the production of two important proinflammatory mediators, IL6 and PGE₂, in IL1 β -stimulated human FLS. The inhibition of PGE₂ production is important due to its central role in triggering pain.

In the present study, we showed that Aqueous and Alcoholic extract of seed coat of *Tamarindus indica* linn could significantly inhibit the disease progression of AIA and markedly protect the affected joints against cartilage destruction and bone erosion in rats, presumably by suppressing the abundant production of pro-inflammatory cytokines TNF- α , IL-1 β , and IL-6 in the blood serum. Therefore, aqueous extract and alcoholic extract of seed coat of *Tamarindus indica* Linn significantly inhibit ($P < 0.01$) the arthritis and have potent anti-arthritis activity.

CONCLUSION

These results suggest that *Tamarindus indica* linn has anti-inflammatory, antinociceptive, and antiarthritic effects in an arthritis animal model. Thus, *Tamarindus indica* linn should be further studied with regard to use either as a pharmaceutical or as a dietary supplement for the treatment of arthritis.

Abbreviations

AIA :- Adjuvant-induced arthritis , RA : Rheumatoid arthritis ; IL: interleukin; IFN -interferon ; FCA- freunds complete adjuvant ; RA: rheumatoid arthritis; TNF- α : Tumor Necrosis Factor- α ; % Percentage; gp: Group.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

VMM and PB participated in the literature survey, data analysis and the design of the study . PB, VMM & JG, performed the experiments. VMM and DMA participated in the preparation of manuscript. All authors read and approved the final manuscript.

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