

FREE RADICAL SCAVENGING ACTIVITY OF *MANGIFERA INDICA* 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 *Mangifera indica* (MI) which exhibited significant anti-inflammatory activity, was evaluated for the possible mode of action by studying its antioxidant potential in Freund's 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 *M. indica* 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 subplanterly. The Methanolic extract of *M. indica* (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 observation was carried out along paw edema with rheumatoid factor and arthritic index. It can be conclude that *M. indica* possesses strong anti-arthritic and anti-oxidant property.

Key-words: *Mangifera indica*, Freund's adjuvant, Arthritis, Antioxidant, Anti-arthritic, Dexamethasone

INTRODUCTION

The adjuvant arthritic 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 in human are joint swelling associated with cellular and pannus invasion of the joint space and bone resorption.¹⁻² Rheumatoid arthritis is a chronic, inflammatory, multisystem, autoimmune disorder. It commonly affects the joints in a polyarticular manner. The symptoms that distinguish rheumatoid arthritis from other forms of arthritis are inflammation and soft-tissue swelling of many joints at the same time (polyarthrititis).²⁻³ *M. indica*, also known as mango, aam, belong to genus *Mangifera* which consists of about 30 species of tropical fruiting trees in the flowering plant, belonging to family Anacardiaceae. Various parts of plant are traditionally used as a dentifrice, antiseptic, astringent, diaphoretic, stomachic, vermifuge, tonic, laxative and diuretic and to treat diarrhea, dysentery, anemia, leucorrhoea, and piles in various condition. Mangiferin, an active constituent of mango being polyphenolic, antioxidant and glucosylxanthone. It has strong antioxidant and antimicrobial activity,⁴ antiviral activity,⁵ antitumor and

anti-HIV activity,⁶ immunomodulation,⁷ anti-inflammatory activity,⁸ antidegenerative and antidiabetic,⁹ anthelmintic and antiallergic activity,¹⁰ hepatoprotective activity.¹¹

MATERIALS AND METHODS

Collection and Authentication of Plant Material

The *M. indica* was collected in month of April from Junaghadh district, Gujarat, India. Authentication of plant was done by Dr. H. A. Solanki, botany department of Gujarat University, Ahmedabad, Gujarat, India.

Preparation of extract

Freshly collected bark washed and pulverized to coarse powder. The powder was extracted with methanol in a Soxhlet apparatus. The extract was concentrated under reduced pressure by a rotary vacuum evaporator. Preliminary qualitative analysis of methanol extract showed the presence of polyphenolics, flavonoids, triterpenoids. Mangiferin, a xanthone glycoside major bio-active constituent, isomangiferin, tannins and gallic acid derivatives. 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.

Animals

Female albino rats of Wistar strain in the weight range of 200-300 gm were housed under standard conditions of temperature (23±1°C), 12 h light/dark cycle and fed with standard pellet diet and water *ad libitum* at 55% relative humidity. The study protocol was approved by institutional animal ethical committee, SKPCPER, Kherava, India.

Complete Freund's adjuvant arthritis

Adult wistar female rat with an initial body weight of 200 to 300gm 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 Complete Freund's adjuvant (CFA). This consist of Mycobacterium tuberculosis suspended in heavy paraffin oil by thorough grinding with motor and pestle to give a concentration of 6mg/ml. Dosing with the test or standard compounds was started on the first day after immunization and continued for 12 days according to the following schedule: Group I: Normal control (Distilled water), Group II: Disease control (CFA suspension of 1% CMC), Group III: Dexamethasone (5 mg/kg, p.o., standard), Group IV: Methanolic extract of *M. indica* (200 mg/kg, p.o.), Group V: Methanolic extract of *M. indica* (400 mg/kg, p.o.), Group VI: Methanolic extract of *M. indica* (600 mg/kg, p.o.). From day 13th to 21st, the animals were not dosed with the test or the standard compound. The following parameters were measured.¹²

Paw edema

Paw edema and paw volume of both hind limbs were recorded on day 1st, 3rd, 6th, 9th, 13th, 21st using mercury column plethysmometer. The 6th day measurement was indicative of primary lesions and 13th day measurement was 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 complete Freund's adjuvant.¹²

Arthritic index

All the animals were closely observed for organs like ears, nose, tail, fore paws and hind paw and on that base arthritic index was calculated.¹³

Rheumatoid factor (RF)

The latex turbidimetry method was used to measure RF factor by using RF turbilatex kit of SPINREACT Company. Calibration was carried out for linear range up 0 to 100 IU/ml. The reading of RF factor of all the groups obtained were compared with the control animals and were expressed as IU/ml RF.¹⁴

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 was rapidly removed and washed with ice-cold saline. The tissue was cut into small pieces and homogenized using Tris buffer (0.01 M, pH 7.4) at 4°C to give 10% homogenate. The haemolysate was extracted.¹⁵ 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 protein¹⁶, lipid peroxide¹⁷ and enzyme SOD superoxide dismatase¹⁸ were estimated.

RESULT AND 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.²⁰ Therapeutic efficiency of herbal drug like *glycerhiza glebra* and *moschus moschipus* were mainly investigated in the rat adjuvant arthritis model.²¹ Evaluation of the inflammatory stratus in RA is reflected inflammation in the hind paw. The hind paw injected with complete Freund's adjuvant became gradually swollen and reached its peak at 21st day. Fig. 1 showed the results obtained for the different formulation of MI and the standard drug (Dexamethasone 5mg/kg) in the complete Freund'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 was increase in time dependent manner and all treatment administration groups significantly inhibited the development of joint swelling induced by complete Freund's adjuvant.

Symmetric involvement of small hand joints (especially proximal interphalangeal and metacarpophalangeal), foot joints (metatarsophalangeal), wrists, elbows, and ankles was typical, but initial manifestations might occur in any joint. Inflammation and / or nodules were observed on ears, nose, and tail, fore paws and hind paws. Arthritic index was the average of the score given to severity of the lesions in these places. This gives full picture of the disease.¹⁵ Prominent immunological abnormalities that may be important in pathogenesis of RA include immune complexes are found in joint fluid cells and in vasculities. 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.²² Serum rheumatoid factor (RF) measures the amount of antibody IgM titer present in the serum.²³ Arthritic index and rheumatoid factor were significantly decreased in treatment with MI (200 mg/kg, 400mg/kg and 600mg/kg) and Dexamethasone (5mg/kg) treated animal as compare to disease control treatment.

In arthritic condition, the granulocytes and macrophages accumulate in the effected area and produce large amount of super oxide and H₂O₂ radical.²⁴ 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 are 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.²⁵ 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.²⁶ H₂O₂ may be reduced by enzymes glutathione peroxidase, 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.²⁷ 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.²⁸

In present study, complete Freund's adjuvant induced arthritic rats showed significant increase in malendehyde level and decrease in SOD and catalase levels in the rat spleen homogenates compared to normal control animals

indicating dysfunction in antioxidant system in RA. Treatment with The Methanolic extract of *M. indica* significantly reduced RA induced increase in malendehyde level, and there was little increased in SOD and Catalase levels in arthritic rats.

CONCLUSION

Data suggests that *M. indica* possess significant anti-arthritic activity. The possible mode of anti-arthritic activity of methanolic extract of *M. indica* possessing anti-inflammatory activity show in arthritic parameters like Paw edema, Arthritic index, Rheumatoid factor and by normalization of pro-oxidant and improving anti-oxidant parameters indicating its anti-oxidant potency.

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TABLE 1: THE EFFECT OF *MANGIFERA INDICA* ON SPLEEN LEVEL OF SOD, CATALASE AND MDA CONTENT IN COMPLETE FREUND'S ADJUVANT INDUCED ARTHRITIS IN RAT.

Treatment	MDA level (nmoles/mg tissue protein)	CATALASE level (units/mg tissue protein)	SOD level (U /mg protein or/ml RBC)
NC	47.67 ± 1.498	78 ± 6.527	1.783 ± 0.060
DC	87.5 ± 1.544 [@]	35.33 ± 0.421 [@]	0.666 ± 0.055 [@]
DEXA	56.83 ± 1.424 [#]	64.67 ± 0.557 [#]	1.433 ± 0.114 [#]
MMI 200	67.33 ± 0.666 [#]	50.33 ± 0.666 [#]	0.9333 ± 0.194 [#]
MMI 400	65.33 ± 0.666 [#]	55 ± 0.577 [#]	1 ± 0.139 [#]
MMI 600	63.33 ± 0.666 [#]	60.83 ± 0.600 [#]	1.133 ± 0.152 [#]

Data are presented as Mean±SEM (n = 6) [@] P < 0.001 Compared to disease control, [#] P < 0.001, when compared with disease control. (One way ANOVA).

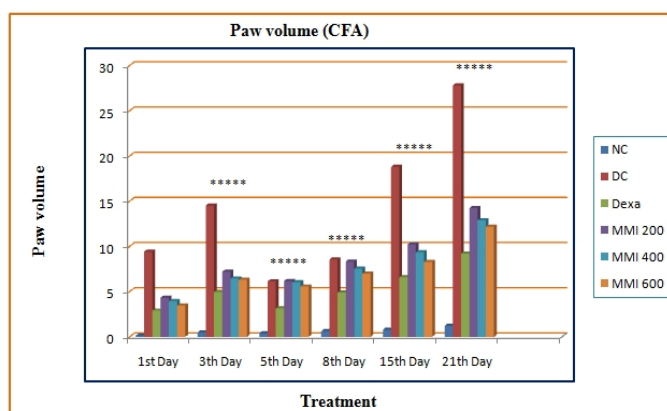


Fig. 1: Effect of *Mangifera indica* methanolic extract (MI) on paw edema in complete freund's adjuvant induced arthritis in rat. Data are presented as Mean ± SEM (n=6), * P < 0.001, when compared with Disease control. (One Way ANOVA).

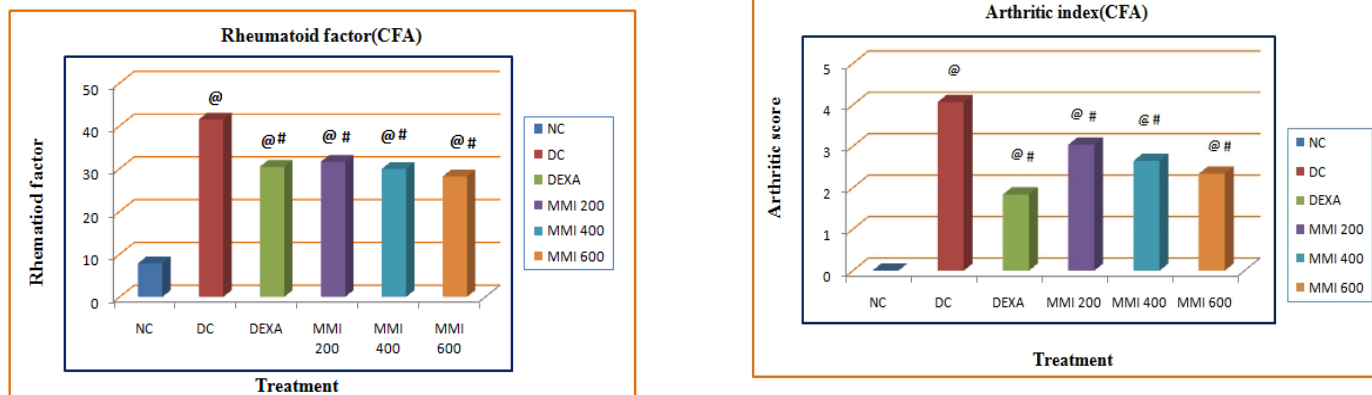


Fig. 2: Effect of *Mangifera indica* methanolic extract (MI) on arthritic index and rheumatoid factor in complete Freund's adjuvant induced arthritis in rat. Data are presented as Mean ± SEM (n=6), @ P < 0.001, when compared with normal control, # P < 0.001, when compared with disease control. (One Way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

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