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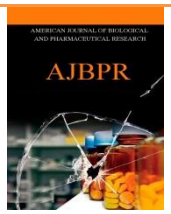


# EVALUATION OF ANTI-RHEUMATIC POTENTIALS OF *PREMNA TOMENTOSA* WILLD ON CHRONIC IMMUNOLOGICAL COMPLETE FREUND'S ADJUVANT -INDUCED ARTHRITIS IN RATS

**S.M.Shaheedha[1*] and K.Bhaskar Reddy[1]**

[1]Sri Venkateswara College of Pharmacy, RVS Nagar, Tirupati Road, Chittoor - 517127, Andhra Pradesh, India.

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**ABSTRACT**

*Premna tomentosa* Willd belongs to the family Verbenaceae. *Premna tomentosa* commonly called as *Krishnapalai* is a medicinal plant. Anti-inflammatory activities of the *Premna tomentosa* have been reported in acute inflammation, but there is lack of evidence regarding anti-inflammatory properties of this plant in chronic inflammation. Therefore the present research evaluated the anti-rheumatic potentials of *Premna tomentosa* Willd on chronic immunological Complete Freund's Adjuvant (CFA)-induced arthritis in rats. Methanolic extract of the whole plant of *Premna tomentosa* Willd. (MEPT 200 & 400mg/kg body weight p.o) and standard drug (Diclofenac sodium, 100mg/kg) suspended in 1% v/v tween 80 was prepared and administered to CFA arthritic rats. One day before the complete freund's adjuvant was injected and daily treatment continued for 21days. The CFA arthritic model was created by the injection of 0.5ml complete freund's adjuvant into the synovial cavity of the right knee joint of hind leg of rats. Oral dosage of MEPT was found to be significantly decreasing the humoral immune response by inhibiting the acute inflammatory reaction by reducing vascular permeability or other inflammatory mediators. The secondary arthritis lesions were reported to presume that,due to delayed hypersensitivity reaction ., MEPT exerted a marked significant effect on this stage. Haematological parameters also showed a significant improvement from the arthritic condition. These observations suggest the potency of MEPT in therapy for rheumatoid arthritis.

## INTRODUCTION

Rheumatoid Arthritis is a chronic auto immune-mediated disease which affects humans and animals [1]. This joint disorder also affects tissues and organs such as the heart, lungs, eye and neuromuscular system. In the joint, Rheumatoid Arthritis is characterized by profuse inflammatory reaction in the synovial membrane and subchondral bone which results in progressive erosion of articular cartilage and synovitis [2]. In advanced cases, ankylosis, subluxation, soft tissue destruction, disuse osteoporosis and pain may be noticed. There is no known cure for Rheumatoid Arthritis but several drugs such as anti-inflammatory and disease modifying anti- rheumatoid drugs are used in mono or combination therapies to inhibit the disease process. However, prolonged use of these drugs is associated with deleterious side effects such as gastric

Corresponding Author

**S.M.Shaheedha**
Email:- mahigold81@gmail.com

ulceration, haemorrhage, anaemia and kidney dysfunction. Thus in recent times, researches have been directed towards the use of biologics and plant derived drugs in the treatment of Rheumatoid Arthritis [3].

*Premna tomentosa* Willd belongs to the family Verbenaceae. *Premna tomentosa* commonly called as "Krishnapalai" is a medicinal plant. It is moderately sized deciduous tree with shoots, leaves and inflorescence densely clothed with a tawny yellow stellate tomentum. The bark is light grayish colour, greenish yellow [1]. The genus premna comprised of 50-200 species, is distributed in tropical and subtropical Asia, Africa, Australia and Pacific islands [2]. The leaves of *P.tomentosa* possess diuretic, anti-inflammatory, antinociceptive, hypnotic effects, cytoprotective and immunomodulatory activities [3].

Therefore the present research was to evaluate the anti-rheumatic potentials of *Premna tomentosa* Willd on chronic immunological Complete Freund's Adjuvant - induced arthritis in rats.

**Animals used**

Wistar strain of albino rats (150-200g) were obtained from the animal house in Sri Venkateswara College of Pharmacy, Chennai. The animals were maintained in a well-ventilated room with 12:12 h light/dark cycle in polypropylene cages. The animals were fed with standard pellet feed (Hindustan Lever Limited., Bangalore) and water was given *ad libitum*. Ethical committee clearance was obtained from IAEC (Institutional Animal Ethics Committee) of CPCSEA.

**Drugs and chemicals**

Complete freund's adjuvant was purchased from S.D. Fine Chemicals Ltd. (Mumbai, India) and Diclofenac sodium received as gift sample from Dr.Reddy's laboratories Limited (Hyderabad, India).

The reference anti-inflammatory drug diclofenac was dissolved in normal saline for the study. The drug solution was freshly prepared and administered orally at dose 4 mg/kg in volumes not exceeding 10 mL/kg.

**CHRONIC IMMUNOLOGICAL ARTHRITIS**

**Chronic immunological CFA-induced arthritis in rats**

Experimental immunological arthritis was induced in rats according to the method of Newbould [8]. The left footpad of each rat was injected subcutaneously with 0.05ml of (0.5%w/v) of complete Freund's adjuvant.

**Group I** - Received vehicle (Normal control) 1% v/v tween 80, 1ml/100 g

**Group II** - Received vehicle (Arthritis control) 1% v/v tween 80, 1ml/100 g

**Group III** - Received methanol extract of whole plant of *Premna tomentosa* Willd. (MEPT) (200mg/kg body weight p.o) suspended in 1% v/v tween 80

**Group IV** - Received methanol extract of whole plant of *Premna tomentosa* Willd. (MEPT) (400mg/kg body weight p.o) suspended in 1% v/v tween 80

**Group V**- Received standard drug (Diclofenac sodium, 100mg/kg) p.o, for 21 days respectively.

1day before the complete Freund's adjuvant injection and daily treatment continued for 21days. The oedema of the left and right hind paws was evaluated at 4, 8, 14 and 21days post injection of complete freund's adjuvant using micrometer screw gauge. After the 21$^{st}$day, animals were sacrificed by cervical dislocation and their legs were amputated at knee joints. These knee joints kept in formalin for histopathological evaluation.

**Histological processing and assessment of arthritis damage**

After the 21$^{st}$day, animals were sacrificed; knee joints were removed and fixed for 4 days in 5% formaldehyde. After decalcification in 5% formic acid, processed for paraffin embedding tissue sections (7 µm thick) were stained with haematoxilin and eosin.

Histopathological changes of arthritis damage was scored as follows: (none, mild, moderate, severe)

- Inflammatory cells in the synovial tissues scored, 0-3;
- Destruction of articular cartilage, 0-3 (ranging from the appearance of dead chondrocytes to complete loss of the articular cartilage);
- Bone erosion, 0-3 (ranging from no abnormalities to complete loss of cortical and trabecular bone of the femoral head);
- Cartilage and bone destruction by pannus formation and vascularity, 0-3 [9-12].

**Estimation of haematological parameters**

On the 21$^{st}$day after arthritis induction, rats were sacrificed by cervical dislocation and blood samples were collected into ethylene diamine tetraacetic acid coated tubes by cardiac puncture. Estimation of RBC count, WBC count, neutrophils, easinophils, lymphocytes and haemoglobin by manual techniques established in the laboratory. The estimation of rheumatoid factor (RF) by turbidimetric method (BTR-810, Ranbaxy) was determined [13].

**Statistical analysis**

The datas were expressed as mean ± standard error mean (S.E.M). The Significance of differences among the group was assessed using one way and multiple way analysis of variance (ANOVA). The test was followed by Dunnett's test, p values less than 0.05 were considered as significance.

**ANTI-RHEUMATIC ACTIVITY OF MEPT & MEPL ON IMMUNOLOGICAL ARTHRITIC RATS**

**Effect of oral administration of MEPT on Complete**

**Freund's Adjuvant (CFA)-induced arthritis in rats**

Observations of paw volume were recorded on the 4, 8, 14 and 21days post injection of complete Freund's adjuvant using micrometer screw gauge. The CFA-induced arthritis control group animals paw volume was increased; it showed signs of arthritis development. Other indications, such as a decreased body weight, also showed induction of arthritis in the CFA-treated control group rats. The assessment made on the 21st day showed that the MEPT (200 & 400mg/kg, body wt.) treatments significantly reduced ($P < 0.01$) the CFA-induced arthritis lesions in the respective treatment groups as was compared with the arthritis control group (Table 1).

Oral dosage of MEPT significantly inhibited joint inflammation on CFA-induced arthritis in rats (62.15, 64.51 & 63.35, 69.72%) respectively. The positive control Diclofenac sodium (100 mg/kg) also produced significant ($P < 0.01$) inhibition in the CFA-induced arthritis in rats (71.31%) (Table 2).

**Effect of oral administration of MEPT on Body weight in CFA-induced arthritis in rat**

The average gain and lose in the body weight on day 21 as compared with the initial body weight in each treatment group has been given in Table 3. The rats in the arthritis control group lost body weight as compared with the MEPT (200 & 400mg/kg b.wt) treated groups. The positive control Diclofenac sodium (100 mg/kg) also showed significantly ($P < 0.01$) increased in body weight in the CFA-induced arthritis in rats.

**Effect of oral administration of MEPT on haematological parameters in CFA-induced arthritis in rats**

The CFA-induced arthritis rats haematological perturbations, such as an increase the percentage of Neutrophils (Table 4), decrease the percentage of Eosinophil (Table 5) & lymphocytes (Table 6), increase in the WBC count (Table 7), a decreased RBC count (Table 8), a decreased Hb count (Table 9) and an increased ESR (Table 10) & rheumatoid factor (Table 11) were also significantly altered by oral administration of MEPT (200 & 400mg/kg b.wt.) treated groups.

**Effect of oral administration of MEPT on histopathological analysis in CFA-induced arthritis in rats**

In normal control animals showed no lesions in articular cartilage and vascularity formation into the joint space Arthritis control showed edematous synovium, destructive lesions in articular cartilage and vascularity formation into the joint space in adjuvant-treated animals. Arthritis rats treated with oral administration of MEPT & MEPL (200 & 400mg/kg b.w, *p.o*) showed well protected synovium, articular cartilage into the joint space with normal cellular characteristics like standard drug Diclofenac sodium treated group (Fig 1).

**Table 1. Effect of oral administration of MEPT on CFA-induced arthritis in rats**

| Groups | Design of treatment | Joint diameter (cm) | | | |
|---|---|---|---|---|---|
| | | $4^{th}$ day | $8^{th}$ day | $14^{th}$ day | $21^{st}$ day |
| I | Normal Control (1% v/v tween 80, 1ml/100g) | 0.44±0.01**[a] | 0.46±0.0070**[a] | 0.47±0.01**[a] | 0.49±0.01**[a] |
| II | Arthritis Control (1% v/v tween 80, 1ml/100g) | 1.35±0.01 | 1.57±0.01 | 2.23±0.01 | 2.51±0.02 |
| III | MEPT (200mg/kg b.w, *p.o*) | 1.25±0.02**[b] | 1.16±0.01**[b] | 0.99±0.0161**[b] | 0.95±0.01**[b] |
| IV | MEPT (400mg/kg b.w, *p.o*) | 1.10±0.02**[b] | 1.00±0.01**[b] | 0.97±0.01**[b] | 0.89±0.02**[b] |
| V | Diclofenac sodium (100mg/kg b.w, *p.o)* | 0.86±0.01**[b] | 0.78±0.01**[b] | 0.74±0.001**[b] | 0.72±0.01**[b] |

Values are expressed as mean ± SEM of six observations. Statistical significant test for comparison was done by ANOVA, followed by Dunnett's test. Comparison between a - Group I Vs Group II. b - Group II Vs Group III, IV, V.

**Table 2. Percentage inhibition of oral administration of MEPT on CFA-induced arthritis in rats**

| Groups | Design of treatment | % Inhibition of Joint inflammation |
|---|---|---|
| I | Normal Control (1% v/v tween 80, 1ml/100g) | - |
| II | Arthritis Control (1% v/v tween 80, 1ml/100g) | - |
| III | MEPT(200mg/kg b.w, *p.o*) | 62.15 |
| IV | MEPT (400mg/kg b.w, *p.o*) | 64.51 |
| V | Diclofenac sodium(100mg/kg b.w, *p.o)* | 71.31 |

Values are expressed as mean ± SEM of six observations. Statistical significant test for comparison was done by ANOVA, followed by Dunnett's test. Comparison between a - Group I Vs Group II; b - Group II Vs Group III, IV, V.

**Table 3. Effect of oral administration of MEPT on Body weight in CFA-induced arthritis in rat**

| Groups | Design of treatment | Body weight in gms (±SEM) | | |
|---|---|---|---|---|
| | | On Day 1 | On Day 21 | Change in body weight |
| I | Normal Control (1% v/v tween 80, 1ml/100g) | 177.83±3.42[a] | 193.33±2.95[**a] | 15.50±1.69**[a] |
| II | Arthritis Control (1% v/v tween 80, 1ml/100g) | 178.37±2.94 | 168.50±2.73 | -9.67±0.84 |
| III | MEPT (200mg/kg b.w, *p.o*) | 180.33±2.67[b] | 186.17±2.47**[b] | 6.67±0.76**[b] |
| IV | MEPT (400mg/kg b.w, *p.o*) | 178.33±3.06[b] | 186.50±2.42*[b] | 8.17±1.28**[b] |
| V | Diclofenac sodium (100mg/kg b.w, *p.o)* | 182.17±3.96[b] | 190.50±5.82**[b] | 10.52±1.43**[b] |

Values are expressed as mean ± SEM of six observations. Statistical significant test for comparison was done by ANOVA, followed by Dunnett's test. Comparison between a - Group I Vs Group II; b - Group II Vs Group III, IV, V.

**EFFECT OF ORAL ADMINISTRATION OF MEPT ON HAEMATOLOGICAL PARAMETERS**

**Table 4. Effect of oral administration of MEPT on Neutrophils in CFA-induced arthritis in rats**

| Groups | Design of treatment | Neutrophil % |
|---|---|---|
| I | Normal Control (1% v/v tween 80, 1ml/100g) | 22.67±0.67**[a] |
| II | Arthritis Control(1% v/v tween 80, 1ml/100g) | 50.50±0.89 |
| III | MEPT (200mg/kg b.w, *p.o*) | 28.83±0.48**[b] |
| IV | MEPT (400mg/kg b.w, *p.o*) | 26.50±0.43**[b] |
| V | Diclofenac sodium (100mg/kg b.w, *p.o)* | 23.50±0.50**[b] |

Values are expressed as mean ± SEM of six observations. Statistical significant test for comparison was done by ANOVA, followed by Dunnett's test. Comparison between a - Group I Vs Group II; b - Group II Vs Group III, IV, V.

**Table 5. Effect of oral administration of MEPT on Eosinophils in CFA-induced arthritis in rats**

| Groups | Design of treatment | Eosinophil % |
|---|---|---|
| I | Normal Control (1% v/v tween 80, 1ml/100g) | 4.10±0.10**[a] |
| II | Arthritis Control (1% v/v tween 80, 1ml/100g) | 1.33±0.07 |
| III | MEPT (200mg/kg b.w, *p.o*) | 2.16±0.05**[b] |
| IV | MEPT (400mg/kg b.w, *p.o*) | 2.53±0.03**[b] |
| V | Diclofenac sodium (100mg/kg b.w, *p.o)* | 3.46±0.05**[b] |

Values are expressed as mean ± SEM of six observations. Statistical significant test for comparison was done by ANOVA, followed by Dunnett's test. Comparison between a - Group I Vs Group II; b - Group II Vs Group III, IV, V.

**Table 6. Effect of oral administration of MEPT on Lymphocytes in CFA-induced arthritis in rats**

| Groups | Design of treatment | Lymphocyte % |
|---|---|---|
| I | Normal Control (1% v/v tween 80, 1ml/100g) | 70.50±0.50**[a] |
| II | Arthritis Control (1% v/v tween 80, 1ml/100g) | 43.17±1.01 |
| III | MEPT (200mg/kg b.w, *p.o*) | 57.33±0.95**[b] |
| IV | MEPT (400mg/kg b.w, *p.o*) | 64.17±0.79**[b] |
| V | Diclofenac sodium (100mg/kg b.w, *p.o)* | 69.50±0.34**[b] |

Values are expressed as mean ± SEM of six observations. Statistical significant test for comparison was done by ANOVA, followed by Dunnett's test. Comparison between a - Group I Vs Group II; b - Group II Vs Group III, IV, V.

**Table 7. Effect of oral administration of MEPT on Erythrocyte Sedimentation Rate (ESR) in CFA-induced arthritis in rats**

| Groups | Design of treatment | ESR |
|---|---|---|
| I | Normal Control (1% v/v tween 80, 1ml/100g) | 10.97±0.09**[a] |
| II | Arthritis Control (1% v/v tween 80, 1ml/100g) | 12.93±0.23 |
| III | MEPT (200mg/kg b.w, *p.o*) | 11.2±0.09**[b] |
| IV | MEPT (400mg/kg b.w, *p.o*) | 10.78±0.08**[b] |
| V | Diclofenac sodium (100mg/kg b.w, *p.o)* | 9.23±0.11**[b] |

Values are expressed as mean ± SEM of six observations. Statistical significant test for comparison was done by ANOVA, followed by Dunnett's test. Comparison between a - Group I Vs Group II; b - Group II Vs Group III, IV, V.

**Table 8. Effect of oral administration of MEPT on Haemoglobin in CFA-induced arthritis in rats**

| Groups | Design of treatment | Hb (g/dl) |
|---|---|---|
| I | Normal Control (1% v/v tween 80, 1ml/100g) | 13.70±0.09**[a] |
| II | Arthritis Control (1% v/v tween 80, 1ml/100g) | 8.48±0.15 |
| III | MEPT (200mg/kg b.w, *p.o*) | 10.68±0.09**[b] |
| IV | MEPT (400mg/kg b.w, *p.o*) | 11.77±0.06**[b] |
| V | Diclofenac sodium (100mg/kg b.w, *p.o)* | 12.60±0.04**[b] |

Values are expressed as mean ± SEM of six observations. Statistical significant test for comparison was done by ANOVA, followed by Dunnett's test. Comparison between a - Group I Vs Group II; b - Group II Vs Group III, IV, V.

**Table 9. Effect of oral administration of MEPT on RBC count in CFA-induced arthritis in rats**

| Groups | Design of treatment | RBC ($\times 10^6$ /mm$^3$) |
|---|---|---|
| I | Normal Control (1% v/v tween 80, 1ml/100g) | 6.95±0.04**[a] |
| II | Arthritis Control (1% v/v tween 80, 1ml/100g) | 4.61±0.05 |
| III | MEPT (200mg/kg b.w, *p.o*) | 5.86±0.04**[b] |
| IV | MEPT (400mg/kg b.w, *p.o*) | 6.25±0.08**[b] |
| V | Diclofenac sodium (100mg/kg b.w, *p.o)* | 6.52±0.12**[b] |

Values are expressed as mean ± SEM of six observations. Statistical significant test for comparison was done by ANOVA, followed by Dunnett's test. Comparison between a - Group I Vs Group II; b - Group II Vs Group III, IV, V.

**Table 10. Effect of oral administration of MEPT on WBC count in CFA-induced arthritis in rats**

| Groups | Design of treatment | WBC ($\times 10^3$/mm$^3$) |
|---|---|---|
| I | Normal Control (1% v/v tween 80, 1ml/100g) | 6.37±0.12**[a] |
| II | Arthritis Control (1% v/v tween 80, 1ml/100g) | 9.25±0.09 |
| III | MEPT (200mg/kg b.w, *p.o*) | 7.30±0.17**[b] |
| IV | MEPT(400mg/kg b.w, *p.o*) | 6.97±0.08**[b] |
| V | Diclofenac sodium (100mg/kg b.w, *p.o)* | 6.17±0.08**[b] |

Values are expressed as mean ± SEM of six observations. Statistical significant test for comparison was done by ANOVA, followed by Dunnett's test. Comparison between a - Group I Vs Group II; b - Group II Vs Group III, IV, V.

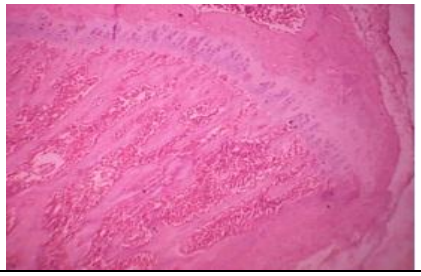
**Table 11. Effect of oral administration of MEPT on Rheumatoid factor in CFA-induced arthritis in rats**

| Groups | Design of treatment | Rheumatoid factor (RF) IU/ml |
|---|---|---|
| I | Normal Control (1% v/v tween 80, 1ml/100g) | 15.32±1.33**[a] |
| II | Arthritis Control (1% v/v tween 80, 1ml/100g) | 54.21±2.17 |
| III | MEPT (200mg/kg b.w, *p.o*) | 32.22±2.14**[b] |
| IV | MEPT (400mg/kg b.w, *p.o*) | 24.28±2.52** [b] |
| V | Diclofenac sodium (100mg/kg b.w, *p.o)* | 24.46±2.62**[b] |

Values are expressed as mean ± SEM of six observations. Statistical significant test for comparison was done by ANOVA, followed by Dunnett's test. Comparison between a - Group I Vs Group II; b - Group II Vs Group III, IV, V.

**Figure 1. Effect of oral administration of MEPT on histopathological analysis in CFA-induced arthritis in rats**

**A**



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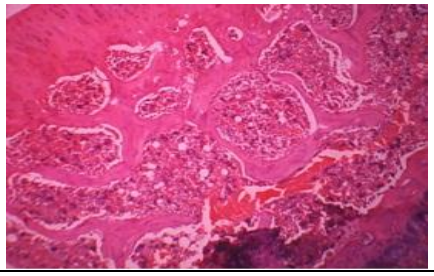

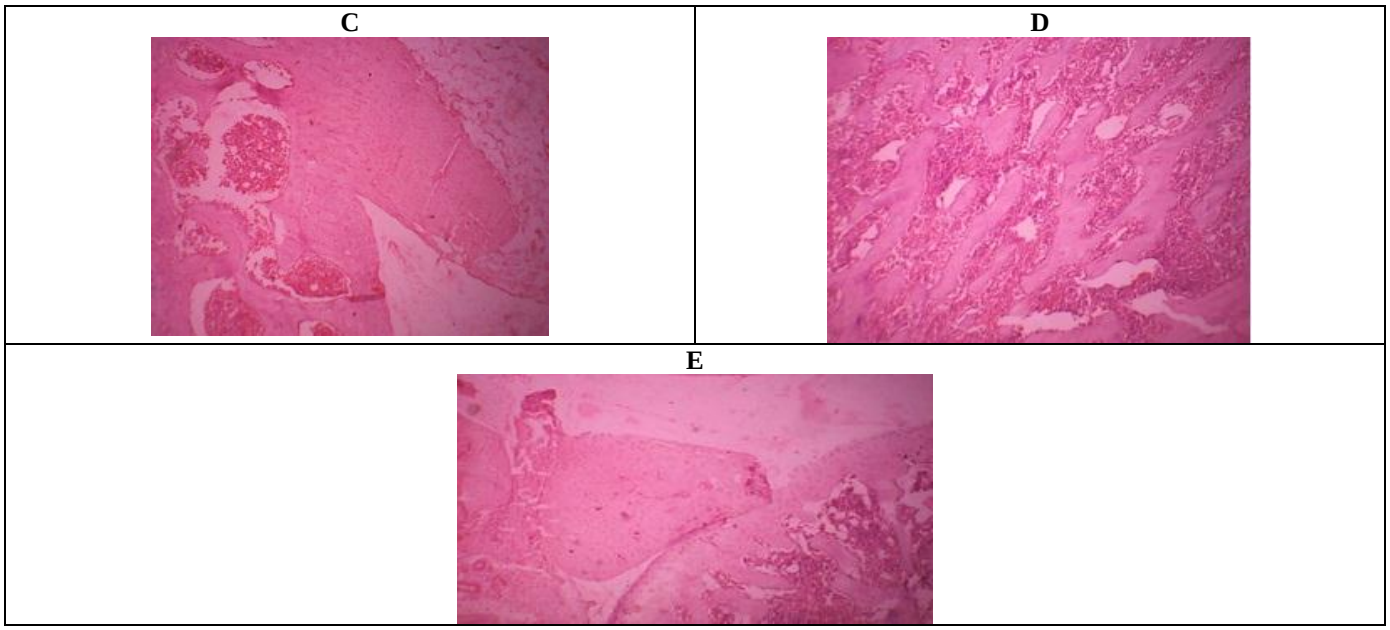


**Histopathological Observation**

(A) Normal control: No lesions in articular cartilage and vascularity formation into the joint space;

(B) Arthritis control: Edematous synovium, destructive lesions in articular cartilage and vascularity formation into the joint space in adjuvant-treated animals;

(C & D) Arthritis rats treated with MEPT (200 & 400mg/kg b.w, *p.o*): moderate edematous synovium, slight destructive lesions in articular cartilage;

(E) Arthritis rats treated with Diclofenac sodium 100mg/kg b.w, *p.o*: observed well protected synovium, articular cartilage into the joint space with normal cellular characteristics.

## DISCUSSION AND CONCLUSION

The present study evaluated the antiarthritic activity of methanolic extract of whole plant of *Premna tomentosa* Willd. (MEPT) by using CFA (Chronic immunological arthritis) induced arthritis.

A large number of studies have indicated that anti-inflammatory and anti-arthritic activities of plants may be attributed to their natural phenolic components, flavanoids and steroids [14].

The phytochemical screening of methanol extract of whole plant of *Premna tomentosa* Willd. (MEPT) confirmed the presence of steroids, phenols/tannins and flavonoids. It was found to have high anti-inflammatory and anti-arthritic effects. It is considered to be the most important anti-inflammatory constituents obtained from MEPT and its anti-arthritic activity is attributed to the inhibition of microtubules in proinflammatory cells including macrophages [15].

The antiarthritic potential of MEPT was confirmed against complete freund's adjuvant induced chronic immunological cellular and proliferative arthritis which is similar to the clinical signs and symptoms. The complete Freund's adjuvant induced arthritis was characterized by progressed swelling (primary reaction) in the hind paw which persisted for few weeks. The primary reaction was followed by swelling in the front paw and contralateral along with appearance of arthritis lesions in ear and tail (secondary reaction) [16-21].

Oral dosage of MEPT was found to be significantly decreasing the humoral immune response by inhibiting the acute inflammatory reaction by reducing vascular permeability or other inflammatory mediators. The secondary arthritis lesions were reported to presume due to delayed hypersensitivity reaction and MEPT exerted a marked significant effect on this stage. Haematological parameters also showed a significant improvement from the arthritic condition. These observations suggest the potency of MEPT in therapy for rheumatoid arthritis [22,23].

Histological studies suggested that Arthritis rats treated with MEPT (200 & 400mg/kg b.w, *p.o*) showed well protected synovium, articular cartilage into the joint space with normal cellular characteristics like standard drug Diclofenac sodium treated group.

In respect of the identification of phytoconstituents, methanolic extract of whole plant of *Premna tomentosa* Willd. (MEPT) was found in this study to contain sterols, phenols and flavonoids. One or a combination of these phytoconstituents may be responsible for the observed anti-inflammatory and anti-arthritic activities of methanol extract of whole plant of *Premna tomentosa* Willd. (MEPT). Overall, these data validated the traditional uses of both plant to assuage pain as well as inflammatory diseases like rheumatism.

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