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EVALUATION OF ANTI-RHEUMATICPOTENTIALS OF *PREMNA LATIFOLIA* ROXB. ON CHRONIC IMMUNOLOGICAL COMPLETE FREUND'S ADJUVANT-INDUCED ARTHRITIS IN RATS

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ABSTRACT

Premna latifolia Roxb.belongs to the family Verbenaceae. *Premna latifolia* Roxb.is widespread in India along the coastal regions of plains and hills. Traditionally the paste of *Premna latifolia* Roxb.bark is applied to cure boils; tender leaves are diuretic, anti-inflammatory, anticancer, anti-rheumatic and used in acute dropsy. Therefore the present research evaluated the anti-rheumatic potentials of *Premna latifolia* Roxb. on chronic immunological Complete Freund's Adjuvant (CFA)-induced arthritis in rats. Methanolic extract of whole plant of *Premna latifolia* Roxb. (MEPL 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 injection 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 MEPL 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 MEPL 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 MEPL in therapy for rheumatoid arthritis.

Keywords: *Premna latifolia* Roxb., Complete Freund's Adjuvant, Chronic Immunological Model.

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 [3]. 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 ulceration, haemorrhage, anemia 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 [4].

Premna latifolia Roxb. is widespread in India along the coastal regions of plains and hills. Traditionally the paste of *P. latifolia* bark is applied to cure boils; tender leaves are diuretic, anti-inflammatory, anticancer, anti-rheumatic and acute dropsy. In Tamil Nadu it is mentioned as a sacred tree and is used to cure swellings [5,6].

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

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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 were 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 foot pad 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 latifolia* Roxb. (MEPL) (200mg/kg body weight p.o) suspended in 1% v/v tween 80

Group IV – Received methanol extract of whole plant of*Premna latifolia* Roxb. (MEPL) (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 were evaluated at 4, 8, 14 and 21days post injection of Complete Freund's adjuvant using micrometer screw gauge. After the 21stday, animals were sacrificed by cervical dislocation and their legs were amputated at knee joints. These knee joints were kept in formalin for histopathological evaluation.

Histological processing and assessment of arthritis damage

After the 21stday, 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 21stday after arthritis induction, rats were sacrificed by cervical dislocation and blood samples were collected into ethylene diaminetetraacetic acid coated tubes by cardiac puncture. Estimation of RBC count, WBC count, neutrophils, easinophils, lymphocytes and haemoglobin were performed by manual techniques established in the laboratory. The rheumatoid factor (RF) was estimated by turbidimetric method (BTR-810, Ranbaxy) [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 MEPL ON IMMUNOLOGICAL ARTHRITIC RATS

Effect of oral administration of MEPL 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 MEPL (200 & 400mg/kg, body wt.) treatments significantly reduced ($P < 0.01$) the CFA-induced arthritis lesions in the respective treatment groups as compared with the arthritis control group (Table 1).

Oral dosage of MEPL 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 MEPL on Body weight in CFA-induced arthritis in rat

The average gain and loss in the body weight on day 21 was 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 MEPL (200 & 400mg/kg b.wt) treated groups. The positive control Diclofenac sodium (100 mg/kg) also showed significant ($P < 0.01$) increase in body weight in the CFA-induced arthritis in rats.

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

The CFA-induced arthritis rats haematological perturbations, such as an increased percentage of Neutrophils (Table 4), decreased percentage of Eosinophil (Table 5) & lymphocytes (Table 6), increased 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 MEPL (200 & 400mg/kg b.wt.) treated groups.

Effect of oral administration of MEPL 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 MEPL & 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 MEPL on CFA-induced arthritis in rats

Groups	Design of treatment	Joint diameter (cm)			
		4th day	8th day	14th day	21st 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	MEPL (200mg/kg b.w, *p.o*)	1.17±0.02**[b]	1.05±0.01**[b]	1.00±0.04**[b]	0.92±0.01**[b]
IV	MEPL (400mg/kg b.w, *p.o*)	0.94±0.01**[b]	0.86±0.01**[b]	0.82±0.04**[b]	0.76±0.01**[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 MEPL 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	MEPL(200mg/kg b.w, *p.o*)	63.35
IV	MEPL(400mg/kg b.w, *p.o*)	69.72
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 MEPL 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	MEPL (200mg/kg b.w, *p.o*)	180.83±2.71[b]	184.67±4.41**[b]	8.83±1.74**[b]
IV	MEPL (400mg/kg b.w, *p.o*)	180.17±3.27[b]	190.00±2.96**[b]	9.83±0.60**[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]

EFFECT OF ORAL ADMINISTRATION OF MEPL ON HAEMATOLOGICAL PARAMETERS

Table 4. Effect of oral administration of MEPL 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	MEPL(200mg/kg b.w, *p.o*)	25.17±0.54**[b]
IV	MEPL(400mg/kg b.w, *p.o*)	23.83±0.56**[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 MEPL 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	MEPL(200mg/kg b.w, *p.o*)	2.48±0.03**[b]
IV	MEPL(400mg/kg b.w, *p.o*)	2.96±0.06**[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 MEPL 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	MEPL(200mg/kg b.w, *p.o*)	59.83±0.60**[b]
IV	MEPL(400mg/kg b.w, *p.o*)	68.83±0.31**[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 MEPL 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	MEPL(200mg/kg b.w, *p.o*)	10.70±0.13**[b]
IV	MEPL(400mg/kg b.w, *p.o*)	9.33±0.12**[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 MEPL 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	MEPL(200mg/kg b.w, *p.o*)	11.63±0.11**[b]
IV	MEPL(400mg/kg b.w, *p.o*)	12.05±0.18**[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 MEPL 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	MEPL(200mg/kg b.w, *p.o*)	6.50±0.07**[b]
IV	MEPL(400mg/kg b.w, *p.o*)	6.78±0.04**[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 MEPL 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	MEPL(200mg/kg b.w, *p.o*)	6.58±0.11**[b]
IV	MEPL(400mg/kg b.w, *p.o*)	6.08±0.04**[b]
V	Diclofenac sodium(100mg/kg b.w, *p.o)*	6.17±0.08**[b]

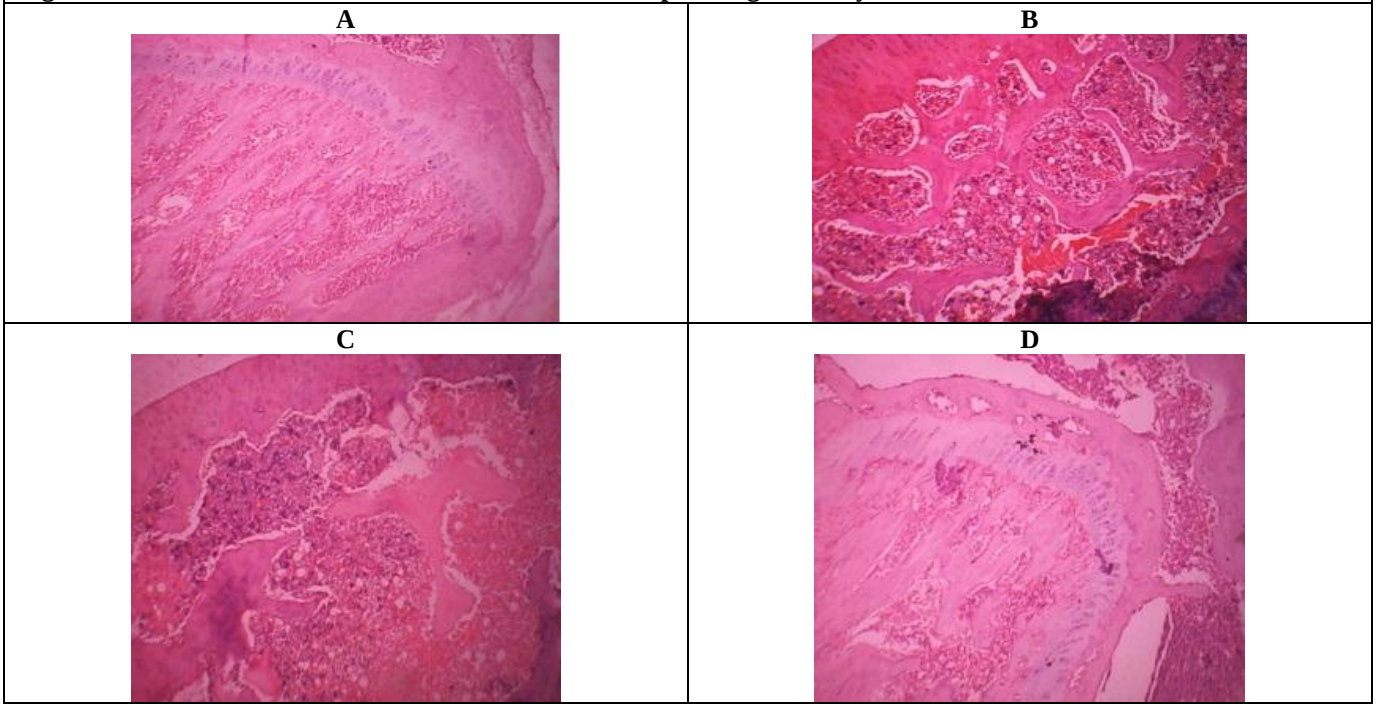
Values are expressed as mean ± SEM of six observations. Statistical significant test for comparison was done by ANOVA, followed by Dunnett's test. Comparison between a - Group I Vs Group II; b - Group II Vs Group III, IV, V.

Table 11. Effect of oral administration of MEPL 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	MEPL(200mg/kg b.w, *p.o*)	35.33±2.41**[b]
IV	MEPL(400mg/kg b.w, *p.o*)	29.52±2.19** [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 MEPL on histopathological analysis in CFA-induced arthritis in rats



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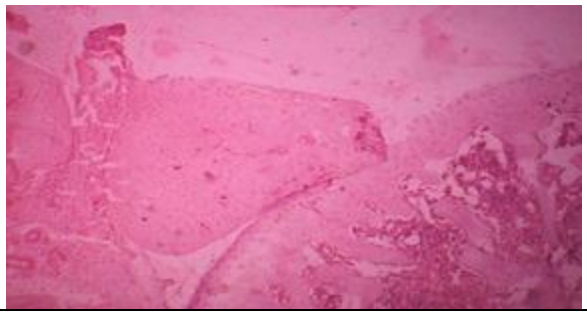

Histopathological Observation

(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 MEPL (200 & 400mg/kg b.w, *p.o*): observed well protected synovium, articular cartilage into the joint space with normal cellular characteristics;

(E) Arthritis rats treated with Diclofenac sodium100mg/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 latifolia* Roxb. (MEPL) 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 methanolic extract of whole plant of *Premna latifolia* Roxb.(MEPL) 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 MEPL and its anti-arthritic activity is attributed to the inhibition of microtubules in proinflammatory cells including macrophages [15].

The antiarthritic potential of MEPL was confirmed against Complete Freund's adjuvant induced chronic immunological cellular and proliferative arthritis which was 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 MEPL 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 MEPL 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 MEPL in therapy for rheumatoid arthritis [22,23].

Histological studies suggested that Arthritis rats treated with 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.

In respect of the identification of phytoconstituents, methanolic extract of whole plant of *Premna latifolia* Roxb. (MEPL) was found 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 latifolia* Roxb. (MEPL).Overall, these data validated the traditional uses of *Premna latifolia* Roxb to assuage pain as well as inflammatory diseases like rheumatism.

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