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Research Article

ANTI ARTHRITIC ACTIVITY OF LEAVES OF *MICHELIA CHAMPACA L. BY COMPLETE FREUND'S ADJUVANT*

M. Dhanalakshmi*[1], T. Jayasree[1] and Sharmila Shaik[2]
Department of pharmacology, Krishna Teja Pharmacy College, Chadalawada Nagar, Renigunta Road, Tirupathi-517 507, Andhra Pradesh, India.
Email: lakshmi.dhana17@gmail.com

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Abstract:

In the present study was carried out to evaluate the protective effect of ethanolic extract of leaves of *Michelia champaca* against Freund.s adjuvant induced arthritis in rats. The ethanolic extract was administered orally at dose of 250 and 500 mg/kg body weight. Indomethacin at dose of 10 mg/kg body weight was used as standard drug. The paw volume was measured on days 4, 8, 14, and 21. At the end of day 21 the blood was collected from retro-orbital route to all the groups of animals and various hematological parameters such as hemoglobin content, total WBC, RBC and erythrocyte sedimentation rate were estimated. The results indicate that at dose of 500 mg/kg body weight of ethanolic extract protects rats against the primary and secondary arthritic lesions, body weight changes and hematological perturbations induced by CFA. Daily treatment of rats with ethanolic extract, standard drug Indomethacin effectively inhibits paw edema in rats. The extract significantly ($p < 0.01$) altered the parameters which were estimated when compared to control group rats.At the end of studies. The phytochemical analysis of extracts revels the presence of sterols, flavonoids, phenols, tannins and saponins. However additional clinical investigations are needed to prove the efficacy of *Michelia champaca L.*in the treatment of various immuno-inflammatory disorders.

Key words: Freund.s adjuvant, arthritic, *Michelia champaca*, Magnoliaceae.

Introduction:

Rheumatoid arthritis (RA) is a chronic, progressive, systemic inflammatory disorder affecting the synovial joints and typically producing symmetrical arthritis that leads to joint destruction, which is responsible for the deformity and

disability. The consequent morbidity and mortality has a substantial socio- economic impact[1]. Adjuvant induced arthritis (AIA) in rats is a chronic inflammatory disease characterized by infiltration of synovial membrane in association with destruction of joints resembles Rheumatoid arthritis in humans[2]. Rheumatoid arthritis progress in three stages. The first stage is the swelling of the synovial lining, causing pain, warmth, stiffness, redness and swelling around the joints. Second is the rapid division and growth of cell, or pannus, which causes the synovium to thicken. In the third stage, the inflamed cell releases enzyme that may digest the bone and cartilage, often causing the joints to loses its shape and alignments, more pain and loss of movements[3]. The most commonly used drugs for the treatment of Rheumatoid arthritis is steroidal, non-steroidal anti-inflammatory, disease modifying anti rheumatic and immunosuppressant drugs. Though the goal of these drugs have been to relieve pain and to decrease joint inflammation, to prevent joint destruction and to restore function of disabled joints, these drugs are known to produce various side effects including gastrointestinal disorders, immunodeficiency and humoral disturbances. Accordingly, reducing side effects should be considered while designing improved therapeutics for Rheumatoid arthritis, besides enhancing medicinal effectiveness. The Siddha and Ayurvedic systems of treatment are being increasingly recognized as an alternate approach to arthritic treatment.

Michelia champaca is a member of family Magnoliaceae. The tree is native from Asia-Tropical (Bangladesh, Arunachal pradesh, Assam, Bihar) and Indo China (Myanmar, Thialand, Vietnam, Sumatra, Malaysia) [4]. It is well known and widely used in traditional medicine such as fever, colic, leprosy, post partum protection, eye disorder and many more[5].It has been reported to possess antipyretic, anti-inflammatory[6] insecticidal[7], antimicrobial[8] and leishmanicidal activities[9].Juice of the leaves of *Michelia champaca* is given with honey in cases of colic. The flower oil is useful in cephalalgia, opthalmia and gout. The flowers and fruits are considered stimulant, antispasmodic, tonic, stomachic, bitter and cool remedies and are used in dyspepsia, nausea and fever. The bark is used as a stimulant, expectorant, astringent and febrifugal properties[10]. The methanol extracts of leaves, seeds, stem and root barks, stem and root heart-woods of *Michelia champaca* and the obtained fractions (petrol, dichloromethane, ethyl acetate and butanol) exhibited a broad spectrum of antibacterial activity. Fractionation drastically enhanced the level of activity particularly in all fractions of the stem bark and dichloromethane fraction of the root bark. Some fractions of the leaves

stem and root bark demonstrated antifungal activity against some of the tested moulds. Several compounds of this plant were also characterized and identified such as alkaloids, saponins, tannins, sterols, flavonoids and triterpenoids. Liriodenine was the active constituent of the root bark, with a broader and, in some cases, better level of activity as compared to the standard[8].

On the basis of these facts, our present investigation was undertaken to study the anti-arthritic effect of *Michelia champaca* leaves extracts on the adjuvant induced arthritis model.

Material and Methods:

Plant Material:

The leaves of *Michelia champaca* was collected from the hills of tirumala region, chittoor (dist).A.P, India. The plant was identified and auntheticated by Dr.Madhava chetty, Assisatant professor, Departmant of botony, S.V. University, Tirupathi. The leaves of the plant were stored in herbarium at the college for further reference.

Preparation of Extract:

The leaves of *Michelia champaca* were collected washed, cleaned and shade dried. The dried leaves were powdered with the help of mechanical mixer and passed through a 40 –mesh sieve to obtain coarse powder. The weighed quantity of coarsely powdered material was extracted by hot continuous extraction using soxhlet apparatus with 70% ethanol at a temperature (40-60^{0}C) upto 72 hr. After completion of extraction, it was undergone distillation under reduced pressure and the remaining solvent was removed by evaporation to dryness on a water bath. Residue was obtained and it was kept in a dessicator and used for further experiment [11].

Phytochemical Screening:

The presence of phytochemical constituents in the ethanolic extract of leaves of *Michelia champaca* was tested by using the standard methods[12].These standard methods revealed the presences of glycosides, flavanoids, steroids, tannins, saponins, triterpenoids and alkaloids.

Animal: Albino rats (150-200g) of either sex were used for the experiment. They were kept in the animal house in a controlled room temperature at 25+2 c, relative humidity 44-56%, light and dark cycles of 10 and 14 hr, respectively

for 1 week before the experiment. The animals were grouped and housed in polyacrylic cages for the further experiment.

Drugs and Chemicals:

- Extract: Required quantity of extract was weighed according to rat body weight and dissolved in 1% CMC solution (250 and 500mg/kg b.w).
- CFA (complete freund's adjuvant): This consists of 5 mg mycobacterium tuberfculosis (Difco) being suspended in heavy paraffin oil (Merck) by thoroughly grinding with mortar and pestle to give a concentration of 5 mg/ml.
- Indomethacin: Required quantity of Indomethacin (10mg/kg b.w) was weighed according to rat body weight and dissolved in 1% CMC (carboxy methyl cellulose) solution.

Acute toxicity studies:

The Acute Toxicity studies were performed in order to establish the therapeutic index of a test drug. The experiment was conducted according to the OECD, 423 guidelines. It was administered as 5,100, 1000 and 2000 mg/kg.

Anti-arthritic Activity:

Freud's adjuvant induced arthritis (14) model was used to assess the anti-arthritic activity in albino rats. Male Wistar rats weighing between 150-200gm were selected for the experiment. They were grouped in a group of six animals each in to five groups. The treatment schedules of rats belonging to the different groups are shown below

Group 1: Normal (1% CMC)

Group 2: Control (Complete Freund.s adjuvant 0.1ml)

Group 3: Indomethacin (10mg/kg p.o)

Group 4: Ethanol extract (250mg/kg p.o)

Group 5: Ethanol extract (500mg/kg p.o)

Arthritis was induced by injecting 0.05 *ml* of suspension of killed *Mycobacterium tuberculosis* bacteria (0.5% *w/w*) homogenized in liquid paraffin into the left hind paw. Drug treatment was started from the initial day i.e. from the day of adjuvant injection (0 day), 30 *min* before adjuvant injection and continued for 12 days. From day 13th to 21st, the

animals were not dosed with the test compound or the standard. The following parameters were measured Paw volume was measured on 4th, 8th, 14th and 21st day with the help of Plethysmometer.The mean changes in injected paw edema with respect to initial paw volume, were calculated on respective days and percentage inhibition of paw edema with respect to untreated group (control) was calculated using the formula: Percentage inhibition of paw edema = [1- (mean change in paw volume of treated rat/ mean change in paw volume of untreated rat)] x 100.The changes in body weight were recorded daily. On the 22nd day, blood was withdrawn through retro-orbital vein puncture of all groups by anaesthetizing the animals with diethyl ether and the biochemical parameters such as hemoglobin content, total WBC count, ESR and RBC were analyzed.

Statistical Analysis:

The experimental results are represented as Mean ±SEM. The data were statistical analyzed by one way analysis of variance (ANOVA) followed by Dunnett.s test. P values< 0.05 were considered as significant.

Results and Discussion:

Preliminary phytochemical screening of leaf extracts revealed the presence of sterols, flavonoids, phenols, tannins and saponins. From the acute toxicity study it was found that the ethanolic extract was safe up to 2000 mg/kg body weight so from this, select the dose (i.e. 250 mg/kg and 500mk/kg) was considered as the evaluation dose for pharmacological studies. Observations such as the paw volume, body weight and hematological parameters were recorded after the injection of CFA. The CFA induced arthritis control group showed sign of arthritis development, as seen by the increase in the paw volume. Table 1 shows the time course of edema and inhibition rate after the administration of CFA and extract. The hind paw developed edema in the footpad. Edema value of the injected footpad significantly increased and reached a peak at 21 days. Administration of ethanolic extract at a dose of 500 mg/kg body weight significantly ($p<0.01$) inhibited the development of swelling induced by CFA. Standard drug Indomethacin at a dose of 10 mg/kg body weight significantly decreases the paw volume from the 1st day after the induction of CFA, where as the extract significantly decreases the paw volume after 4th day. From the table2 a loss of body weight was observed during the arthritis condition. Standard drug and ethanolic extract significantly increases the body weight of the animals as compared to control group animals. The CFA induced haematological perturbations, such as an increase in the WBC

count, a decreased RBC count; decreased hemoglobin (Hb) content and an increased erythrocyte sedimentation rate (ESR) were also favorably altered by *Michelia champaca* treatment (Table-3).CFA induced arthritis is the most widely used chronic test model in which the clinical and pathological changes are comparable with those seen in human rheumatoid arthritis[13]. The Freund.s adjuvant model is chosen as, it develop chronic swelling in multiple joints with influence of inflammatory cells with erosion of joint cartilage and bone destruction. Chronic inflammation involves the release of number of mediators like cytokines (IL-1B and TNF-á), GM-CSF, interferon.s and PGDF. These mediators are responsible for pain, destruction of bone and cartilage that can lead to severe disability (Eric et al. 1996.)[14]. Prostaglandins are mediator for acute inflammation but chronic inflammation is mediated by proinflammatory cytokine such as TNF-á. The articular cartilage destruction, circumarticular fibrosis, and ankylosis are the pathological changes found in chronic inflammation[15]. However standard drug, ethanolic extract significantly suppressed the swelling of the paw in both acute and chronic phase which may be due to the suppression of inflammatory mediator released due to induction of Freund.s adjuvant. Though the actual mechanism of suppressing inflammation is not known but it can be correlated with the presence of phytoconstituents such as flavonoids and tannins[16.] Changes in body weight have also been used to assess the course of the disease and the response to therapy of anti-inflammatory drugs[17]. A report by Patil *et al*. suggests that the decrease in body weight during inflammation is due to deficient absorption of nutrients through the intestine and that treatment with anti-inflammatory drugs normalizes the process of absorption[18]. The evident restoration of the body weight of rats in the *Michelia champacas* and Indomethacin treated groups may involve improvement of intestinal absorption of the nutrients and a reduction in the distress caused by the severity of the arthritis.

Table-1: Mean changes in paw volume using plethysmometer in Adjuvant-induced arthritis in rats.

S.NO	Treatment and Dose	Mean changes in Paw Volume			
		0 week	**1st week**	**2nd week**	**3rd week**
1.	-ve Control	0.216±0.1430	0.226±0.0105	0.201±0.0130	0.213±0.0095
2.	+ve Control	0.225±0.1708	0.825±0.0381**	0.783±0.0247**	0.706±0.0147**

3.	Indomethacin (10mg/kg)	0.283± 0.0210	0.583±0.0401**	0.441±0.0351**	0.366±0.0307**
4.	MCEE-250mg/kg	0.216± 0.0278	0.671±0.0258**	0.566±0.0333**	0.458±0.0238**
5.	MCEE-500mg/kg	0.225±0.0214	0.608±0.0238**	0.466±0.0333**	0.433±0.0278**

MCEE: *Michelia champaca* ethanolic extract.

Values are expressed in mean ± SEM, (n=6), when compared with control, *P<0.05, **P<0.01, ***P<0.001 one-way ANOVA followed by Dunnet's t – Test.

Table-2: Changes in body weight in Adjuvant-induced arthritis in rats.

S.NO	Treatment and Dose	Mean changes in Body Weight (gms)			
		0 week	**1st week**	**2nd week**	**3rd week**
1.	-ve Control	189.17±8.002	192.50±7.610	195.00±6.708	198.33±5.110
2.	+ve Control	162.67± 7.839	149.67±5.011	155.33±6.184	156.67±6.280
3.	Indomethacin (10mg/kg)	187.50± 6.677	180.83±7.236	186.33±5.136	195.17±4.214
4.	MCEE-250mg/kg	192.50± 5.439	187.50±3.594	188.83±3.060	191.67±2.472
5.	MCEE-500mg/kg	182.17±5.199	175.83±4.729	178.83±4.729	183.33±4.595

MCEE: *Michelia champaca* ethanolic extract.

Values are expressed in mean ± SEM, (n=6), when compared with control, *P<0.05, **P<0.01, ***P<0.001 oneway ANOVA followed by Dunnet's t – Test.

Table-3: Effect of hematological parameters in Adjuvant-induced arthritis in rats.

S.NO	TREATMENT	Mean changes in haematological parameters			
		RBC ($X10^6$ cells/mm^3)	**WBC ($X10^3$ cells/mm^3)**	**Hb (gm %)**	**ESR (mm/hr)**
1.	-ve Control	6.84 ± 0.069	7.00 ± 0.035	14.77 ± 0.031	3.87 ± 0.078

2.	+ve Control	5.26±0.455**	9.34 ± 0.043**	10.75±0.038**	6.63 ± 0.140**
3.	Standard	6.16±0.031**	7.44 ± 0.120**	13.80±0.063**	4.27 ± 0.081**
4.	MCEE- 250mg/kg	5.66±0.141**	8.26 ± 0.086**	11.70±0.098**	5.79 ± 0.045**
5.	MCEE- 500mg/kg	6.04±0.072**	7.84 ± 0.070**	12.00±0.041**	4.84 ± 0.060**

MCEE: *Michelia champaca* ethanolic extract.

Values are expressed in mean ± SEM, (n=6), when compared with control, *P<0.05, **P<0.01, ***P<0.001 oneway ANOVA followed by Dunnet's t – Test.

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Corresponding Author:

M. Dhanalakshmi*[1],

Email: *Lakshmi.dhana17@gmail.com*