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Original Article

Effect of *Ficus benghalensis* Linn. Root Extracts on Freund's Adjuvant Induced Arthritis in Rats

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ABSTRACT

Aim: The present study was carried out to evaluate the anti-arthritic activity of *Ficus benghalensis* root extracts on Freund's adjuvant induced arthritis in rats. **Method:** The crude root extracts was administered orally at does of 300mg/kg body weight for 28 days. Indomethacin at does of 10mg/kg body weight was used as standard drug. The paw volume was measured on days 7, 14, 21 and 28. At the end of day 28th the animals were anaesthetized with anesthetic ether and blood was collected from retro-orbital route to all the groups of animals and various haematological parameters such as hemoglobin content, total WBC, RBC and erythrocyte sedimentation rate (ESR) were estimated. The body weight of the animals was measured by digital balance to access the course of the disease at the initial day before induction and the end of 28th day. **Results:** The results indicate that all the extracts at the does of 300mg/kg b.w. protect the rats against primary and secondary arthritic lesions, body weight changes and haematological perturbations induced by FCA. Daily treatment with crude extracts and standard drug effectively inhibits paw edema in rats. All the extracts significantly ($p<0.01$) altered the parameters which were estimated, when compared to control group rats. The observations showed that, ethanol and chloroform extract inhibits rat paw edema by 63.64% and 61.69% when compared to standard drug 62.34% on 28th day. **Conclusion:** At the end of study the ethanol and chloroform extract show more pronounce effect then aqueous and pt-ether extract when compared to standard drug. Our findings showed a significant anti-arthritic activity of *Ficus benghalensis* root extracts against FCA induced arthritis in rats.

Key words: Freund's adjuvant, arthritis, *Ficus benghalensis*, root.

1. INTRODUCTION

Rheumatoid arthritis (RA) is an autoimmune systemic disease with chronic inflammation of the synovial joint and progressive destruction of cartilage and bone. Rheumatoid arthritis is a chronic systemic inflammatory disorder that may affect many tissue and organs- skin, blood vessels, heart, lungs and muscles-

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but principally attacks the joints, producing a nonsuppurative proliferative and inflammatory synovitis that often progresses to destruction of the articular cartilage and ankylosis of the joints.[1] Rheumatoid arthritis is characterized by the infiltration of a variety of inflammatory cells in to the joint. The synovial membrane becomes highly vascularized, synovial fibroblasts proliferate and inflammatory cells release numerous cytokines and growth factors into the joint. These agents subsequently cause synovial cells to release proteolytic enzymes resulting in destruction of bone and cartilage.[2] As a symmetric disease, RA usually involves the same joints on both sides of the body. Angiogenesis and microvascular lesions are common features of RA inflammation, which leads to abnormal serum protein infiltration into the synovia. Clearance of synovial fluid and its constituents was reported to be increased in inflamed joints as a result of increased lymphatic drainage, though damaged or depleted lymphatics have been observed in the synovium of RA patients as well. [3]

Ficus benghalensis Linn (Family-Moraceae) is commonly known as Banyan tree or vata or vada tree in ayurveda. *Ficus benghalensis* is a remarkable tree from India that sends down its branches and great number of shoots, which take root and become new trunks. This tree is considered to be sacred in many places in India.[4] Traditionally all parts of the plant are astringent, acrid, sweet, refrigerant, anodyne, vulnerary, depurative, anti-inflammatory, ophthalmic, styptic, antiarthritic, diaphoretic, antidiarrhoeal, antiemetic and tonic.[5] It is used in Ayurveda for the treatment of diarrhea, dysentery and piles, teeth disorders, rheumatism, skin disorders like sores and to boost immune system, as a hypoglycemic.[6] Bark contains tannins, wax, esters and glucoside, 20-tetratriaconthene-2-one, 6-heptatriacontene-10-one, pentatriacontan-5-one, beta sitostirol-alpha-D-glucose and meso-inositol. Two flavonoid compounds, viz. 5,7-dimethylether of leucopelargonidin 3-0-alpha-L-rhamnoside and 5,3-dimethyl ether of leucocyanidin 3-0-alpha-D galactosyl cellobioside were present in the bark of *Ficus benghalensis* [6,7]. Pharmacological evaluation has shown the various extract of *Ficus benghalensis* has shown anthalmintic,[6] analgesic & anti-inflammatory,[7] antioxidants,[8] antidiabetic,[9] immunomodulatory [10] and antimicrobial [11] activity in experimental animals. The ethno-medicinal use of the roots of *Ficus benghalensis* in arthritic disorders has not been systematically investigated so far. Therefore the present study was an effort to evaluate the anti-arthritic activity of root extracts of *Ficus benghalensis* on Freund's adjuvant induced arthritis model in rats.

2. MATERIALS AND METHODS

2.1 Plant material

The aerial roots of *Ficus benghalensis* were collected from khurja, District Bulandshahar, U.P in the mounth of July-August. The plant material was identified and authenticated by Prof. A.K.Sharma, Head, Department of Dravyaguna, Vaidya Yagya Dutt Sharma Ayurvedic Medical College Bulandshahar, U.P. The Voucher specimen YDS/0159 has been deposited in Department of Dravyaguna and Sir Madan Lal Institute of Pharmacy, Etawah U.P.

2.2 Preparation of Extracts

The aerial roots were collected and shade dried for 4-5 week and the material was subjected to pulverization, made course powder. Successive solvent extraction was performed with Pt-Ether (60-80), Chloroform and 95% Ethanol. The dried root powder about 90 g was exhaustively extracted by hot continuous extraction using soxhlet apparatus with Pt-Ether (60-80), chloroform, and 95 % ethanol in increasing order of polarity up to 48-50 siphons separately. The extracts were filtered and concentrated by distillation process.

The concentrated mass was dried under vacuum till constant weight for each of extract. For aqueous extract the dried root powder 200 g was macerated with 1000 ml chloroform water (1:9) for seven days. Chloroform water was used to prevent the growth of microorganism in the extract. The extractive was filtered and concentrated over a water bath at 40-45 ^{0} C and further dried in vacuum oven till constant weight.

2.3 Experimental animals

Wister albino rats of either sex weighing between 150-200gm were selected for the present study and received from centralized animal house, Sir Madan Lal Institute of Pharmacy, Etawah U.P. They were housed in polypropylene cages and fed with standard diet and water *ad libitum*. All the animals were kept under standard laboratory conditions in a 12 h : 12 h light and dark cycles and maintained under controlled temperature 27±2^0 C for acclimatization. The experiment was conducted in accordance with the direction of Institutional animal ethical committee (IAEC) CPCSEA Government of India. The study was approved by Institutional animal ethical committee for Sir Madan Lal Institute of Pharmacy, Etawah U.P.

2.4 Acute toxicity study

Acute toxicity study was carried out by up and down method. Crude extracts were administered orally to overnight fasted animals. The rats were observed continuously for 2h for behavioral, neurological and autonomic profiles and after 24h and 72h for any lethality. [12] None of the animal died even at a dose of 3000mg/kg b.w. of each extract. Hence one tenth (1/10th of LD_{50}) cut off dose (i.e. 300mg/kg) was selected for each extract for the subsequent study.

2.5 Induction of Arthritis

Male Wistar rats weighing between 150-200gm were selected for the experiment. They were grouped in a group of six animals each in to six groups. The treatment schedules of rats belonging to the different groups are shown below

- Group 1: Control (Complete Freund's adjuvant 0.1ml plus normal saline)
- Group 2: Indomethacin (10mg/kg p.o)
- Group 3: Pt-Ether extract (300mg/kg p.o)
- Group 4: Chloroform extract (300mg/kg p.o)
- Group 5: Ethanol extract (300mg/kg p.o)
- Group 6: Aqueous extract (300mg/kg p.o)

On the 0th day, the basal paw volume of left hind paw of each animal was measured using mercury plethysmometer. On the 1st day all the animals of all groups were once anaesthetized, they were injected in to the ankle joint of left hind paw with 0.1 ml of Complete Freund's adjuvant (Sigma Aldrich, USA) containing 0.1 mg of heat killed *Mycobacterium tuberculosis* cells in liquid paraffin and were allowed to recover to serve as control. Dosing with standard drug Indomethacin (10 mg/kg body weight) and extracts (300 mg/kg body weight) was started on the same day i.e. 1st day and continued for 28th day. Arthritic control group rats receive normal saline through out study while the rest experimental groups animals receives respective treatment once daily by oral route. The 1% Tween 80 was used as vehicle for suspended the extracts. Paw volume of injected paw was measured on 7th, 14th, 21st and 28th day of study period. At the end of day 28th, the animals were anaesthetized with anesthetic ether and blood was isolated from the retro orbital route to all the groups of animals and various haematological parameters such as Haemoglobin content, Total WBC, RBC, and Erythrocyte Sedimentation Rate (ESR) were estimated using routine laboratory methods. The body weight of the animals was measured by digital balance to access the course of the disease at the initial day before induction and at the end of 28th day.

2.6 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 multiple comparison test. P values< 0.05 were considered as significant.

3. RESULTS AND DISCUSSION

From the acute toxicity study it was found that all the extracts were safe up to 3000 mg/kg body weight, so one tenth of this dose (i.e. 300 mg/kg) was considered as the evaluation dose for pharmacological studies. Observations such as paw volume, body weight and hematological parameters were recorded after the injection of CFA. The Freund's adjuvant induced arthritic control group showed sign of arthritis development, as seen by the increase in the paw volume. Table - 01 shows the time course of edema and inhibition rate after the administration of Indomethacin and extracts. The hind paw developed edema in the footpad. Edema value of the injected footpad significantly increased and reached a peak at 28 days. All the extracts and standard drug showed significant decreases paw swelling on 28th day as compared to control group. Standard drug Indomethacin at a does of 10mg/kg b.w. significantly ($p<0.01$) decreases the paw volume from 1st day after the induction of CFA, where as the extracts significantly decreases the paw volume after the 4th day. From the Table – 02 it was found that the ethanol extract has got highest percentage of inhibition 63.64% of paw volume as compared to Indomethacin, which is 62.34% at the end of 28 day. Chloroform extract, Pt-ether extract and aqueous extract decreases the paw edema by 61.69%, 58.43% and 31.82% respectively on 28th day. From the Table – 03, a loss of body weight was observed during the arthritis condition. The significant increases in the body weight of animals during the treatment of Indomethacin, Pt-ether extract, Chloroform extract and ethanol extract was found when compared to control group animals but aqueous extract not show significant increases in the body weight. From the Table – 04, in the CFA induced arthritic animals the haematological perturbations such as an increase in the WBC count, a decreased RBC count and an increased erythrocyte sedimentation rate (ESR) were also favorably altered by Indomethacin and extracts treatment.

Table 1: Mean changes in Paw Edema (ml) in FCA induced Paw Edema in Rats

Treatment Groups (n=6) (Dose mg/kg)	Paw Edema (ml) (Mean ±SEM)				
	0 week	1st week	2nd week	3rd week	4th week
Control	0.017 ±	2.450 ±	2.417 ±	2.650 ±	2.567 ±
FCA+ Saline	0.0067	0.0500	0.0477	0.0428	0.0803
Indomethacin	0.018 ±	1.333 ±	1.367 ±	1.117 ±	0.967 ±
10 mg/kg	0.0048	0.0494***	0.0954***	0.1352***	0.1256***
Pet Ether extract	0.050 ±	1.717 ±	1.683 ±	1.383 ±	1.067 ±
300 mg/kg	0.0306	0.0601***	0.1078**	0.0792***	0.0989***
Chloroform extract	0.012 ±	1.567 ±	1.533 ±	1.283 ±	0.983 ±
300 mg/kg	0.0056	0.0989***	0.0760***	0.1108***	0.1249***
Ethanol extract	0.015 ±	1.433 ±	1.383 ±	1.200 ±	0.933 ±
300 mg/kg	0.0037	0.0667***	0.0477***	0.0931***	0.0882***
Aqueous extract	0.022 ±	2.200 ±	1.900 ±	1.800 ±	1.750 ±
300 mg/kg	0.0048	0.1528†	0.2898†	0.3246**	0.3364**

Values are presented as mean ±SEM for n=6 †Non significant (P>0.05), *Significant (P<0.05), **More significant (P<0.01), ***Higher significant (P<0.001)

Table 2: Percentage inhibition of Paw Edema (ml) in FCA induced Paw Edema in Rats

Treatment Groups (n=6) (Dose mg/kg)	% Inhibition of Paw Edema (ml)				
	0 week	1st Week	2nd week	3rd week	4th week
Control	-	-	-	-	-
Indomethacin	-	45.59	43.44	57.84	62.34
Pet Ether extract	-	29.91	30.36	47.81	58.43
Chloroform extract	-	36.04	36.57	51.58	61.69
Ethanol extract	-	41.51	42.78	54.71	63.64
Aqueous extract	-	10.20	21.39	32.07	31.82

Table 3: Changes in Body Weight of animals in FCA induced arthritis in Rats.

Treatment Groups (n=6)	Mean Body Weight (gm)					Mean changes in Body Weight (±SEM)
	Initial week	1st week	2nd week	3rd week	4th week	
Control	172.8 ±	171.2 ±	169.0 ±	154.3 ±	150.8 ±	-22±0.660
FCA+ Saline	6.789	6.580	5.882	6.086	6.129	
Indomethacin	169.3 ±	169.3 ±	173.0 ±	174.7 ±	176.2 ±	6.9±1.089
10 mg/kg	4.161	4.161	5.317	4.828	5.250	
Pet Ether	169.2 ±	170.0 ±	172.2 ±	175.8 ±	177.0 ±	7.8±1.130
extract	6.655	6.703	6.554	7.774	7.785	
300 mg/kg						

Chloroform extract 300 mg/kg	174.0 ± 7.849	174.0 ± 7.849	176.2 ± 7.423	180.0 ± 8.311	180.5 ± 8.690	**6.5±0.841**
Ethanol extract 300 mg/kg	168.0 ± 4.872	168.3 ± 4.674	171.0 ± 4.740	174.0 ± 4.147	177.5 ± 5.271	**9.5±0.399**
Aqueous extract 300 mg/kg	165.3 ± 2.929	165.3 ± 2.929	161.3 ± 3.509	158.8 ± 2.960	155.2 ± 3.902	**-10.1±0.973**

Values are presented as mean ±SEM for n=6

Table 4: Effect of Hematological Parameters on FCA induced arthritis in Rats.

Treatment Groups (n=6)	Changing in Hematological Parameters (Mean ± SEM)			
	WBC ($\times10^3$ cells/mm^3)	RBC ($\times10^6$ cells/mm^3)	Hb %	ESR (mm/h)
Control	11.38 ±	6.733 ±	10.15 ±	12.20 ±
FCA+ Saline	0.1922	0.191	0.316	0.3276
Indomethacin	10.80 ±	6.933 ±	13.08 ±	3.617 ±
10 mg/kg	0.2206	0.105	0.322***	0.5212***
Pet Ether extract	10.83 ±	6.783 ±	12.45 ±	4.750 ±
300 mg/kg	0.2824	0.149	0.469***	0.3547***
Chloroform extract	10.75 ±	6.800 ±	12.98 ±	4.200 ±
300 mg/kg	0.2884	0.106	0.508***	0.3347***
Ethanol extract	10.72 ±	7.000 ±	13.13 ±	4.000 ±
300 mg/kg	0.1956	0.073	0.508***	0.749***
Aqueous extract	11.30 ±	6.850 ±	10.27 ±	8.833 ±
300 mg/kg	0.1461	0.138	0.206*	0.4958**

Values are presented as mean ±SEM for n=6 †Non significant (P>0.05), *Significant (P<0.05), **More significant (P<0.01), ***Higher significant (P<0.001)

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-α), 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 are mediated by proinflammatory cytokine such as TNF-α. The articular cartilage destruction, circumarticular fibrosis, and ankylosis are the pathological changes found in chronic inflammation. [15] However standard drug, ethanol and chloroform 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.[7, 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 *Ficus benghalensis* 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.

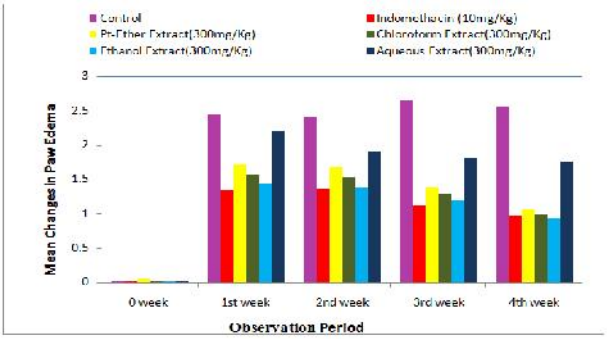

Fig 1: Mean changes in Paw Edema (ml) in FCA induced Paw Edema in Rats

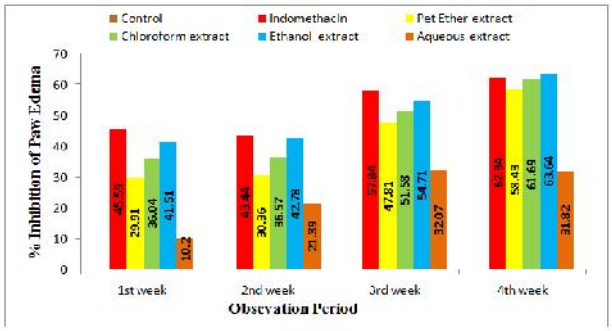

Fig 2: Percentage inhibition of Paw Edema (ml) in FCA induced Paw Edema in Rats

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