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STUDY ON EFFICACY OF TREATMENT WITH *FICUS BENGHALENSIS* LEAF EXTRACTS ON FREUND'S ADJUVANT INDUCED ARTHRITIS IN RATS

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ABSTRACT

In view of the well-established anti-inflammatory properties of bark and leaf of Ficus benghalensis, the present study was carried out to evaluate the protective effect of ethanol and aqueous leaf extracts against Freund's adjuvant induced arthritis in rats. The ethanol and aqueous leaf extracts was administered orally at dose of 300 mg/kg body weight for 21 days. 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 haematological parameters such as haemoglobin content, total WBC, RBC and erythrocyte sedimentation rate were estimated. The results indicate that at dose of 300 mg/kg body weight, both the extracts protects rats against the primary and secondary arthritic lesions, body weight changes and haematological perturbations induced by CFA. Daily treatment of rats with ethanol and aqueous leaf extracts, standard drug Indomethacin effectively inhibits paw edema in rats. Both the extracts significantly ($p< 0.01$) altered the parameters which were estimated when compared to control group rats. At the end of studies the ethanol extract shows more pronounce effect 66.88% than the aqueous extract 63.82% as compared to standard drug Indomethacin 75.42%. 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 Ficus benghalensis L.in the treatment of various immuno-inflammatory disorders.

Key words: *Freund's adjuvant, arthritic, Ficus benghalensis, Moraceae, leaf.*

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- 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

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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[7], anti-inflammatory[7], antioxidants[8], antidiabetic[9], immunomodulatory[10] and antimicrobial[11] activity in experimental animals. The ethnomedicinal use of the leafs of *Ficus benghalensis* in arthritic disorders, has not been systematically investigated so far. Therefore the present study was designed to determine the anti-arthritic activity of leafs in the Complete Freund's adjuvant induced arthritis model in rats.

Materials and Methods

Plant material

The Fresh leafs of *Ficus benghalensis* were collected from the Jawaharlal Nehru Medical college campus (JNMC), Belgaum in the month of May-June. The plant was authenticated by Dr. Harsha Hegde, Scientist B, Regional Medical Research Center, Indian Council of medical Research (ICMR) Belgaum. The voucher specimen (RMRC-508) has been deposited in ICMR herbaria and Department of Pharmacognosy, KLES College of Pharmacy, Belgaum, India.

Preparation of Extracts

The collected fresh leafs about 2kg were dried under shade for two weeks than powdered and passed through 40 meshes and stored in air tight plastic jar for further use. The leafs powder 450g was exhaustively extracted by hot continuous extraction using soxhlet apparatus with 95% ethanol at a temp. 70^0 C up to 72 h. The total ethanolic extract was filtered and concentrated by distillation process. The concentrated mass was dried under vacuum till constant weight. For aqueous extract the leafs powder 200g was macerated with 1000ml 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 and further dried in vacuum oven till constant weight.

Experimental animals

Wistar albino rats of either sex weighing between 150-200gm were selected for the present study and received from centralized animal house, KLES College of Pharmacy, Belgaum. 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 study protocol was approved by Institutional animal ethical committee with CPCSEA registration number

221/CPCSEA for KLES College of Pharmacy Belgaum, Karnataka.

Acute toxicity study

An acute toxicity study was carried out by up and down method. Drugs 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 animals died even at a dose of 3000mg/kg b.w. of each extract. Hence one tenth ($1/10^{th}$ of LD_{50}) cut off dose (i.e. 300mg/kg) was selected for the subsequent study.

Experimental design

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 group. The treatment schedules of rats belonging to the different groups are shown below

Group 1: Normal (Normal saline)

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

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

Group 4: Ethanol extract (300mg/kg p.o)

Group 5: Aqueous extract (300mg/kg p.o)

On the 0^{th} day, the basal paw volume of left hind paw of each animal was measured using mercury plethysmometer. On the 1^{st} day all the animals except normal group 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 and extracts was started on the same day i.e. 1^{st} day and continued for 21^{st} day. Normal and arthritic control groups rats receives normal saline through out study while the rest experimental groups animals receives respective treatment once daily by oral route. The gum acacia 2%w/w was used as vehicle for suspended the extracts. Paw volume of injected paw was measured on 4^{th}, 8^{th}, 14^{th} and 21^{st} day of study period. At the end of day 21^{st}, 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 Hemoglobin 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 21^{st} day.

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.

Results and Discussion:

The leafs were green and odourless with a slightly bitter taste. Leafs were 7.2-16.5 cm in length, lanceolate in shape, have a glabrous surface, acute, apex, equal base, entire margin and were petioled. From the extraction the yield was found to be 2.6 % for ethanol and 5.8 % for aqueous extract. 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 both 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 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 extracts. 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 ethanol and aqueous extract at a dose of 300 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 decrease the paw volume from the 1st day after the induction of CFA, where as the extracts significantly decreases the paw volume after 4th day. From the table 2 it was found that the ethanol extract has got highest percentage of inhibition 66.88 % of paw edema as compared to aqueous extract which is 63.82 % at the end of 21 days. Standard drug Indomethacin decreases the paw edema by 75.42 % respectively. From the table 3 a loss of body weight was observed during the arthritis condition. Standard drug, ethanol and aqueous 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, a decreased hemoglobin (Hb) content and an increased erythrocyte sedimentation rate (ESR) were also favorably altered by *Ficus benghalensis* treatment (Table-4).

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 aqueous 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.

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

Treatment groups	Mean changes in paw volume ±SEM			
	4th day	8th day	14th day	21st day
Arthritis control	4.350±0.0428	4.383±0.0600	4.450±0.0562	4.690±0.1100
Indomethacin (10 mg/kg)	3.817±0.0307*	3.050±0.0223**	1.983±0.0307**	1.040±0.1400**
Ethanol extract (300 mg/kg)	3.952±0.0130*	3.003±0.0157**	2.298±0.0146**	1.540±0.0500**
Aqueous extract (300 mg/kg)	4.033±0.0333†	3.333±0.0210*	2.350±0.0223**	1.700±0.0365**

n=6, values are expressed as mean ± SEM, †Non significant ($P>0.05$), *Significant($P<0.05$), **More significant($P<0.01$) ,when compared to control

Table 2: Percentage inhibition of paw volume in Adjuvant-induced arthritis in rats.

Treatment groups	% inhibition of paw volume			
	4th day	8th day	14th day	21st day
Control	--	--	--	0
Standard	12.41	30.85	55.31	75.42
Ethanol extract	9.74	32.06	48.88	66.88
Aqueous extract	7.43	24.51	47.35	63.82

Table 3: Changes in body weight in Adjuvant-induced arthritis in rats.

Treatment groups	Mean body weight(gm)		Mean changes in body weight
	Before induction 0th day(gm)	On 21st day (gm)	
Normal	166.5	180.8	14.3±1.022
Control	183.7	179.5	– 4.2±1.100
Standard	170.2	178.5	8.5±0.220**
Ethanol extract	165.2	172.5	7.3±1.400**
Aqueous extract	169.0	173.8	4.8±2.900*

n=6, values are expressed as mean ± SEM, †Non significant (P>0.05), *Significant(P<0.05), **More significant(P<0.01)

Table 4: Effect of haematological parameters in Adjuvant-induced arthritis in rats.

Treatment groups	Changes in haematological parameters mean ± SEM			
	WBC ($\times10^3$cells/mm^3)	RBC ($\times10^6$cells/mm^3)	Hb (gm %)	ESR (mm/hr)
Normal	7.00±0.10	5.88±0.110	14.08±0.1030	2.78±0.20
Control	7.85±0.080	5.13±0.284	11.37±0.1453	4.05±0.17
Standard	7.02±0.011**	5.20±0.033†	13.20±0.1528**	3.25±0.12**
Ethanol extract	7.03±0.082**	5.33±0.272†	12.87±0.5207**	3.22±0.15**
Aqueous extract	7.15±0.028**	5.23±0.088†	12.70±0.1528*	3.50±0.17*

n=6, values are expressed as mean ± SEM, Comparison of all parameters of control group with standard and test group, †Non significant (P>0.05), *Significant(P<0.05), **More significant(P<0.01)

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