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### EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF *FICUS RETUSA* (MORACEAE)

N. Jaya Raju<sup>\*1</sup>, N. Sreekanth<sup>2</sup>

<sup>1</sup>Dept. of Pharmacy, College of Public Health & Medical Sciences, Jimma University, Jimma, Ethiopia <sup>2</sup>Dept. of Pharmacy, J.N.T. University, Hyderabad, Andhra Pradesh, India

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### ABSTRACT

The study was designed to evaluate the anti-inflammatory effect of leaves of *Ficus retusa* (Moraceae) in Sanskrit, it is known as 'Kantalaka', 'Kshudra' and in Telugu it is well known as 'Yerrajuvvi'. It is also called as "Indian Laurel Fig" of ethyl acetate and methanolic extracts in carrageenan induced albino wistar rats of either sex (175-250g). The anti-inflammatory effects of ethyl acetate extract of *Ficus retusa* 200, 400 mg/kg p.o were found to be significant (P<0.05) in reducing rat paw oedema induced by carrageenan, where as methanolic extract produced significant reduction of paw oedema at 400 mg/kg p. o. Diclofenac sodium(50 mg/kg) was used as the reference anti-inflammatory agent for comparison. The ethyl acetate extract was highly significant in reducing rat paw oedema. **KEYWORDS**: Anti-inflammatory, *Ficus retusa*, Carrageenan, Diclofenac sodium.

### \*Corresponding Author

N. Jaya Raju, Email: Jayaraju123@yahoo.com, raju8859@rediffmail.com

### **INTRODUCTION**

The word inflammation, a defensive reaction to injury with classical signs of warmth, reddening, pain, swelling and loss of function, which is of a acute or chronic type<sup>1</sup> this inflammation is also observed in cancer<sup>2</sup>, bowel syndrome, hepatic and Alzheimer's diseases. The characteristics of inflammation are humorous like reddening (visible), swelling (oedema), soreness (pain) and corresponding histological changes.

*Ficus retusa* (Moraceae) is distributed through out Western peninsula and also found in Chota Nagpur, Bihar, Central India, Andamans, Sundribuns, Malaya islands and Australia. In Sanskrit, it is known as 'Kantalaka', 'Kshudra' and in Telugu it is well known as 'Yerrajuvvi'. It is also called as "Indian Laurel Fig". Root bark and the leaves boiled in oil form good applications for wounds and bruises. Adventitious roots, dried and powdered and mixed with salt are applied to decaying or aching tooth. Bark is used in treatment of liver diseases<sup>3-5</sup>.

*Ficus* (Moraceae) species are used in folk medicine for the treatment of various diseases, such as biliousness, ulcers, vomiting, vaginal complains, fever, diabetes, inflammations, Wound, lucoderma, ulcer itching, diuretic, liver diseases and leprosy, liver<sup>6</sup>. *Ficus retusa* is a rapidly growing tree in India but originated from Ceylon. Roots are adventitious, occasionally hanging. Bark is gray and smooth. Branchlets are brown and glabrous<sup>7-8</sup>. The golden yellow leaves of *Ficus retusa* contain high amounts of flavonoids and carotenoids, triterpenoids, fatty alcohol, steroids, coumarins, flavane, 4-hydroxybenzoate and isoflavones<sup>9-11</sup>.

The present study aimed to investigate the possible antiinflammatory effects of two solvent extracts of leaves of *Ficus retusa*, using in vivo experimental model.

### MATERIALS AND METHODS

### Plant Material Collection

*Ficus retusa* (Moraceae) leaves were collected from the Andhra University region of Visakhapatnam, Andhra Pradesh in the month of January 2009 and Authenticated by the taxonomist, Dept of Botany, Andhra University and the specimen Voucher No JNTUCP/2009/F75 has been preserved in the Department.

### **Extraction of plant material**

Dried powdered plant material (200 g) was extracted in 1000 ml of each solvent using a Soxhlet apparatus. A successive solvent extraction method was employed with two different solvents, viz. ethyl acetate and methanol. The extracts were poured into the evaporating dishes and the solvents were evaporated under reduced pressure. The solvent was then evaporated to dryness to give the dried crude extract, which was stored in air-tight bottles

## at 4°C. Two extracts were named. The extractive yield of FREE and FRME was 6.48 and 9.28%, respectively.

### Animals

Wistar albino rats of either sex weighing between 175-250 gm were obtained from M/s. Mahavir Enterprises, Hyderabad, Andhra Pradesh, India. The animals were housed under standard environmental conditions (temperature of  $22 \pm 1^{\circ}$ C with an alternating 12 h light– dark cycle and relative humidity of  $60 \pm 5$  %), one week before the start and also during the experiment as per the rules and regulations of the Institutional Animal Ethics committee and by the Regulatory body of the government. They were fed with standard laboratory diet supplied by M/s. Rayans biotechnologies Pvt. Ltd., Hyderabad, Andhra Pradesh, India. Food and water was allowed *ad libitum* during the experiment.

### Acute toxicity studies

Acute toxicity studies were performed for extracts of selected plant according to the toxic classic method as per guidelines. None of these extracts showed mortality even at a dose of 1000mg/kg and therefore considered safe.

Toxicological studies were conducted in mice (N=6) for all the extracts as per the Irvin's method<sup>12</sup> at the doses of 100, 300 and 1000 mg/kg, no mortality was observed.

### Anti-inflammatory activity

The rats were divided into different groups (each contains 6) as follows: Group -I received drug vehicle 1% sodium CMC. Group -II received standard drug Diclofenac sodium at the dose of 50 mg/kg. Group- III, IV and V received ethyl acetate extract of leaves of Ficus retusa at doses 100, 200 and 400 mg/kg body weight p. o. Group - VI, VII and VIII received methanolic extract of leaves of Ficus retusa at the doses of 100, 200 and 400 mg/kg respectively. Two hours after these administrations, each rat received in its right hind paw sub plantar region injection of 0.1 ml of 1% carrageenan in N-saline to the left hind paw. The initial paw volume of each rat paws measured by using Plethysmometer before and at 1, 2 and 3 hrs after carrageenan injection<sup>13</sup>. **Statistical analysis** 

# All values were expressed as mean $\pm$ S.E.M. The differences were compared using one way analysis of variable (ANOVA) followed by students t-tests. P-values (<0.05) were considered statistically significant<sup>14</sup>.

### **RESULTS AND DISCUSSION**

Sub plantar injection of 1% carrageenan(0.1 ml) produced was marked, sustained and time related increase in the rat hind paw oedema of the control group. The ethyl acetate extract of leaves of *Ficus retusa* at the doses of 100, 200 and 400 mg/kg produced time related, sustained and dose dependent significant reduction

(P<0.05-0.001) of carrageenan induced inflammation of the rat hind paw (**Table-1 & Fig-1**). A methanolic extracts of leaves of *Ficus retusa* at the doses of 100, 200 mg/kg were not able to produce significant reduction, where as doses of 400 mg/kg produced significant reduction (P<0.05) in the inflammation produced by carrageenan (**Table-2 & Fig-2**).

From the observed values, the percentage of maximal paw oedema produced during 3 hours was calculated for two extracts of the plant. The effect of different concentrations of both extracts was taken to study its anti-inflammatory activity. The present study was conducted to evaluate the anti-inflammatory activity of *Ficus retusa*, which is very new herbal drug that was firstly identified by us to get a berth in the group of antiinflammatory herbal drugs. In the methanolic extract treated groups, a significant percentage inflammation reduction was produced by the extract at 400 mg/kg in inflammatory groups is highly significant (P<0.05), when compared to the percentage reduction observed in Diclofenac sodium (standard) treated groups.

Prostaglandins and bradykinins were suggested to play an important role in carrageenan induced oedema and analgesia<sup>15,16</sup>. As phytochemical tests showed the presence of sterols, alkaloids, flavonoids<sup>17,18</sup> and tannins in both methanolic and ethyl acetate extracts they might suppress the formation of prostaglandins and bradykinins or antagonize their action and exerts its activity. It can be concluded that all the extracts have potential to be explored as anti-inflammatory agents. Further studies may reveal the exact mechanisms of action responsible for the anti-inflammatory activities of *Ficus retusa*.

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 Table 1: Percentage inhibition of Carrageenan induced paw oedema in rats by prophylactic treatment with the ethyl acetate extract of *Ficus retusa* and Diclofenac sodium

Treatments	1st hour	2nd hour	3rd hour
(mg/kg)	Paw volume	Paw volume	Paw volume
Control	25.08±3.17	36.61±3.58	48.51±4.16
Diclofenac sodium (50)	18.21±2.96*	22.02±1.34*	24.21±3.00***
FREE (100)	27.11±6.16	29.11±3.41	31.70±2.98**
FREE (200)	24.03±3.41	30.56±2.21	32.34±7.87**
FREE (400)	22.01±2.11	33.21±4.58	22.02±3.21***

P: \*<0.05, \*\*<0.01, \*\*\*<0.001, ns=not significant

Table 2: Percentage inhibition of Carrageenan induced paw oedema in rats by prophylactic treatment with the methanolic extract of *Ficus retusa* and Diclofenac sodium

Treatments	1 <sup>st</sup> hour	2 <sup>nd</sup> hour	3 <sup>rd</sup> hour
(mg/kg)	Paw volume	Paw volume	Paw volume
Control	25.08±3.17	36.61±3.58	48.51±4.16
Diclofenac sodium (50)	18.21±2.96*	22.02±1.34*	24.21±3.00***
FRME(100)	30.01±5.11	34.20±1.25	39.70±1.11**
FRME(200)	28.31±1.52	39.16±3.16	42.34±5.33**
FRME(400)	25.22±3.21	36.32±1.34	25.32±2.42***

P: \*<0.05, \*\*<0.01, \*\*\*<0.001, ns=not significant



