



## Evaluation of Anti-Inflammatory Activity and Toxicity Studies of *Feronia Limonia* in Acute Inflammatory Model in Rats

Jayashree V Hanchinalmath, Ramesh Londonkar\*

Dept of Biotechnology, Gulbarga University, Gulbarga 585106, Karnataka, India.

\*Corresponding author's E-mail: [londonkarramesh53@gmail.com](mailto:londonkarramesh53@gmail.com)

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### ABSTRACT

The methanol extract of *Feronia limonia* fruits (MEFL) was evaluated for anti-inflammatory activity using *in vivo* and *in vitro* methods. In the *in vivo* method, MEFL significantly inhibited edema produced by carrageenan in rats. In the *in vitro* method, the probable supporting mode by which MEFL mediates its effects on inflammatory conditions was studied on thermally induced protein denaturation. MEFL exhibited significant protein denaturation property. The effect was compared with the activity of indomethacin as reference standard. Toxicity study indicates that the extract is non-toxic at the tested doses. Thus, the present study revealed that the MEFL exhibited significant anti-inflammatory activity in the tested models.

**Keywords:** Carrageenan, anti-inflammatory, *Feronia limonia*, antioxidant.

### INTRODUCTION

Inflammation is the complex biological response of vascular tissues to harmful stimuli including pathogens, irritants, or damaged cells. It is a protective mechanism by the organism to remove the injurious stimuli as well as initiate the healing process for the tissue.<sup>1</sup> The process of inflammation is required in healing of wounds. However, if inflammation is unchecked, leads to onset of diseases like rhinorrhoea, rheumatoid arthritis and atherosclerosis. Acute inflammation is characterized by classical signs like edema, erythema, pain, heat, and primarily loss of function. The typical signs are triggered by the infiltration of the tissues by serum and white blood corpuscles (leukocytes). Early inflammatory changes in damaged tissues are known to release various biologically active materials from polymorph nuclear leukocytes, lysosomal enzymes and others. The vascular effects are mediated by prostaglandins, kinins and vasoactive amines (histamine) released by mast cells. Acute models are designed to test drugs that modulate erythema, measurement of local pain, antipyretic activity, local analgesic action and rat paw edema, changes in vascular permeability, leukocyte migration and chemotaxis, phagocytosis-polymorphonuclear leucocytes and other phagocytic cells.<sup>2</sup> These responses to inflammation include increased total leukocyte count (TLC) and differential leukocyte count (DLC), hyperpyrexia, elevated erythrocytic sedimentation rate (ESR) and proteins. These are referred to as markers of acute inflammation.<sup>3</sup> All the steroidal and non-steroidal anti-inflammatory drugs (NSAIDs) although effective, cause undesirable and side effects. Thus, the development of a safer and efficacious alternative is needed. Natural products serve as an important source of therapeutically effective medicines. Carrageenan-induced local inflammation is commonly used to evaluate NSAIDs.

*Feronia limonia* is one of the medicinally important plants belonging to Rutaceae, commonly known as wood apple. Various parts have been used against various ailments in ethnomedicine. Ripe fruit of *F. limonia* is effective in dysentery, stomatitis, diarrhea, tumors, cough, asthma, leucorrhoea, wounds and ulcers. In this view, the present study has been undertaken to investigate the anti-inflammatory and toxicity studies of methanol extract of *Feronia limonia* (MEFL) in rats.

### MATERIALS AND METHODS

#### Plant material

The *Feronia limonia* fruits were collected in the month of March 2011 from the market, Bellary district, Karnataka, India. The fruit pulp was dried under shade and then powdered with a mechanical grinder. The powder was passed through sieve no 40 and stored in an airtight container for further use.

#### Chemicals and reagents

The chemicals used in the present study were carrageenan (S. D. Fine Chemicals Limited, Bombay), Indomethacin (Sigma Aldrich, Bengaluru).

#### Preparation of extract

The dried powdered plant material was extracted with methanol in a Soxhlet extraction apparatus. The solvent was removed under reduced pressure and semi solid mass was obtained (yield 12.25%). The extract showed positive test for alkaloids, steroids, and tannins. The extract at the different doses of 200 and 400 mg/kg was suspended in aqueous Tween 80 solution (2%) and Indomethacin (12.5 mg/kg) in saline was used for the present study.

#### Animals



Wistar strain Albino male rats of inbred colony weighing about 150 – 175 g were used. The protocol was approved by the Institute's Animal Ethical Committee (IAEC Reg No. 34800/ CPCSEA Dated: 19.08.2001). They were maintained under standard environmental conditions and were fed with standard pellet diet (procured from CFTRI, Mysore) and water *ad libitum*.

### Investigation of acute anti-inflammatory effects by carrageenan induced paw oedema

The rats were divided into 5 groups (n = 6). Animals were deprived of food and water for 24 hours before the experiment. On the day of the experiment they were assigned to 5 groups of six animals each. They were marked and numbered for identification. Paw oedema was induced by injecting 1% carrageenan into the rat hind paw (group 2,3,4). 0.1 ml sterile saline was given to group 1 (control group). A group of rats were treated with test compounds (group 2 and 3) and standard drug Indomethacin (group 4) was administered orally. The volume of the paw was measured by a vernier caliper immediately after the injection as previously described.<sup>4,5</sup> Successive readings were carried out at one-hour intervals up to 4h and compared to the initial readings. The increase in paw volume was taken as oedema volume. The percentage of inhibition of inflammation was calculated for comparison.

The anti-inflammatory effect of MEFL was calculated by the following equation: Anti-inflammatory activity (%) =  $(1 - D/C) \times 100$ , where D is the percentage difference in paw volume after MEFL administration, and C is the percentage difference in volume of the control group.

### Toxicity study

Albino rats were divided into control and test groups (6 animals each). Control group received the vehicle (3% Tween 80) while the test groups got graded doses (100–4000 mg/kg) of MEFL orally and were observed for mortality till 48 h and the LD<sub>50</sub> was calculated. No death was seen till the dose 4000 mg/kg of the extract.

### Biochemical assays and hematological studies

Blood was obtained from the tail vein, 24 h after the last dose, centrifuged at 3000 rpm at 4°C for 10 minutes to separate serum. The activities of serum glutamate oxaloacetate transaminase level (SGOT) and serum glutamate pyruvate transaminase (SGPT) were assayed by the method (Bergmeyer and Bernt).<sup>6</sup> The alkaline phosphatase activity in the serum was measured according to the procedure of King.<sup>7</sup> The blood was also used to estimate other hematological parameters of normal, control and treated animal groups.<sup>8,9</sup> The liver was excised, rinsed in ice-cold normal saline followed by cold 0.15 M Tris-HCl (pH 7.4), blotted and weighed. The homogenate was processed for estimation of lipid peroxidation and GSH.<sup>10,11</sup> Proteins were estimated by using bovine serum albumin as the standard.<sup>12</sup>

### In vitro studies

#### Effect on protein denaturation

Effect on protein denaturation of MEFL was assayed by the method of Elias and Rao.<sup>13</sup> Test solution (1ml) containing different concentrations (100 - 400 µg/ml) of plant extract and indomethacin (100 µg/ml) was mixed with 1ml of egg albumin solution (1mM) and incubated at 27°C for 15 min. Denaturation was induced by heating the reaction mixture at 70°C in water bath for 10 min. After cooling, the turbidity was measured at 660 nm spectrophotometrically. Percentage inhibition of denaturation was calculated from control where no drug was added. Each experiment was carried out in triplicate and the average was taken.

### RESULTS AND DISCUSSION

The present study establishes the potential of the MEFL for its anti-inflammatory effect and short-term toxicity was investigated in acute experimental animal models and was found no mortality upto 4000 mg/kg. The methanol extract exhibited significant anti-inflammatory activity at the tested doses of 200 and 400 mg/kg in a dose dependant manner. The methanol extract showed maximum inhibition of 54.60% at the dose of 400 mg/kg, whereas the standard drug showed 61.30% of inhibition after 3 h of drug treatment in carrageenan induced paw oedema. The results are summarized in table 1. The rats were observed for the behavioral changes such as loss of appetite, inactiveness, dizziness, erection of hairs and hypothermia. After oral administration of a single dose of the extract at the doses of 200 and 400 mg/kg, none of the rats exhibited any abnormal behavioral responses. Administration of repeated daily doses of 200 and 400 mg/kg for 7 days did not influence the body weight of the rat. The weight of liver remained same. Carrageenan is a sulphated polysaccharide acquired from sea weed (Rhodophyceae) and is generally used to induce acute inflammation and is assumed to be bi-phasic. The first phase is caused by the release of histamine and serotonin. The second phase is due to the release of bradykinin, protease, prostaglandin and lysosome<sup>14</sup>. It is reported that the second phase of oedema is sensitive to most clinically effective anti-inflammatory drugs, which has been frequently used to access the anti-oedematous effect of natural products. Prostaglandins play a key role in the development of second phase of reaction measured at 3 hours time. These mediators take part in the inflammatory response and are able to stimulate nociceptor and thus induce pain.<sup>15</sup> Based on these reports; it can be assumed that the inhibitory effect of the extract of *Feronia limonia* on carrageenan-induced inflammation in rats may be due to the inhibition of the mediators responsible for inflammation.



**Table 1:** Effect of the *Feronia limonia* methanol extract on carrageenan induced paw oedema

Treatment	Dose (mg/kg)	Oedema volume (ml)	% of Inhibition
Saline control	0	0.732 ± 0.063	-
MEFL	200	0.403 ± 0.028	44.90
MEFL	400	0.327 ± 0.017	54.60
Indomethacin	10	0.283 ± 0.024	61.33

Values are mean ± SEM (n = 6). Experimental groups were compared with control p < 0.001.

The hematological profile and biochemical parameter are shown in table 2. Slight elevations in blood parameters were seen. Oral administration of MEFL (200 and 400 mg/kg body weight) for 7 days resulted in increased

glutathione, LPO in liver (Table 2) which clearly shows the antioxidant property of MEFL in acute induced oedema model and also indicates that for the safety use of the extract. Serum enzymes elevation has been reported to be linked with number of inflammatory disorders.<sup>16</sup> It has also been reported that the low hemoglobin concentration with increased level of serum alkaline phosphatase are seen in inflammatory disease such as rheumatoid arthritis, which is usually associated with anorexia and weight loss.<sup>17,18</sup> Our findings suggest that the anti-inflammatory effect of MEFL in carrageenan induced paw oedema is due to its ability to decrease capillary permeability which results in decrease in fluid exudation. It is possible that MEFL inhibits the release of mediators like histamine, prostaglandins, bradykinin and leukotrienes which play a significant role in increasing vascular permeability.<sup>19-23</sup>

**Table 2:** Effect of *Feronia limonia* methanol extract on hematological and different biochemical parameters in rats

Parameters	Control (Normal saline 5 ml /kg)	MEFL (200 mg/kg)	MEFL (400 mg/kg)	Indomethacin (10 mg/kg)
Hemoglobin (g %)	12.4 ± 1.10	12.3 ± 0.83	12.5 ± 1.04	12.4 ± 1.21
Erythrocyte volume fraction (EVF)%	37.7 ± 1.94	35.7 ± 1.43	37.7 ± 1.23 <sup>b</sup>	36.9 ± 1.24
Mean Cell Hemoglobin Concentration (MCHC)%	33.4 ± 2.83	32.2 ± 2.17	33.4 ± 0.77	33.5 ± 0.71
Platelets (10 <sup>3</sup> × mm <sup>3</sup> )	1358 ± 41.2	1346.0 ± 74.1	1355 ± 57.13 <sup>a</sup>	1257.4 ± 42.2
<b>Liver function test</b>				
SGOT (U/L)	114.9 ± 8.57	110.8 ± 6.44 <sup>a</sup>	114.5 ± 1.62	98.8 ± 1.34 <sup>a</sup>
SGPT (U/L)	20.7 ± 1.72	19.5 ± 2.21 <sup>b</sup>	20.1 ± 1.74	19.7 ± 1.88
ALP (U/L)	60.2 ± 3.04	59.9 ± 4.8 <sup>b</sup>	67.4 ± 4.65	43.7 ± 3.95 <sup>b</sup>
Protein (g/dL)	7.2 ± 0.04	6.92 ± 0.53	7.23 ± 0.65	6.85 ± 0.54
Lipid peroxidation (n moles MDA/g of tissue)	0.97 ± 0.03	0.97 ± 0.04	0.98 ± 0.02	0.99 ± 0.02
GSH (mg/g of tissue)	2.36 ± 0.03	2.39 ± 0.12 <sup>*</sup>	2.46 ± 0.17 <sup>*</sup>	2.54 ± 0.21

Values are mean ± SEM (n=6). Groups were compared with normal control group <sup>a</sup> p < 0.001, <sup>b</sup> p < 0.01

### In vitro studies

#### Inhibition of protein denaturation

The inhibitory effect of different concentrations of MEFL on protein denaturation is shown in Table 3. MEFL extract (100-400 µg/ml) showed significant (p < 0.05) inhibition of denaturation of egg albumin in concentration dependent manner. MEFL at concentration of 400 µg/ml and indomethacin at concentration of 100 µg/ml showed significant (p < 0.01) inhibition (66.25% and 80.74% respectively) of protein denaturation when compared with control. It is possible that the crude MEFL possesses more than one compound responsible for its anti-inflammatory effect. The fruits of *Feronia limonia*, have been shown to contain alkaloids, glycosides, tannins, flavonoids, sterols and/or Triterpenes.<sup>24</sup>

**Table 3:** Effect of methanol extract of *Feronia limonia* fruits on protein denaturation

Samples	Concentration	% inhibition
Control	-	-
MEFL	100 µg/ml	34.36
	200 µg/ml	56.62
	300 µg/ml	64.42
	400 µg/ml	66.25
Indomethacin	100 µg/ml	80.74

Each value represents the mean ± SEM (n=3)

### CONCLUSION

In conclusion, our results indicate that the extract exhibited significant *in vivo* and *in vitro* anti-inflammatory activity which is comparable to standard anti-inflammatory drug. Thus this study substantiates the use of the plant as an anti-inflammatory folklore medicine.



Further detailed investigation is ongoing to determine the exact phytoconstituents responsible for the anti-inflammatory activity and the investigations on biochemical pathway may yield in the development of a potent anti-inflammatory agent with a low toxicity and better therapeutic index.

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