

## ***In-Vivo* Anti-Inflammatory Activity of an Methanolic Extract of *Fraxinus Micrantha***

**\*Suresh Kumar, Priya Kashyap**

\*Associate Professor, University School of Biotechnology  
GGS Indraprastha University, Dwarka, Sector 16C, New Delhi  
sk222ind@yahoo.com

---

### **Abstract:**

**Introduction:** Inflammation is the response of living tissues towards an injury which involves a complex array of enzyme activation, mediator release and extravasations of fluid, cell migration, tissue breakdown and repair. Various anti-inflammatory synthetic drugs available in the market are of least interest due to their side effects. In the local medicines the bark infusion of *Fraxinus micrantha* is used for the treatment of liver enlargement and jaundice.

**Aim:** In the present study, the in-vivo anti-inflammatory effect of an methanolic extract of *Fraxinus micrantha* investigated.

**Methods:** The anti-inflammatory effect was evaluated using the Carrageenan-induced mice pedal (paw) oedema model.

**Results:** Administration of 10mg/kg of an methanolic extract of the aerial parts of *Fraxinus micrantha* produced significant anti-inflammatory effects against carrageenan-induced acute inflammation in mice that was comparable to one of the standard drug (Phenylbutazone) used in the treatment.

**Conclusions:** The present findings indicate that *F. micrantha* has genuine anti-inflammatory properties, lending pharmacological support to the folklore or anecdotal use of the plant in the treatment and/or management of painful inflammatory conditions.

**Keywords:** *Fraxinus micrantha*; Anti-inflammation; Carrageenan; Oedema.

---

## **1. INTRODUCTION**

Inflammation is a complex biological response in which vascular tissues responds to harmful stimuli such as irritants, pathogens and damaged cells [1]. Inflammation is response of a damage repair process through mediators where the injurious stimuli are removed and the healing process initiated. Despite the progress of medical science, the anti-inflammatory drugs available are a cause of concern due to their moderate to severe side effects such as gastric lesions caused by non-steroidal anti-inflammatory drugs (NSAID), tolerance and dependence induced by opiates [2]. Therefore, there is a constant need to search new anti-inflammatory drugs lacking these side effects as alternatives to NSAID and opiates. Herbal plant-based drugs are focused these days because they are economical, have no or little side effects. The genus *Fraxinus* is in the olive family, *Oleaceae*. *Fraxinus micrantha* is one of the ashes found in Asia mainly in India and Nepal. In India it is found in Himachal Pradesh and Uttar Pradesh region [3]. *Fraxinus micrantha* have been studied for its medicinal and economical value worldwide since ancient times. Local inhabitants of Dharchula, Himalayas use inner bark infusion for the treatment of liver enlargement, jaundice and other liver diseases [4].

## **2. MATERIALS AND METHODS**

### **2.1. Plant Material, Extraction and Preparation**

The dried barks of *Fraxinus micrantha* were purchased from the local market in Delhi around February-March, 2012 and authenticated by a certified botanist. A voucher specimen (USBT#101-19012012FM) was submitted to the herbarium in the laboratory. The dried barks were grinded using electric grinder and subjected to extraction (1:10) by using methanol as solvent system by decoction method for 3-4 days. The solvent thus obtained was subjected to rota-evaporation for 24 hours to get an methanolic extract of *Fraxinus micrantha* (MeFM). The fraction was reconstituted in saline

(pH7.1) and stock concentration of 20mg/kg was made. The stock of an MeFM was filtered using 0.2 micron syringe filter before making different concentrations (2.5-20mg/kg).

## 2.2. Chemicals and Solvents

The following chemicals and solvents were purchased: Carrageenan (Spectrochem), Prednisolone (intervet), Aspirin (Procter & Gamble), Phenylbutazone (Astra pharmaceuticals) and Sodium chloride (CDH). The other reagents were of analytical grade and obtained from different commercial sources.

## 2.3. Animals

Male Balb/c mice weighing 25-30g were used for the experiments and housed under standard laboratory conditions and fed commercial mice feed and tap water *ad libitum*. All animal experiments were conducted in accordance with the institutional ethical committee accepted laboratory animal use, care and guidelines. The animals submitted to oral administration of the extract or drugs were fasted for 12 hours. The mice were divided into 5 groups, with 5 mice in each group. In the first set of experiment, the first group of mice was orally administered with Phenylbutazone, second group with Prednisolone, third group with Aspirin, fourth group with an MeFM and the fifth group was taken as control and was injected with Carrageenan alone. In second set of experiment, all the groups of mice were orally administered with different doses of an MeFM, except one group which was taken as control, was orally given saline and injected with carrageenan alone.

## 2.4. Carrageenan-Induced Mice Paw Oedema Test

*In-vivo* anti-inflammatory activity was evaluated on the basis of the inhibition of carrageenan-induced mice hind paw oedema, with some modifications [5]. The mice were fasted for 12 hours with free access to water until the experiment started. Drugs used in the experiment were diluted in a way to obtain concentration of 20mg/Kg (animal weight) in water for prednisolone, 100mg/Kg in water for phenylbutazone and aspirin and 10mg/kg in water for an MeFM. Animals in the reference group received an MeFM (10 mg/kg), while control animals received distilled water (10 ml/kg). One hour later, oedema was induced by injecting 50µl of 1% carrageenan solution in normal saline (0.9%) subcutaneously into the plantar surface of right hind paw of each mouse. The paw volume up to fixed mark at the level of lateral malleolus, were measured by recording the volume displacement of saline solution (0.9% NaCl) with the help of Plethysmometer (model LE 7500, Letica scientific instruments) just before zero hour reading and one, two, three, four and five hours after induction of inflammation, using the Plethysmometer (model LE 7500, Letica scientific instruments). To analyse the dose dependent association, various concentrations of an MeFM, ranging from 2.5mg/kg to 20mg/kg were administered orally 1hour before carrageenan induced inflammation and the paw volume was measured at one, two, three, four and five hours after induction of inflammation, using the Plethysmometer.

## 2.5. Data Analysis

Anti-inflammatory activity of an MeFM was measured through reduction in the paw volume were compared between the control group animals and that of the test groups and the anti-inflammatory activity were carried out on the basis of the percentage inhibition of oedema.

$$\text{Percentage inhibition of oedema} = (V_c - V_t / V_c) \times 100$$

Where,  $V_t$  = paw volume in the test group animals

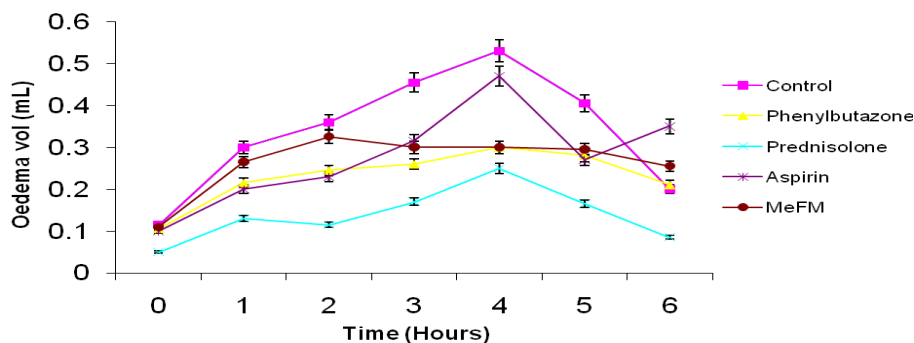
$V_c$  = paw volume in the control group

The data obtained was expressed as percentage inhibition of inflammation and analyzed using one way ANOVA. P values less than 0.05 ( $P < 0.05$ ) were considered to be statistically significant.

## 3. RESULTS AND DISCUSSION

### 3.1. Anti-Inflammatory Response of the Paw to Different Standard Anti-Inflammatory Drugs in Comparison with an MeFM in A Time Dependent Manner

Carrageenan when injected into the hind paws of mice produced a severe inflammatory response in time dependent manner and the maximum effect was attained in control group at 4hr time interval (Figure1).



**Figure1.** Anti-inflammatory effect of an MeFM and its comparison with standard anti-inflammatory drugs

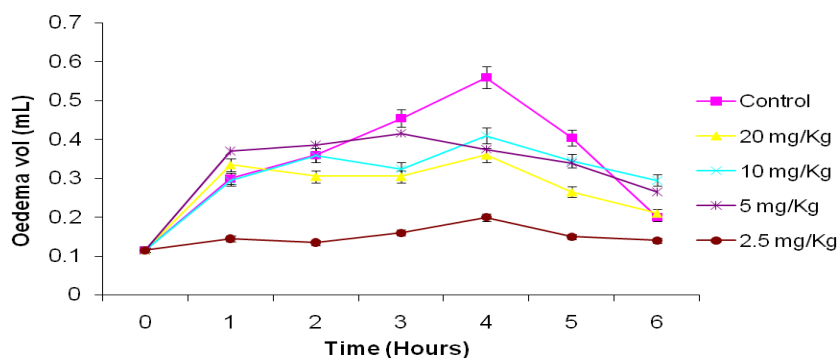
The comparative study of anti-inflammatory effect of different standard drugs and an MeFM at 1,2,3,4,5 hours showed that an MeFM was significantly reduced inflammatory response. At four hour duration the comparative inflammation was reduced to almost 43% by MeFM as compared to control ( $p < 0.05$ ) which is found to be statistically significant. The maximum inhibition was showed by Prednisolone (Percentage inhibition 68%) as compare to control ( $p < 0.01$ ). An MeFM showed significant inhibition (43%) which is comparable to percentage inhibition shown by standard drug Phenylbutazone (43%) and slightly less than Predisolone (53%) (Table 1).

**Table1.** The percentage inhibition of anti-inflammatory response in various treatment groups

Hour	Percentage inhibition of various treatment groups			
	Phenylbutazone	Prednisolone	Aspirin	MeFM
1	28%	57%	33%	12%
2	32%	68%	36%	10%
3	43%	63%	31%	34%
4	43%	53%	11%	43%
5	30.8%	59%	33%	27%

### 3.2. Anti-Inflammatory Response of the Paw to Different Doses of an MeFM in a Time Dependent Manner

The five assigned groups of Balb/c mice were given orally different concentration of an MeFM (20, 10, 5 and 2.5mg/kg) in the volume of 100µl. The entire group was injected with 50µl of 1% carrageenan. The control group was given equal volume 0.9% saline. The group of mice treated with 2.5mg/Kg showed maximum anti-inflammatory response in terms of minimum paw oedema volume (Figure2).



**Figure1.** Anti-inflammatory effect of various concentrations of an MeFM in terms of minimum paw oedema volume

At 4 hours, which has the peak inflammatory response an MeFM at concentration 2.5mg/Kg had 68% inhibition which is maximum as compared to various other concentrations (20, 10, 5mg/Kg) of an MeFM.

**Table 2.** The percentage inhibition of anti-inflammatory response in various concentrations of an MeFM treatment groups

Hour	Percentage inhibition of various concentrations of an MeFM			
	20mg/Kg	10mg/Kg	5mg/Kg	2.5mg/Kg
1	15%	1.7%	9%	33%
2	33%	29%	33%	47%
3	36%	27%	16%	52%
4	35%	15%	12%	68%
5	23%	5%	11%	62%

Inflammation is one of the series of events which leads to redness, heat, swelling, pain and dysfunction of the organs. It is the first response of the immune system to infection or irritation and may be referred to as the innate cascade [6]. Various anti-inflammatory drugs are commercially available used for treatment of inflammation such as, prednisolone, phenylbutazone and aspirin. The drawback associated with these drugs have their number of side effects such as colitis, Crohn's disease, diverticulitis, stomach ulcer, diabetes mellitus, hemorrhoids and hepatitis [7]. In the present study, the anti-inflammatory activity of an MeFM on pedal oedema model of mice was demonstrated. The carrageenan induced inflammation resulted in increased vascular permeability which reaches within half an hour of carrageenan administration [8]. These are biphasic effects in carrageenan induced oedema. The first phase begins immediately after injection and diminishes in 1 hour and the second phase begins after 1 hour [9]. In order to validate the anti-inflammatory effect of an MeFM various standard anti-inflammatory drugs such as phenylbutazone, prednisolone, aspirin were used. The percentage inhibition showed by an MeFM was comparable to phenylbutazone and slightly less than prednisolone in a time dependent manner. The plausible explanation might be due to greater inhibition of capillary permeability. The small concentration of an MeFM (2.5mg/Kg) has much more pronounced anti-inflammatory action as compared to higher doses (20mg/Kg). This might be due to the phenomenon of tolerance at higher dosage. The use of plant extract used in this study that is an MeFM might be useful in future in alleviation of pain associated with other inflammatory conditions.

#### 4. CONCLUSION

In conclusion, MeFM possesses potential anti-inflammatory activity as demonstrated from this study although further studies are required to isolate, purify and characterize the active compound from an MeFM which may give new lead that might be used in treatment of various anti-inflammatory diseases.

#### REFERENCES

- [1] Phanse M.A., Patil M.J., Chaudhari K.A.P.D. and Patel B., In-vivo and in-vitro screening of medicinal plants for their anti-inflammatory activity: an overview, J Appl Pharm Sci. 02 (06), 19 (2012).
- [2] Winter C.A., Nonsteroid anti-inflammatory therapy II, Gac Med Mex. 97(5), 543(1967).
- [3] Kumar S. and Kashyap P., Antiproliferative activity and nitric oxide production of a methanolic extract of *Fraxinus micrantha* on Michigan Cancer Foundation-7 mammalian breast carcinoma cell line, J Intercult Ethnopharmacol. 4(2), 109 (2015).
- [4] Garbyal SS, Aggarwal KK, Babu C. Traditional phytomedicinal knowledge of *Bhotias* of Dharchula in Pithoragarh. Indian J Traditional Knowledge. 2005; 4(2): 199-207.
- [5] Winter C.A., Risley E.A. and Nuss G.W., Carrageenin-induced oedema in hind paw of the rat as an assay for anti-inflammatory drugs, Proc Soc Exp Biol Med. 111(02), 544 (1962).
- [6] Tazawa H., Okada F., Kobayashi T., Tada M., Mori Y. *et. al.*, Infiltration of neutrophils is required for acquisition of metastatic phenotype of benign murine fibrosarcoma cells: implication of inflammation-associated carcinogenesis and tumor progression, Am J Pathol. 163(6), 2221(2003).
- [7] Gabriel S.E., Jaakkimainen L. and Bombardier C., Risk for Serious Gastrointestinal Complications Related to Use of Nonsteroidal Anti-inflammatory Drugs: A Meta-analysis, Ann Intern Med. 115(10), 787 (1991).
- [8] Leme J.G., Hamamura L., Leite M.P. and Silva M.R., Pharmacological analysis of the acute inflammatory process induced in rat's paw by local injection of carrageenan and by heating, Brit J Pharma. 48, 88 (1973).
- [9] Andersson J., The inflammatory reflex-introduction, J Intern Med. 257(2), 122 (2005).