

The Journal of Phytopharmacology

(Pharmacognosy and phytomedicine Research)

Research Article

ISSN 2230-480X
JPHYTO 2013; 2(3): 39-43
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Evaluation of Carrageenan induced anti-inflammatory activity of ethanolic extract of bark of *Ficus virens* Linn. in swiss albino mice

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Abstract

The aim of the present study was to explore the probable anti-inflammatory activity of ethanolic extract of *Ficus virens* bark using Carrageenan induced inflammation in the mice. Swiss albino mice were treated orally with normal saline (as control group) and *Ficus virens* extract (200 and 400 mg/kg), 60 min before 0.1 mL 1% carrageenan injection. Paw volume was measured before and 1, 2, and 3 h after the injection of carrageenan. The results were expressed as the Mean \pm SEM and the statistical significance of differences between groups was analyzed by One Way Analysis of Variance (ANOVA) followed by Dunnett's test. The sub plantar injection of carrageenan caused a time-dependent paw edema in the mice. Oral administration of *Ficus virens* extract (200 and 400 mg/kg) inhibited paw swelling dose-dependently at 1, 2, and 3, h after Carrageenan injection. We can conclude from the outcome of the present work that *Ficus virens* extract exert an excellent anti-inflammatory effect in the mice.

Keywords: *Ficus virens*, Anti-inflammatory, Carrageenan, Ibuprofen, Ethanolic extract

Introduction

Medicinal plants constitute a source of raw materials for both traditional systems of medicine (e.g. Ayurvedic, Chinese, Unani, Homeopathy, and Siddha) and modern medicine. Nowadays, plant materials are employed throughout the industrialized and developing world as home remedies, over-the-counter drugs, and ingredients for the pharmaceutical industry. As such, they represent a substantial proportion of the global drug market. Most rural populations, especially in the developing world, depend on medicinal herbs as their main source of primary health care. Although most medicinal herbs are not, in their natural state, fit for administration, preparations suitable for administration are made according to pharmacopeia directions. The therapeutic potential of a herbal drugs depends on its form: whether parts of a plant, or simple extracts, or isolated active constituents. Herbal remedies consist of portions of plants or unpurified plant extracts containing several constituents, which often work together synergistically.¹

Inflammation is defined as the local response of living mammalian tissues to injury due to any agent. It is a body defence reaction in order to eliminate or limit the spread of injurious agent, followed by removal of the necroses cells and tissues.

Agents causing inflammation may be as under:

1. Infective agents like bacteria, viruses, and their toxins, fungi, parasites.
2. Immunological agents like cell mediated and antigen antibody reactions.
3. Physical agents like heat, cold, radiation, mechanical trauma.
4. Chemical agents like organic and inorganic poisons.
5. Inert materials such as foreign bodies.²

Inflammation is characterized in acute phase by increased blood flow and vascular permeability along with the

accumulation of fluid, leukocytes and inflammatory mediators such as cytokines. In the sub acute/chronic phase it is characterized by the development of specific humoral and cellular immune responses to pathogens present at the site of tissue injury.³

Ficus virens (Moreaceae), commonly known as Pakad tree (Figure 1) is distributed throughout India, Pakistan, China and other tropical countries.⁴ The bark contains Methyl ricinolate, Caffeic acid, Bergenin, β -sitosterol and lanosterol (Figure 2).⁵ The bark is reported to possess antibacterial and antifungal activities. The leaf extract shows the presence of glycosides and tannins from the phytochemical analysis.^{6, 7} Botanical classifications of *Ficus virens* is given in table 1.



Figure 1: Leaves and bark of *Ficus virens* plant

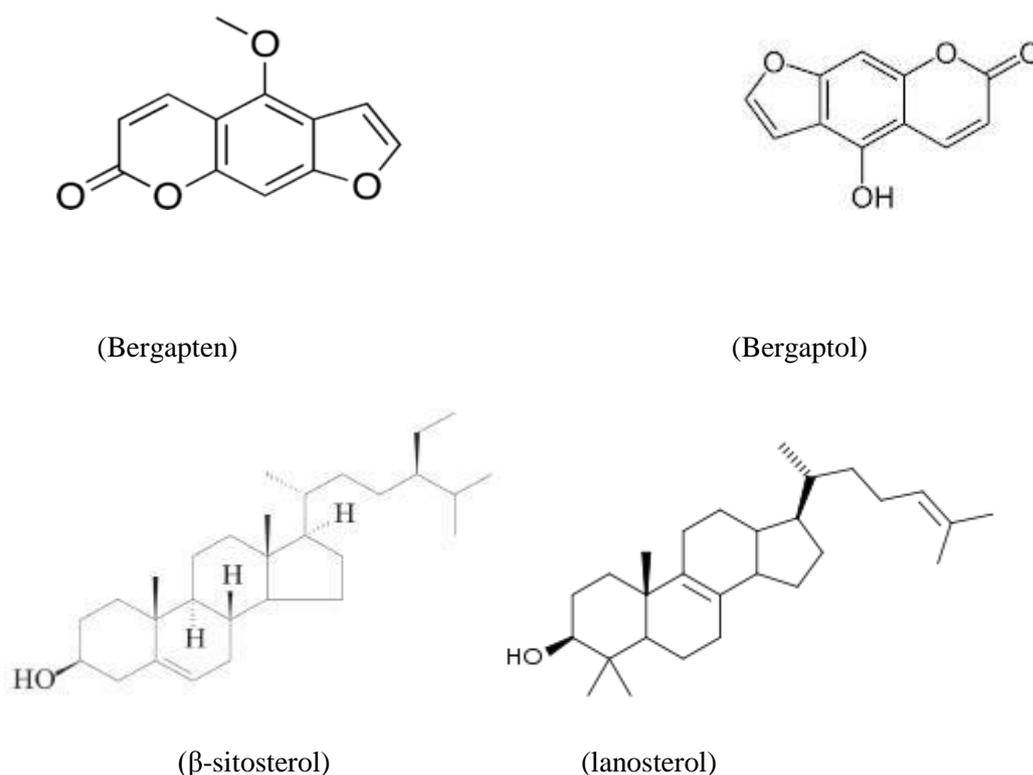


Figure 2: Structures of some phytoconstituents isolated from *Ficus virens* plant

Table 1: Botanical classification of *Ficus virens*

Kingdom	Plantae
Phylum	Tracheophyta
Class	Magnoliopsida
Order	Rosales
Family	Moraceae
Genus	<i>Ficus</i>
Species	<i>virens</i>

Material and methods

Plant material and Authentication

The fresh bark of *Ficus virens* plant was collected from Saharanpur district, Uttar Pradesh, India. The plant was authenticated and a specimen sample of the same was preserved in the herbarium section of the Doon College of

Pharmacy, Saharanpur. Initially bark were washed with fresh water to remove adhering dirt and foreign particles and dried at 35 - 400C in an oven. The dried bark was crushed and grinded to get powder and weighed. The weighed powder was then used for extraction procedure.

Preparation of Herbal Extract

The powdered drug was repeatedly extracted in a Soxhlet apparatus using ethanol solvents for 48 hours. The extract were collected and concentrated by evaporation and dried in vacuum and used for anti-inflammatory activity.

Chemicals

All the chemicals were of analytical grade and were either Sigma or Merk chemicals.

Experimental Animals

Swiss albino mice (25-30g) of either sex were used in the entire study. They were housed in standard polypropylene cages and kept under controlled room temperature (24 ± 2 °C; relative humidity 60 -70 %) in a 12 h light -dark cycle. The animals were fed with standard laboratory diet and water ad libitum. Food was withdrawing 12h before and during the experimental hours. The experimental protocol was approved by Institutional Animal Ethics Committee.

Screening of Anti-inflammatory Activity

Carrageenan induced paw edema in mice

The effect of oral administration of 200 and 400 mg/kg of the extract of *Ficus virens*, 40 mg/kg ibuprofen or vehicle (Saline, 10ml/kg) on the hind-paw oedema induced by sub plantar injection of 0.1ml Carrageenan (1% w/v) was

evaluated according to the method described by Winter et al., (1962).⁸ In short, 0.1 mL of 1 % w/v carrageenan was injected into the sub plantar tissue of left hind paw of each rat. Swelling of carrageenan injected foot was measured at 0, 1, 2, 3 h using Plethysmometer (UGO Basile, Italy). Animals were treated with test extract 1hour before the carrageenan injection. Measurement was carried out immediately before and 3hrs following carrageenan injection. Percent inhibition of test drugs was calculated in comparison with vehicle control (100%).

Statistical analysis

Results were analyzed using One way analysis of variance (ANOVA) and expressed as Mean \pm SEM. Data was further subjected to Dunnett's test and differences between means were regarded significant at $P < 0.01$ and $P < 0.05$.

Results

The anti-inflammatory activity of the extract *Ficus virens* bark against acute pedal oedema has been shown in Tables 1 and Table 2 which showed significant anti-inflammatory activity and the results were comparable to that of control. It was observed that the ethanolic extract of *Ficus virens* (400 mg/kg, p.o.) exhibits maximum anti-inflammatory activity against Carrageenan induced hind paw edema. The inhibition obtained with *Ficus virens* was 66.46 % (Table 2).

Table 2

Treatment (mg/kg)	Mean increase in paw volume (mL)				% Decrease in paw volume at 3 h
	0 h	1 h	2 h	3 h	
Control	0.94 \pm 0.01	1.53 \pm 0.007	1.89 \pm 0.004	2.49 \pm 0.007	-
IBH (40)	0.90 \pm 0.008	1.05 \pm 0.01*	1.30 \pm 0.002**	1.61 \pm 0.001*	52.31
EE (200)	0.96 \pm 0.037	1.25 \pm 0.035**	1.51 \pm 0.32**	1.71 \pm 0.049**	50.31
EE (400)	0.95 \pm 0.046	1.19 \pm 0.061**	1.32 \pm 0.037**	1.46 \pm 0.035**	66.46

N= 6, treatment, mg/kg, data were analyzed using ANOVA and expressed as Mean \pm SEM followed by Dunnett's test and differences between means were regarded significant at * [$P < 0.05$], ** $P < 0.01$ IBU – Ibuprofen, EE-Ethanolic extract

Discussion

In order to provide a scientific explanation for the folk use of *Ficus virens*, we have investigated the biological effects of its extracts, mainly the ones related to the inflammatory process. The present data clearly showed that extracts of dried bark *Ficus virens* have anti-inflammatory activity by the highly significant responses of some extracts on inhibiting the edema formation after carrageenan subplantar injection.

The extracts which showed the highest anti-inflammatory activity, presented also highly significant statistic values ($P < 0.01$) for carrageenan induced edema inhibition after the treatment with the phlogistic agent. The present study establishes the anti-inflammatory activity of extracts of *Ficus virens*. It is evident that carrageenan is a sulphated polysaccharide obtained from sea weed (Rhodophyceae) and is commonly used to induce acute inflammation and is believed to be bi-phasic.

The first phase is due to release of histamine and serotonin. The second phase is caused by the release of bradykinin, protease, prostaglandin and lysosome. Based on this, it would be argued that suppression of Ist phase may be due to inhibition of release of early mediators, such a histamine, serotonin and action in IInd phase may be explained by an inhibition of cyclo-oxygenase. These mediators take part in inflammatory response and are able to stimulate nociceptive and thus reduce pain. It has been reported that 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.⁹ Based on these reports, it can be inferred that the inhibition effect of the extract of *Ficus virens* on carrageenan induced inflammation in mice may be due to inhibition of the mediators responsible for inflammation.

Conclusion

From the above study it can be suggested that the bark extract of *Ficus virens* promising anti-inflammatory

activity. This effect may be beneficial for the management of pain.

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