

## Evaluation of anti-inflammatory activity of ethanolic extract of vitex negundo flowers in experimental animals

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### Abstract

**Introduction:** The objective of the present study was to evaluate the anti-inflammatory activity of ethanolic extract of vitex negundo flowers (EEVNF) in experimental animals.

**Methods:** Acute toxicity test of extract was done following Organization for Economic co-operation and development guidelines (OECD guidelines). Wistar albino rats of either sex weighing 150-250 grams were used for the study. Animals were randomly grouped into 5 groups consisting of 6 animals each. The in vivo acute anti-inflammatory activity was studied using the Carrageenan induced paw edema method. Normal saline was administered to the first group, diclofenac sodium (25mg/kg body weight{b.w} of rat ) was administered to the second group and doses of 100mg/kg, 200mg/kg and 400mg/kg b.w of rat of EEVNF were administered to the third, fourth and fifth groups respectively. All the drugs were given orally. 0.05ml of 1 % w/v carrageenan was used to induce paw edema. After 1 hr interval all the animals were given 0.05ml of 1 % w/v carrageenan in normal saline solution into the subplantar aspect of the left hind paw. Right hind paw served as the control for the same animal. The paw volume was measured by using digital plethysmometer by measuring fluid displacement at 1hr, 3hrs & 5hrs after Carrageenan injection.

**Results:** Test drug produced reduction in paw volume which was significant ( $p < 0.05$ ) at 3<sup>rd</sup> and 5<sup>th</sup> hr respectively at doses 100mg/kg, 200mg/kg and 400mg/kg b.w of rat when compared to diclofenac sodium.

**Conclusion:** EEVNF has significant anti-inflammatory activity.

**Keywords:** Anti-inflammatory, Ethanolic extract, Carrageenan, Vitex negundo flowers.

### Introduction

Inflammation is a physiological mechanism of the response of the organism to the injury. This mechanism is phylogenetically highly conserved among humans and many other animal species.<sup>(1)</sup>Inflammation has the essential primary purpose of restoring tissue homeostasis. The cardinal signs that identify the inflammation are rubor (redness), tumor (swelling), calor (heat), dolor (pain) and loss of function (functio laesa). These are intrinsically associated with events which include vasodilatation, edema and leukocyte trafficking into the site of inflammation. Inflammation itself can lead to further tissue damage and give rise to chronic inflammatory diseases and autoimmunity with eventual loss of organ function if uncontrolled or unresolved.<sup>(2)</sup> Inflammatory diseases are major cause of morbidity throughout the world. The drugs which are used to suppress inflammation like non-steroidal anti-inflammatory drugs and corticosteroids have adverse effects such as epigastric distress, peptic ulceration, osteoporosis, and iatrogenic Cushing's syndrome which have limited their use.<sup>(3,4)</sup> Due to adverse effects with the development of synthetic drugs, it is time that indigenous herbal plants should be looked for possible remedies. Since long time medicines which are derived from plant origin have been used to treat various disorders. So necessary efforts have to be made for introduction of new plants with medicinal value having beneficial effects with less adverse effects.<sup>(5)</sup> One

among them is Vitex negundo Linn which belongs to the family Verbenaceae.

Vitex negundo Linn is a large aromatic, evergreen to semi evergreen shrub or small tree. It is commonly called five leaved chaste tree. The description of leaves is that the leaves are opposite 3 to 5 foliate, leaflets are lanceolate, entire or crenate, glabrate, above which are dark green, beneath pale greenish tomentose and central leaflets are larger. Flowers are described as small, bluish purple in lateral cymes, forming an elongated terminal thyrus. Fruits are described as drupaceous and black when ripe and seeds are described as obovate or oblong. It is generally found at warmer zones throughout greater part of India and in outer Western Himalayas ascending to an altitude of 15,00metres.<sup>(6)</sup> It is commonly found in deciduous forests, river banks, villages and cultivated as hedge plant. Various phytochemical metabolites are present in the plant which make this plant useful in medical conditions.<sup>(7,8)</sup> Various activities have been evaluated in this plant along with anti-inflammatory activity.<sup>(9-15)</sup> There were previous studies done on other parts of the plant except flowers to evaluate anti-inflammatory activity. Therefore the present study was done to evaluate the anti-inflammatory activity of ethanolic extract of flowers of vitex negundo.

## Materials and Methods

### Plant material

The plant material was supplied by AGHP Enterprises, Adyar, Chennai.

### Drugs and chemicals

Carrageenan, Diclofenac sodium and normal saline were used.

### Instruments

Beaker, Digital weighing balance, Digital plethysmometer, Feeding tube, Glass jar, Glass rod, Hot air oven, Ryle's tube, Soxhlet apparatus, Stop watch and Tuberculin syringe were used.

### Preparation of Plant extract

The extract was made using Soxhlet apparatus and the solvent used was ethanol and later dried in hot air oven. 10grams of crude extract was yielded from 50 grams of flower powder of vitex negundo. The formed residues were preserved<sup>(16)</sup> and fresh preparations were used whenever required.

### Experimental animals

- Animals: Wistar albino rats
- Number of animals used =30
- Animals were obtained from Animal House, S.Nijalingappa Medical College, Bagalkot.

### Exclusion criteria:

- Pregnant rats
- Rats with infection
- Rats with injuries and deformities

### Housing and feeding conditions

Standard animal house conditions were used to maintain animals. Standard laboratory diet with adequate water and feed were given to the animals. These housing and feeding conditions were maintained before and during the study as suggested by Committee for the Purpose of Control and Supervision of experiments on Animals.

Approval was given by Institutional Animal Ethics Committee for starting the experiment.

### Phytochemical screening

Phytochemical screening was done for detection of various constituents.<sup>(17)</sup>

### Acute toxicity study

OECD (Organization for Economic co-operation and development) 423 guidelines were used for acute toxicity studies.<sup>(18)</sup>

- Dosing pattern:** Animals were treated with extract doses 5mg/kg, 50mg/kg, 300mg/kg and 2000mg/kg b.w of rat.
- Observation:** Animals were observed for 14 days for any abnormal or toxic manifestations and mortality.

### Anti-inflammatory activity evaluation by Carrageenan induced paw edema method

30 albino wistar rats were randomly divided into 5 groups which consisted of 6 rats each after 12hrs of fasting. Normal saline, diclofenac sodium and test drug with different doses of 100mg/kg, 200mg/kg and 400 mg/kg body weight of rat were given to the groups. After 1 hour(hr) of oral administration of drugs all the animals were given an injection of 0.05ml Carrageenan(1% carrageenan suspended in 0.9% NaCl into the subplantar aspect of the left hind paw. In the same animal right paw served as control. 0.05ml of 1 % w/v carrageenan was used to induce paw edema. The paw volume was measured by using digital plethysmometer by measuring fluid displacement at 1hr, 3hrs & 5hrs after Carrageenan injection.<sup>(19)</sup>

Percentage inhibition of edema was calculated by following formula:

$$\% \text{inhibition of edema} = \frac{\text{Mean edema in untreated control group} - \text{Mean edema in drug treated group}}{\text{Mean edema in control group}} \times 100$$

### Statistical Analysis

- Tests used:** One way analysis of variance and Dunnett's multiple comparison tests were used.
- Expression of values:** Values were expressed as Mean  $\pm$  Standard Error of Mean
- Statistical significance:** p value <(less than) 0.05.

## Results and Discussion

### Results

#### Phytochemical screening

Alkaloids, flavonoids, phenolic compounds, proteins, sterols, tannins and terpenoids were the phytoconstituents present.

#### Toxicity study results

At dose of 2g/kg of extract no adverse effect or mortality was detected in animals

- Health condition of animals:** All the animals were alive, healthy and active during test study period.
- LD 50 value:** was considered as > 2g/kg.

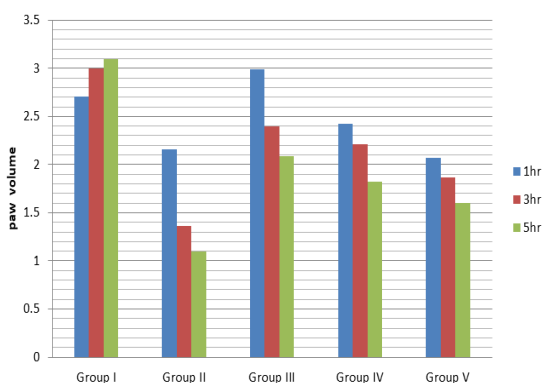
#### Carrageenan induced paw edema method

Test drug EEVNF (ethanolic extract of vitex negundo flowers) in different doses 100mg/kg, 200mg/kg and 400mg/kg produced significant reduction in paw volume ( $p < 0.05$ ) at 3<sup>rd</sup> (2.40 $\pm$ 0.11, 2.21 $\pm$ 0.16, 1.87 $\pm$ 0.04) and 5<sup>th</sup> hr (2.09 $\pm$ 0.08, 1.82 $\pm$ 0.12, 1.60 $\pm$ 0.05) respectively when compared to diclofenac sodium (1.36 $\pm$  0.04, 1.10 $\pm$  0.06, 100mg/kg, 200mg/kg and 400mg/kg b.w doses of ethanolic extract of vitex negundo flowers showed percentage inhibition of edema i.e 32.58%, 41.29% & 48.38% respectively whereas diclofenac sodium showed 64.51% shown in Table 1 and Fig. 1

**Table 1: Carrageenan induced paw edema method showing paw volume in ml**

Groups	1 hr (M ± SEM)	3 hr (M ± SEM)	5 hr (M ± SEM)	Percentage inhibition (%) at 5hrs
<b>Group I</b> (Control)	2.71±0.26	3.00±0.24	3.10 ± 0.20	-
<b>Group II</b> (Diclofenac sodium 25mg/kg)	2.16 ± 0.66	1.36±0.04	1.10±0.06	64.51
<b>Group III</b> (EEVNF 100mg/kg)	2.99 ± 0.22	2.40±0.11*	2.09 ± 0.08*	32.58
<b>Group IV</b> (EEVNF 200mg/kg)	2.42 ± 0.16	2.21±0.16*	1.82±0.12*	41.29
<b>Group V</b> (EEVNF 400mg/kg)	2.07 ± 0.86	1.87±0.04*	1.60±0.05*	48.38

\*p<0.05, All values expressed as Mean ± Standard error of mean (M ± SEM)

**Fig. 1: Graph showing paw volume (ml) in carrageenan induced paw edema method**

## Discussion

Inflammation is the response of living tissue to injury which involves activation of various enzyme, mediators release, cell migration, tissue breakdown and repair. In inflammatory conditions the drugs which are used like non-steroidal anti-inflammatory drugs, steroids, and immunosuppressant drugs are associated with adverse effects like ulceration, perforation, gastric irritation, angioedema, headache, haematochezia, hepatic failure, hemolytic anemia, hyperglycemia, osteoporosis, immunodeficiency-related problems, and others. In view of adverse effects of these drugs medicinal products derived from plant sources that are generally considered safe are being explored for their anti-inflammatory potential. One of the plant from family Verbenaceae i.e. *Vitex negundo* Linn is one amongst them.

Studies were done on other parts of the plant like roots for evaluation of anti-inflammatory activity which showed significant anti-inflammatory activity.<sup>(20)</sup> The present study is the first report regarding anti-inflammatory activity of flowers of *Vitex negundo*. The experimental model which is used as the standard in vivo model for acute inflammation is carrageenan induced paw edema method. Evaluation of anti-inflammatory activity of drugs is done by this method. Carrageenan is used as the phlogistic agent for testing anti-inflammatory drugs as it is not known to be antigenic and is devoid of apparent systemic effects. High degree of reproducibility is exhibited by this experimental model.<sup>(21,22)</sup> Biphasic response is a characteristic of this method wherein hyperemia the earliest feature is due to release of histamine and serotonin and edema which is the late feature is due to release of bradykinin and prostaglandin.<sup>(23)</sup>

In this study at 3<sup>rd</sup> and 5<sup>th</sup> hour significant reduction in paw volume (p<0.05) was found with three doses of EEVNF when compared to diclofenac sodium. Alkaloids, flavonoids, phenolic compounds, proteins, sterols, tannins and terpenoids were found on phytochemical analysis of EEVNF.

Studies suggest that prostaglandin biosynthesis is inhibited by flavonoids.<sup>(24)</sup> There are few studies which have shown tannins play role in anti-inflammatory activity.<sup>(25)</sup> So in the present study, presence of phytochemical constituents like tannins and flavonoids could be attributed to the anti-inflammatory activity of extract.

## Conclusion

In our study in experimental animals we found that EEVNF possess significant anti-inflammatory activity. *Vitex negundo* Linn can be recommended for further studies for isolation of active ingredients.

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**Ethical approval:** Institutional Animal Ethics Committee approved the study

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