WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.045
Volume 4, Issue 3, 902-908. Research Article ISSN 2277– 7105

ANTI-INFLAMMATORY ACTIVITY OF ETHANOLIC EXTRACT OF *VITEX NEGUNDO LINN.* AND *VITEX TRIFOLIA LINN.* LEAVES ON EXPERIMENTAL ANIMAL

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Article Received on
24 Dec 2014,

Revised on 18 Jan 2015,
Accepted on 12 Feb 2015

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ABSTRACT

Inflammation is a part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells or irritants. It is characterized by redness, swollen joints, joint pain, its stiffness and loss of joint function. The present study was conducted to comparative anti-inflammatory study of ethanolic extract of *Vitex Negundo Linn., Vitex Trifolia Linn.* leaves and diclofenac sodium in Carragenan-induced paw edema model Albino Wistar rats. A significant inhibition of carragenan induced rat paw edema comparable to that produced by Diclofenac sodium, the standard anti inflammatory drug. Test extracts was obtained by shoxelation method of extraction of both of the plant leaves with ethanol, tested in the present study. The results from present study indicate the efficacy of the ethanolic extract of *Vitex Negundo Linn., Vitex Trifolia Linn.* as a therapeutic agent in inflammatory conditions. Thus it could be concluded that *Vitex Negundo Linn. and Vitex Trifolia Linn.* leaves extracts possess significant anti-inflammatory properties. A better effect was observed with *Vitex Negundo Linn.* because it effectively reduce the paw edema in Carragenan-induced paw edema model Albino Wistar rats.

KEYWORDS: Anti inflammatory activity, *Vitex Negundo Linn., Vitex Trifolia Linn.*, Carragenan-induced paw edema, Diclofenac sodium.

INTRODUCTION

Inflammation is defined as the local response of living mammalian tissues to injury due to any agent. Such as infection, injury, and exposure to contaminants, such as bacteria, trauma chemicals and heat and is triggered by innate immune receptors that recognize pathogens and damaged cells. It is characterized by pain, swelling and redness. These characteristic features are brought about by complex actions of various inflammogens like Histamines, Bradykinins, Prostaglandins, and Leukotrines etc. By inhibiting these mediators, the inflammatory response could be suppressed.[1,2,3]

In vertebrates, the inflammatory cascade is a complex network of immunological, physiological, and behavioral events that are coordinated by cytokines, immune signaling molecules. Although the molecular basis of inflammation is well studied, its role in mediating the outcome of host-parasite interactions has received minimal attention by ecologists. Inflammation involves two basic processes early inflammatory response later followed by healing. There are two types of inflammation, Acute and Chronic. Acute inflammation is of short duration and represents the early body reaction, resolves quickly and is usually followed by healing. Chronic inflammation is defined as prolonged process in which tissue destruction and inflammation occur at same time.[4]

Inflammation is a part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells or irritants. It is characterized by redness, swollen joints, joint pain, its stiffness and loss of joint function. Inflammation is currently treated by NSAIDs. Unfortunately these drugs cause increased risk of blood clot resulting in heart attacks and strokes. Therefore, the developments of potent anti-inflammatory drugs from the natural products are now under considerations. Natural products are rich source for discovery of new drugs because of their chemical diversity. A natural product from medicinal plants plays a major role to cure many diseases associated with inflammation. The conventional drug available in the market to treat inflammation produces various side-effects. Due to these side-effects, there is need for the search of newer drugs with less or no side-effects. There are hundreds of phytoconstituents reported to have many pharmacological activities.[5]

The anti-inflammatory activity of new substances can be evaluated by using various pre clinical screening method. Here by use of various phlogestic agent like carragenan, brewer's yeast, dextran, egg albumin, kaolin, aerosil, croton oil, and cotton wool inflammation is induced and the amount of decrease in its inducing characteristics is measured.

Vitex Negundo Linn. is also called as Nirgundi. It is a large shrub or sometimes a small slender tree with quadrangular branchlets found in most of the hotter parts of India and up to an altitude of 3000 ft in the north-west Himalayas. It also occurs in Ceylon, Afghanistan, tropical Africa, Madagascar, China and Philippines. The leaf and root of the plant are sold as commercial drugs in India. The leaves are used as hot poultice to inflammatory swellings of joints in acute rheumatism and suppressed gonorrhoea.[6]

The plant *Vitex Trifolia Linn.* is well known in Hindi as 'Pani-ki-Sanbhalu', 'Sufed-Sanbhalu'. It is stout aromatic shrub or a small tree, found from the foot of Himalayas southwards throughout greater part of India,western ghat and in Andamans. Vitex trifolia Linn. is a shrub or small tree growing from 1 to 4 meters in height, etimes prostate or ascending in habit. The leaves are simple or 3-foliolate. *Vitex. Negundo Linn.* closely resembles *Vitex. Trifolia Linn.* but can be distinguished by its long-petioluled median leaflet and 3-5 leaflets.Agroforestry Database 4.0.[7,8]

MATERIALS AND METHOD

Plant Material: The both plants *Vitex Negundo Linn. and Vitex Trifolia Linn.* were collected during the month of May- June from the Indore, District of M.P. The leaf of *Vitex Negundo Linn.* and *Vitex Trifolia Linn.* were authenticated by Dr. Jitendra Singh Pachaya (Asst. Professor Botany) Govt. PG College Alirajpur (M.P.) (Ref. no. 869).

Preparation of Extract: Locally collected plant leaf of *Vitex Negundo Linn. and Vitex Trifolia Linn.* both were shade-dried and then powdered and passed through the sieve (coarse 10/40). Powdered leaves extracted with ethanol (90%) in soxhlet extractor at 40^0C to 50^0C temperature. The extraction was continued for 12 cycles or until the solvent in the thimble was clears. The extracts freed of the solvent under reduced pressure yielding green semi-solid mass. These extracts dissolved or suspended in distilled water, its pH brought to 7.0 and used for the anti-inflammatory activity studies. Extract obtained was stored under refrigerating condition.

Experimental Animals: Wistar albino rats of both sexes weighing between 150-200 gm were obtained from the animal house of ShriRam College of Pharmacy, Gwalior (M.P.), India .The animals were housed in polypropylene cages at 24±2°C and fed with commercial pellet diet and water ad libitum. All the animal experiments were carried out in accordance with the guidelines of Committee for the Purpose of Care and Supervision on Experimental

Animals (CPCSEA) and the study was approved by the Institutional Animal Ethics Committee (IAEC) (Reg.No.891/AC/05/CPCSEA). The animals were fasted prior to dosing by withholding food overnight. Fasted body weight of rats was determined and the dose was calculated according to their body weight.

Experiment Design and Drug Treatment: The rats were divided into four groups of 5 rats each. Group I received 0.9% NaCl and served as control. Group II received Diaclofinac sodium (100 mg/kg) is standard group. Group III, was administered the test drug *Vitex Negundo Linn.* leaf extract (400 mg/kg/orally) one hour before experiment in single dose in carrageenin induced rat paw oedema method. Whereas group IV was given of *Vitex Trifolia Linn.* leaf extract (400 mg/kg/orally) in the above doses in respective anti-inflammatory models.

Carrageenan-Induced Paw Edema in Rats: The rats were divided into 4 groups each containing 5 animals. Group 1 served as Control, Group 2 Standard drug Diclofenac (100 mg/kg) treated, Group 3 trated with ethanolic extract of *Vitex Negundo Linn.* (400mg/kg), and 4 served as treatment with the ethanolic extract of *Vitex Trifolia Linn.* (400mg/kg). Acute inflammation in rats was induced by injecting Carragenan (0.1 ml of 1% suspension in 0.9% saline) in subplantar region of left paw and right paw remained untreated. Than the paw volume was measured at 0, 60, 120, 240 min. with Plethysmometer. A mark was put on the leg at the mallelous region to facilitate the dipping of the leg to the same level. The mean paw volume at different time interval was measured and compared to control and the percentage inhibition was then calculated by following formulas:

% Inhibition = (1- Ct/Co) X 100

Where, Co= edema volume in Control group,

Ct= edema volume in Drug treated group.[9, 10]

RESULT AND DISCUSSION

The results of anti-inflammatory activity of leaves extracts of *Vitex Negundo Linn., Vitex trifolia Linn.* and standard drug Diclofenac sodium (100mg/kg) against carrageenan induced paw edema in rats is shown in **Table 1.** Paw volume was reduced in all treated groups as compared to control group. Ethanolic extract of *Vitex Negundo Linn.* at dose of 400 mg/kg showed more inhibition of edema before 60 min. and ethanolic extract of *Vitex trifolia Linn.* at dose 400 mg/kg showed inhibition of edema after 60 min. Ethanolic extract of *Vitex*

Negundo Linn. at dose of 400 mg/kg showed more inhibition of edema than other treated groups.

At 60 min. Test-I, Test-II and standard drug both cause slightly reduction in paw volume. Value of paw volume reduction was found to be greater in case of Diclofenac sodium treated rats in comparison to test-I, II treated rats at 60 min. It might be due to delay in release of phytoconstituents in case of test treated animals. After 120 min. paw volume reduction was found to be higher as compared to standard treated in case of test-I. At the 180 min. the paw volume reduction of Test-II was higher than standard treated animals. Treated rats after complete release of phytoconstituents from extracts. So the present study shown that the *Vitex Negundo Linn.* (400 mg/kg) have more significant anti-inflammatory activity as compaire to *Vitex Trifolia Linn.* (400mg/kg).

The % of inhibition of inflammation different groups on carragenan-induced paw edema in rats is shown in **Table 2.** The results obtained indicate that the ethanolic leaves extract of *Vitex Negundo Linn.* has significant anti-inflammatory activity in rats. The Ethanolic leaves extract of *Vitex Trifolia Linn.* also reduces the paw edema induce by carragenan but less effective than *Vitex Negundo Linn.*

The standard drug Diclofenac sodium at the dose of 100mg/kg highest % inhibition of inflammation found to be 62.8%. Whereas the Test-I *Vitex Negundo Linn.* extract at dose of 400mg/kg % inhibition of inflammation found to be 66%. and Test-II *Vitex Trifolia Linn.* Extract at same dose shown highest inhibition 62.8%.

Table-1: Paw Volumes of Rats of All Groups at Different Time Interval

GROUP	PAW VOLUME (ml) AT DIFFERENT TIME INTERVAL				
	0 min.	**60 min.**	**120 min.**	**180 min.**	**240 min.**
Control	0.41±0.05	0.50±0.07	0.64±0.05	0.84±0.05	0.94±0.05
Standard	0.42±0.05	0.40±0.00	0.38±0,05	0.38±0.05	0.35±0.04
Test-I	0.42±0.07	0.39±0.05	0.38±0.08	0.36±0.05	0.32±0.05
Test-II	0.41±0.05	0.40±0.44	0.39±0.05	0.35±0.05	0.35±0.04

Data analysis: Data are expressed as MEAN ± SEM

Table-2: % Inhibition of Inflammation in all Groups at Different Time Interval

GROUPS	% INHIBITION OF INFLAMMATION AT DIFFERENT TIME INTERVAL			
	60 min.	120 min.	180 min.	240 min.
Control	-	-	-	-
Standard	20%	40.7%	54.8%	62.8%
Test-I	22	40.7%	57.2%	66%
Test-II	20%	39.1%	58.4%	62.8%

CONCLUSION

In the present study, we observed that *Vitex Negundo Linn*. and *Vitex Tifolia Linn*. leaves extracts were significantly reduced paw edema. Ethanolic extract of *Vitex Negundo Linn*. leaves being more prominent than *Vitex Tifolia Linn*. leaves extracts. This suggests that the extracts modify the effects of prostaglandins which are released into third phase of inflammation. Diclofenac sodium (100 mg/kg) was used as a reference standard. The drug was one of the most extensively used NSAID's which acts by inhibiting prostaglandin synthesis.

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