ANTI-INFLAMMATORY ACTIVITY OF *VITEX NEGUNDO* ROOT EXTRACT

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Summary

The present study is aimed to evaluate the anti-inflammatory activity of ethanolic extract of roots of *Vitex negundo* at low dose level (50 mg/kg b.w) and high dose level (500 mg/kg b.w). The anti-inflammatory activity was evaluated by carrageenan induced rat paw oedema method for acute inflammation and cotton pellet granuloma method for chronic inflammation. The standard drug used was Indomethacin (10 mg/kg b.w) in both the models. The result obtained showed that the ethanolic extract at a dose level of 500 mg/kg p.o. exhibited remarkable anti-inflammatory activity in both models, comparable to the standard reference drug Indomethacin.

Key words: *Vitex negundo*, Anti-Inflammatory Activity, Carrageenan, Albino rats

Introduction

Inflammation is a local response of living mammalian tissues to injury. It is a body defense reaction in order to eliminate or limit the spread of injurious agent. As such, inflammation is also intimately interwoven with repair process. There are various components to an inflammatory reaction that can contribute to the associate symptoms and tissue injury. Oedema formation, leukocyte infiltration and granuloma formation represent such components of inflammation [1]. Oedema formation in the paw is the result of a synergism between various inflammatory mediators that increase vascular permeability and /or the mediators that increase blood flow [2].

Inflammatory diseases including different types of rheumatic diseases are a major cause of morbidity of working force throughout world. This has been called the "King of Human Miseries" [3]. A systemic study of anti-inflammatory effects of Indian medicinal plants began by Gujral and his associates in 1956 and they screened a number of plants for their anti-arthritic effects. Gujral and Saxena (1956), Karandikar et.al (1960) and others mainly used formaldehyde induced arthritis (Brownlee, 1950) and croton oil induced granuloma pouch in rats (Selya, 1958), as the experimental models of inflammation. Later, with the introduction of better and more specific models of experimental inflammation like carrageenan induced paw oedema in rats (Winter et. al. 1950), cotton pellet induced granuloma in rats (Winter et. al. 1958), Freund's complete adjuvant induced arthritis (Newbould, 1968) etc., worked in different laboratories tested their drugs with the help of the later models [4].
A large numbers of Indian medicinal plants are attributed with various pharmacological activities because they contain a diversified class of phytochemicals. It is believed that current analgesia-inducing drugs such as opioids and non-steroidal anti-inflammatory drugs are not useful in all cases, because of their side-effects and potency [5].As a result, a search for other alternatives seems necessary and beneficial.

Traditional and folklore medicines play an important role in health services around the globe. Ayurveda, the traditional medicinal system in India, describes certain plants which strengthen the host immune system.

Vitex negundo (verbenaceae), popularly known as "Nirgundi"in Hindi and "Five leaved chaste tree" in English is widely distributed throughout in India. Almost all parts of plant are used in the Ayurvedic and Unani system of medicines [6, 7]. *Vitex negundo* (verbenaceae), is used for dispelling inflammatory swelling of joints from acute rheumatism, healing wounds, ulcers and different bacterial infections. Hence it was thought worthwhile to investigate the anti-inflammatory activity of ethanolic extract of roots of *Vitex negundo.*

Material and Methods

Plant material: The roots of *Vitex negundo* were collected from Ganeshpur village, Saharanpur (UP -Uttaranchal border) and identified by Dr. Anjula pandey, Taxnomist, National Bureau of Plant Genetic Resources (NBPGR), Pusa campus, New Delhi. A voucher specimen **(HS-19710)** is preserved in the herbarium section of taxonomic department of NBPGR, New Delhi.

Plant extract: The air dried roots (2.5 kg) were coarsely powdered and then about 2.5 kg materials were defatted with petroleum ether (60-80°C) and then extracted with alcohol (95%). The extract was dried under vacuum (yield 12.6%).The ethanolic extract was screened for the anti inflammatory activity.

Preliminary Phytochemical Studies: The ethanolic extract was then subjected to qualitative phytochemical screening for the identification of different phytoconstituents. The ethanolic extract showed the presence of alkaloids, carbohydrates, glycosides, phenolic compounds, saponins and sterols.

Preparation of suspension of extract: Dried ethanolic extract was suspended in a solution of normal saline (0.9% w/v) and Tween 20 (95:5) and subjected for anti inflammatory activity.

Animals: Wistar strains of albino rats (180 to 230 gm) of either sex, maintained under standard animal housing conditions, were used for all sets of experiments performed on 6 rats in each group. The rats were allowed to take the standard laboratory feed with water *ad libitum.* The study was performed according to the protocol approved by the Institutional Ethical Committee and allotted the **CPCSEA no – 385.**

Screening of Anti-inflammatory activity:

1. Carrageenan-induced paw oedema method: Carrageenan induced paw edema is simplest and most widely used model for studying the anti inflammatory activity [8,9,10]. The acute hind paw oedema was produced by injecting 0.1 ml of freshly prepared 1% (w/v) carrageenan solution in normal saline locally in to the plantar region of the left hind paw of rats of each group. Ethanolic extract of roots [50 mg/kg and 500 mg/kg, p.o., [11]. suspended in 0.9% w/v normal saline and Tween 20 (95:5)] were administered orally to two different groups while the two other groups received standard drug Indomethacin (10 mg/kg, p.o.) [12] and normal saline. The extract with two dose levels, Indomethacin and normal saline were administered orally 1 hr. prior to the injection of carrageenan. The rat paw volume up to the ankle joint was measured using Plethysmometer at the interval of 1 hr, 2 hr, 3 hr, 4 hr and 5 hr after injection of carrageenan.
% Inhibition of oedema volume between treated (Indomethacin and test samples) and control as per the given formula-

% Inhibition = Vc-Vt / Vc × 100

Where Vc and Vt represent mean increase in paw volume in control and treated groups respectively.

2. Cotton pellet granuloma pouch method: Cotton pellet granuloma was induced according to the method of D' Arcy et.al. [13, 14]. Sterilized cotton pellets each weighing 10 mg were implanted in both axilla and groin of each rat under light ether anaesthesia. Twenty four rats were divided into four groups as shown in table for various treatments for five days. The test group received Indomethacin (10 mg/kg). Subsequently, on the sixth day all pellets were dissected out under ether anesthesia and weighed. Then dried at 70 °C for 6 hours and weight of each granuloma was determined.

Statistical analysis: Significance of the results of biochemical estimations were calculated by ANOVA followed by Tukey's test by using sigma stat software. The experimental results were expressed as mean ± SEM.

Results

The results obtained from various parameters are summarized in the tables given below.

Figure No. 1:

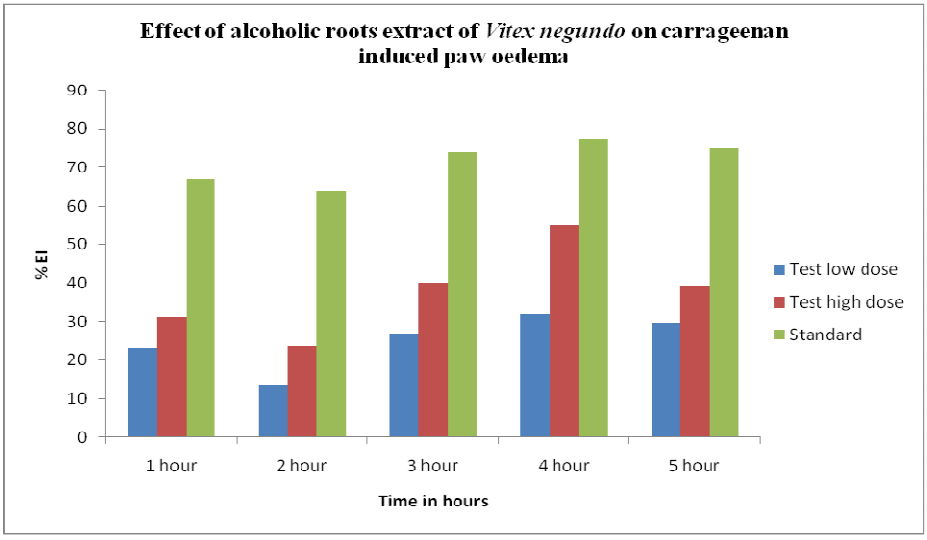

Table 1: Effect of Ethanolic extract of *Vitex negundo* on carrageenan induced rat paw oedema

S. No.	Groups (mg/kg po.)	Paw oedema									
		1 hr		2hr		3hr		4hr		5hr	
		Mean ± SEM	% EI	Mean± SEM	% EI	Mean± SEM	% EI	Mean± SEM	% EI	Mean± SEM	% EI
1.	Control	0.475 ± 0.025	-	0.686 ± 0.017	-	0.810 ± 0.018	-	0.728 ± 0.005	-	0.686 ± 0.004	-
2.	Indomethacin (10)	0.160 ± 0.007*	66.94	0.238 ± 0.004*	63.70	0.195 ± 0.005*	74.00	0.163 ± 0.008*	77.60	0.173 ± 0.008*	74.78
3.	Ethanolic extract (50)	0.365 ± 0.017*	23.16	0.592 ± 0.018	13.70	0.591 ± 0.011*	27.00	0.495 ± 0.009*	32.00	0.482 ± 0.007*	29.70
4.	Ethanolic extract (500)	0.326 ± 0.012*	31.37	0.523 ± 0.021*	23.76	0.487 ± 0.005*	39.87	0.327 ± 0.013*	55.00	0.417 ± 0.008*	39.20

Values are expressed as mean ± SEM. (n=6)
*p < 0.05 when compared with control group.

Table 2: Effect of Ethanolic extract of *Vitex negundo* on cotton pellet granuloma pouch in rats

Treatment dose (mg/kg po.)	Granuloma wet wt. (gm)	Granuloma dry wt. (gm)
Control	0.15±0.004	0.05±0.002
Indomethacin (10)	0.09±0.003**	0.02±0.002**
Ethanolic extract (50)	0.12±0.003**	0.03±0.003**
Ethanolic extract (500)	0.10±0.002**	0.02±0.002**

Values are expressed as mean ± SEM. (n=6)
*p < 0.05, **p<0.001 when compared with control group.

Discussion

The present study shows that ethanolic extract of roots of *Vitex negundo* possesses anti-inflammatory activity on carrageenan induced oedema in rat paw. The activity profile of extract closely resembled to that of Indomethacin.

Carrageenan induced paw oedema was taken as a prototype of exudative phase of acute inflammation. Inflammatory stimuli microbes, chemicals and necrosed cells activate the different mediator systems through a common trigger mechanism. The development of carrageenan-induced oedema is believed to be biphasic. The early phase is attributed to the release of histamaine and serotonin [15, 16, 17].and the delayed phase is sustained by the leukotrienes and prostaglandins [18].

For evaluation of anti-inflammatory activity of ethanolic extract of roots of *Vitex negundo,* doses were selected at two dose levels as low dose (50 mg/kg) and high dose (500 mg/kg) with using Indomethacin (10 mg/kg) as a standard anti-inflammatory agent.

The high dose (500 mg/kg) of ethanolic extract showed 55% oedema inhibition which is comparable with 77.60% of standard Indomethcin after 4 hours of carrageenan injection and attained statistically significant value compared with control group whereas the low dose (50 mg/kg) showed 32.0% oedema inhibition by using the same standard drug (77.60%).

In order to assess its efficacy against proliferative phase of inflammation, we have selected cotton pellet granuloma animal model in which tissue degeneration and fibrosis occurs. During the repair process of inflammation, there is proliferation of macrophases, neutrophils, fibroblasts and multiplication of small blood vessels, which are the basic sourse of forming a highly vascularised reddish mass, termed granulation tissue [19, 20]. The herbal product treatment (50 and 500 mg/kg, p.o.) moderately inhibited the granuloma formation. The effect of herbal product was lower as compared to Indomethacin.

The results of our present study clearly demonstrate the strong anti-inflammatory activity of ethanolic extract of roots of *Vitex negundo*. However, further detailed investigation is underway to determine the exact phytoconstituents that are responsible for this activity.

Conclusion

From the above results, it can be deduced that ethanolic extract has shown comparable activity to that of standard drug. Phytochemical screening has shown the presence of alkaloids, carbohydrates, glycosides, phenolic compounds, saponins and sterols in ethanolic extract. The potent activity may be attributed to the presence of these phytoconstituents. More detailed studies are, however, necessary to identify the active principle(s) and exact mechanism of action.

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