

Preliminary Phytochemical Screening and Evaluation of Anti-Inflammatory Potential of *Vitex leucoxylo* Linn.

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Abstract

The anti-inflammatory activity of ethanolic and aqueous extract of the bark of *Vitex leucoxylo* Linn. was studied in albino wistar rats using the Carageenan induced rat paw edema model. The ethanolic and aqueous extract of *Vitex leucoxylo* (500 mg/kg p.o.) inhibited Carageenan induced rat paw edema. The extract was also studied for its preliminary phytochemical screening and acute toxicity studies. The result indicated that both the extract produced significant ($P < 0.001$) anti-inflammatory activity when compared with the standard drug Indomethacin (10 mg/kg p.o.) and untreated control. Phyto-chemical screening showed the presence of various constituent like phenolic compounds, phytosterol, protein, flavonoids, carbohydrate etc.

Key Words: Anti-inflammatory, *Vitex-leucoxylo*, Carageenan.

Introduction

Vitex leucoxylo Linn. (Verbenaceae) commonly known as Songarphi (Marathi) an excellent herbal crude drug in the nature which has composition of the various essential constituent that are required for normal and good health of human. It is small to large tree with a short thick trunk and a spreading crown and almost throughout the Deccan peninsula of India upto an altitude 900 mt, it extends northwards upto Jhansi and part of Bihar.^(1,2) The trees are generally found on the river bank, stream & ponds. The bark is used as astringent whereas the roots are febrifuge and astringent. The leaves are smoked for relieving headache and catarrh and also used for medicinal baths in fever and anemia.^(3,4)

The purpose of the present study was to evaluate the anti-inflammatory potential of *Vitex leucoxylo* bark extract using carageenan induced rat paw model. The extract was also studied for its acute toxicity effects and preliminary phytochemical screening.

Material and Methods

Plant Material

The bark of *Vitex leucoxylo* Linn. was collected from the bank of pond known as Chennapur (Canara circle, Sirsi division) in Bachagaon village, Karnataka- India and were authenticated by Prof. B.D.Huddar, Head of the Botany Department, HSK College of Arts and Science, Hubli, Karnataka, India where a voucher specimen is deposited for further reference.

Chemicals

Carageenan and Indomethacin was obtained from Sigma-Aldrich Germany. All the solvents were of analytical grade produce from SD fine chemicals Mumbai.

Preparation of Extracts

For preliminary phytochemical screening alcoholic extract, aqueous extract and successive extract (pet ether, ethyl acetate, alcoholic and aqueous extract) of bark were taken.

For the preparation of ethanolic extract of the bark of *Vitex leucoxylo* (EEVL) was collected, shade dried at room temperature, pulverized and extracted with ethanol in a soxhlet extractor for about 80 cycles. The extract was concentrated in a rotary flash evaporator. The extract was divided into two portions one was taken for screening^(5,6) and rest was suspended

in Tween-80 for anti-inflammatory activity. Similarly the aqueous extract (AEVL) was prepared but water selected for extraction instead of alcohol. Here again the extract was divided into two parts one was taken for screening and rest for anti-inflammatory activity. For the preparation of successive extract the dried drug was successively extracted from petroleum ether, ethyl acetate, alcohol and in last with water. The solvents were evaporated in a rotary flash evaporator and the dried residues were taken for screening.

Animals

Albino rats of wistar strain of the either sex (150-200gm) maintained under standard environmental conditions (27±2° C light / dark cycle of 12 hrs) and fed with standard pellet diet (Goldmohor brand, Lipton India Ltd.) and *water ad libitum* was used for the present study. All the experimental protocols were approved by institutional Animal Ethics Committee.

Toxicity Studies

The 50% lethal dose of the EEVL and AEVL was estimated by the up and down stair case method. (7) Doses were administered orally at the dose of 100, 1000, 2000, 3000, 4000 and 5000 mg to six groups of animals. Control group received normal saline (10 ml/kg) orally. Signs and mortality within 24-72 hrs were noted CPCSEA Registration no.126/1999/CPCSEA.

Anti-inflammatory activity

The anti-inflammatory activity of drug extract was assessed by the method described by Winter et al. (8). The rats were divided into four groups where six animals in each group were used for study. Acute inflammation was produced by the sub-plantar administration of 0.1 ml of 1% w/v Carageenan (Sigma-Aldrich Germany) in normal saline solution in the hind paw of rats. The group 1 was treated as control. Group II was treated with Indomethacin in normal saline (10 ml/kg). Group III and Group IV were treated with EEVL (500 mg/kg p.o) and AEVL (500 mg/kg p.o.) respectively. The standard and drug extract were given orally to the animal 30 minute prior to Carageenan injection. The paw volume was measured before the injection and then at intervals of 60 minute for a period of 3 hrs after Carageenan injection by mercury displacement method using plethysmograph. % inhibition of inflammation was calculated using the following formula

$$\% \text{ inhibition} = [1 - V_t / V_c] \times 100$$

Vc represented edema volume in control and Vt edema volume in treated with test extracts.

Statistical analysis – Results are expressed as Mean ± SEM. The statistical analysis was performed by using unpaired student t- test for comparing test groups with control group. P value less than 0.001 were considered statistically significant. (9)

Results

Acute Toxicity Studies – In the acute toxicity test sign of toxicity included lethargy, jerk, convulsion and death. No sign of toxicity are seen at even the dose of 5 gm / kg.

Preliminary Phytochemical screening – As shown in Table 1 preliminary phytochemical screening of different extract showed the presence of Flavonoid, Carbohydrate, Phytosterol, Phenolic, Proteins and glycosides.

Anti-inflammatory activity- The result of EEVL and AEVL against Carageenan induced paw edema is shown in Table – 2. The result showed that the ethanolic extract of *Vitex leucoxylo*n exhibited significant activity at dose of 500 (mg/kg p.o.). As shown in table 2 aqueous extract of *Vitex leucoxylo*n exhibited 46.06% inhibition in rat paw edema whereas standard drug showed 50.6% inhibition of inflammation.

Table 1. Preliminary Phytochemical analysis of the bark of *Vitex leucoxylo*n Linn.

Phyto-constituent	Alcoholic extract	Aqueous extract	Successive extract			
			PE	EA	ALC	AQ
Flavonoids	+	+	-	+	+	+
Carbohydrates	+	+	-	+	+	+
Alkaloids	-	-	-	-	-	-
Phytosterols	+	-	+	-	-	-
Phenolic compounds	+	+	-	+	+	+
Proteins	+	+	-	+	+	+

Glycosides	+	+	-	+	+	+
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Key:

PE = Petroleum ether extract (60-80°C) ALC = Alcoholic

EA = Ethyl acetate AQ = Aqueous

(+) = Present (-) = Absent

Table 2. Effect of Indomethacin, EEVL and AEVL on Carageenan induced paw edema in rats.

S. No.	Groups	Dose (mg/kg p. o.)	Time after Carageenan injection (Minute)							
			30		60		120		180	
			EV (ml)	EI (%)	EV (ml)	EI (%)	EV (ml)	EI (%)	EV (ml)	EI (%)
1.	Control	-	0.61±0.003	-	0.68±0.023	-	0.79±0.015	-	0.89±0.023	-
2.	Indomethacin	10	0.52±0.012	14.7*	0.50±0.013	26.5*	0.49±0.083	37.9*	0.44±0.015	50.60*
3.	EEVL	500	0.55±0.010	9.8	0.54±0.012	20.58*	0.53±0.010	32.91*	0.50±0.01	43.82*
4.	AEVL	500	0.56±0.005	8.19	0.54±0.014	20.58*	0.50±0.008	36.71*	0.48±0.018	46.06*

*Significant at $p < 0.001$, P value was calculated by comparing with control by ANOVA followed by Student T- test, Values are expressed as Mean±SEM

EV: edema volume

EI: Edema inhibition

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Discussion – Indigenous drug system can be source of variety of new drugs which can provide relief in inflammation. The most widely used primary test to screen new anti-inflammatory agent measure the ability of a compound to reduce local edema induced in the rat paw injection of a phlogistic agent. This edema depend on the participation of kinins and polymorphonuclear leucocytes with there pro inflammatory factors including prostaglandins. ⁽¹⁰⁾ The development of edema in the paw of the rat after the injection of Carageenan has been described as a biphasic event. The initial phase, observed around 1 hrs, is attributed to the release of histamine and serotonin, the second accelerating phase of swelling is due to the release of prostaglandin- like substances. It has been reported that the second phase of edema is sensitive to both clinically useful steroidal and nonsteroidal anti-inflammatory agents. ^(10, 11) Significant anti-inflammatory activity was observed for ethanolic and aqueous extract of *Vitex leucoxylo*n in Carageenan induced edema model. Hence the anti-inflammatory activity in *Vitex leucoxylo*n can be attributed to the presence of flavonoid and tannins. So from the above study it is quite apparent that the aqueous extract of *Vitex leucoxylo*n plants possesses significant anti-inflammatory activity. The further study justifies its use in inflammation, pain and wound healing as suggested in the folklore medicines.

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