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ANTI- INFLAMMATORY ACTIVITY OF *VITEX TRIFOLIA* LINN. (VERBANEACEAE) LEAVES EXTRACTS

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ABSTRACT

Keywords:

Vitex trifolia Linn.
Carrageenan,
Xylene,
Ear edema,
Paw volume,
Inflammation

Objectives: The present study was carried out to evaluate the anti-inflammatory effect of extracts of leaves of *Vitex trifolia* Linn. (Verbaneaceae).

Methods: The anti inflammatory activity of plant extracts at different dose intervals was evaluated by using two models Carrageenan induced rat paw edema and Xylene induced ear edema in Mice. Acute inflammation in rats was induced by injecting Carrageenan (0.1 ml of 1% suspension in 0.9% saline) in subplantar region and topical application of xylene (0.03ml) to the anterior and posterior surface of mice ear. The mean paw volume and percent reduction in ear edema were evaluated.

Results: Significant reduction in Paw volume ($P < 0.01$) and ear edema were observed in all treated groups. Alcoholic extract showed more activity than aqueous extract and also in dose dependent manner.

Conclusion: The study showed that the leaves of *Vitex trifolia* Linn. (Verbaneaceae) possess an anti-inflammatory activity.

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INTRODUCTION: Inflammation is a complex localized response of living mammalian tissue to foreign substances such as bacteria, trauma chemicals and heat. 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 Leukotrienes etc. By inhibiting these mediators, the inflammatory response could be suppressed¹.

However, the use of antagonists of some of these mediators to suppress inhibition may not be feasible clinically. Antihistaminics are rarely used as anti-inflammatory agents probably due to their clinical inefficacy, while NSAIDs are clinically effective but are not completely devoid of adverse effects such as gastric or intestinal mucosal damage². *Vitex trifolia* Linn. (Verbaneaceae) is well known 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 and in Western ghats^{3,4}.

The leaves are oppositely arranged along the stems and are usually compound, composed of 3 linear leaflets which range between 1 -12 cm in length⁵. Leaves and twig yields 0.11-0.28% of essential oil of spicy odor and flavones^{6,7}.

The plant is used as fungicidal, cytotoxic and insecticidal⁸. Leaves showed inhibitory action against *Mycobacterium tuberculosis*⁹ and also used as an anti-inflammatory and sedative for headache, rheumatism and in common cold in Asian countries⁶. However no scientific data regarding anti-inflammatory effect of said plant is available.

In this study, the anti-inflammatory effect of alcoholic and aqueous extracts of *Vitex trifolia* leaves was evaluated by using carrageenan induced paw edema in rats; xylene induced ear edema in mice as an inflammatory models.

MATERIALS AND METHODS:

Animals: Swiss Albino Mice of Either Sex (20-25gm) and Male Albino Wistar rats (150-200gm) were housed in a group of 5 in clean polyacrylic cages and were maintained under the natural day night cycle. Animals were acclimatized for one week before experiment.

Plant material: The leaves of *Vitex trifolia* Linn. were collected from the local areas of Belgaum, and were authenticated from Dr. R. S. Gaudar, Botanist & Head, Department of Botany, R.L.S. Institute, Belgaum. The drug was later powdered and used for the extraction process. The ethanolic extract and aqueous extract of leaves of *Vitex trifolia* Linn. obtained were used as the test drug for the evaluation of anti inflammatory activity.

Preparation of plant extract: Hundred grams of dried, coarse powdered leaves were continuously extracted for 48 h with 90% ethanol in a Soxhlet's apparatus. The extract was filtered and concentrated in a rotatory evaporator, at 30-40°C, to obtain semi-solid material. The viscous residue thus obtained was kept in a vacuum desiccator to obtain a completely dry solid mass. The extract yield was 12 %. Aqueous extract was prepared by maceration process (Yield 8 %). For screening purpose, the leaves extracts were weighed and triturated with tween 80 (1%) and then was suspended in distilled water quantity sufficient to produce a suspension of suitable strength. The extracts were administered at two different doses (100mg/kg b.w, 200mg/kg b.w.p.o.)

Drugs and chemicals: Carrageenan (Himedia Ltd. Mumbai, India), Xylene, and Diclofenac sodium as a reference standard were used in the study.

Methodology:

Model 1- Carrageenan induced rat paw edema ^{10, 11}: The rats were divided into six groups each containing 5 animals. Group 1 served as Control, Group 2 Standard drug Diclofenac (100 mg/kg) treated, Group 3, 4, 5 & 6

served as treatment with the extracts at two different doses. Acute inflammation in rats was induced by injecting Carrageenan (0.1 ml of 1% suspension in 0.9% saline) in subplantar region and the paw volume was measured at 0, 1, 2 & 3 hrs with Plethysmometer. A mark was put on the leg at the malleolus 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:

$$\% \text{ Inhibition} = (1 - Ct/Co) \times 100$$

Where, Co= edema volume in Control group, Ct= edema volume in Drug treated group.

Model 2 - Xylene induced ear edema in Mice ¹²: Mice were divided into six groups, each containing 5 animals. After 30 min of administration of standard drug (i.p) and tests compounds (p.o), 0.03 ml of xylene was applied to the anterior and posterior surface of right ear. Left ear was remained untreated. Mice were killed after 2 hrs of application and both ears were removed. Circular disks were taken by using a cork borer with a diameter of 7 mm and weighed. The increase in weight was measured by subtracting the weight of untreated left ear to that of treated right ear.

Data analysis: Data are expressed as MEAN \pm SEM and were analyzed by one way ANOVA followed by Dunnet's post test for multiple comparisons. P<0.01 was considered as significant.

RESULTS: The result of anti-inflammatory activity of leaves extracts of *Vitex trifolia* Linn. against carrageenan induced paw edema is shown in **Table 1**. Paw volume was significantly reduced (P<0.01) in all treated groups as compared to control group. Alcoholic extract at dose 200mg/kg showed more inhibition of edema at 3rd hr (43%) than other treated groups. However the anti inflammatory activity showed by two different doses of both the extracts were found to be less effective than reference standard compound (59.49%). Alcoholic extract showed more activity than aqueous extract and also in dose dependent manner. The percentage inhibition of edema after 3hr for aqueous extract (100, 200mg/kg),

alcoholic extract (100 mg/kg) was found to be 14.55%, 36.07% and 32.91% respectively.

Significant activity by both the extracts was observed against acute inflammation in the xylene ear edema test (**Table 2**). The alcoholic and aqueous extracts (at

100mg/kg dose) showed 40.74% and 34.56% reduction in the edema respectively. In higher doses (200mg/kg), the aqueous (46.91%) and ethanolic (60.49%) extracts showed significant activity ($P < 0.0001$) against the acute inflammatory response which is less than that of reference standard (70.27%).

TABLE 1: EFFECT OF VARIOUS TREATMENTS ON CARRAGEENAN INDUCED PAW EDEMA IN RATS AT DIFFERENT TIME INTERVAL

Treatment Groups	Mean Change in paw volume (ml) at different time interval				Percent Inhibition after 3 rd hr
	0 hr	1 hr	2hr	3 hr	
Normal Control (Saline-1 ml/rat)	1.03±0.011	1.24±0.044	1.42±0.066	1.58±0.055	-
Standard drug treated (Diclofenac 100mg/kg)	0.92±0.058	1.11±0.057	0.98±0.012	0.64***±0.036	59.49%
Alcoholic Extract treated (100mg/kg)	0.97±0.02	1.15±0.05	1.22±0.03	1.06***±0.02	32.91%
Alcoholic Extract treated (200mg/kg)	1.00±0.044	1.03±0.03	1.16±0.05	0.90***±0.04	43.00%
Aqueous Extract treated (100mg/kg)	0.97±0.02	1.16±0.05	1.30±0.04	1.35***±0.04	14.55%
Aqueous Extract treated (200mg/kg)	0.94±0.02	0.99±0.033	1.04±0.05	1.01***±0.02	36.07%

N=5, Data expressed as MEAN±SEM. One way ANOVA followed by Dunnet's test. **P<0.01, ***P<0.0001 Vs Control

TABLE 2: EFFECT OF VARIOUS TREATMENTS ON XYLENE INDUCED EAR EDEMA IN MICE

Treatment Groups	Dose (mg/kg)	Weight of right ear (mg)	Weight of left ear (mg)	Difference (mg)	Inhibition (%)
Control	10ml/kg	32.20±1.428	16.00±1.673	16.2	-
Standard drug treated (Diclofenac)	10	21.60±1.327	16.80±1.497	4.8***	70.27%
Alcoholic Extract treated	100	27.20±1.414	17.60±0.800	9.6**	40.74%
Alcoholic Extract treated	200	27.60±1.720	21.20±1.855	6.4***	60.49%
Aqueous Extract treated	100	24.60±1.720	14.00±1.414	10.6**	34.56%
Aqueous Extract treated	200	26.60±2.135	18.00±1.673	8.6***	46.91%

Values are expressed as MEAN ± SEM. Student's t-test (N = 5). **P<0.01, ***P<0.0001 Vs Control

DISCUSSION: The plant extracts of *Vitex trifolia* Linn. were studied against Carrageenan induced rat paw edema and Xylene induced ear edema formation in mice. Carrageenan induced paw edema is the most widely used screening method for anti-inflammatory agents¹³. Carrageenan is said to mediate its action through the mobilization of PG synthesis. There are three distinct phases in edema, namely an initial phase (Histamine release), a second phase mediated by Kinins and finally third phase mediated by Prostaglandins¹⁴. Xylene is used as a phlogestic agent in the present study because Xylene causes instant irritation to mouse ear which leads to edema and fluid accumulation which are responsible for acute inflammation and the suppression of these indicates antiphlogestic effect¹⁵.

In the present study, we observed that plant extracts significantly reduced paw edema after 3rd hr, alcoholic extract being more prominent than aqueous at two different doses. This suggests that the extracts modify the effects of prostaglandins which are released into third phase of inflammation. The extracts also showed antiphlogestic effect in xylene induced ear edema in mice. Diclofenac sodium is used as a reference standard. The drug is one of the most extensively used NSAID's which acts by inhibiting prostaglandin synthesis¹⁶.

The preliminary phytochemical investigation showed the presence of Carbohydrates, Phenolic compounds, Flavonoids and tannins. It could be suggested that the anti-inflammatory effects of leaves extracts may be

due to presence of flavonoids and tannins. Other studies have demonstrated that various flavonoids such as rutin, quercetin, luteolin, and hesperidin produced significant anti-inflammatory activity^{17, 18}.

Flavonoids inhibit both inflammatory and allergic reaction as well as offer some protection in ulcer development by increasing capillary resistant and improving microcirculation which renders the cell less injurious to precipitating factors¹⁹.

CONCLUSION: These findings concluded that the leaves of *Vitex trifolia* Linn.(Verbanaceae) possess anti-inflammatory activity which is due to the presence of flavonoids, which may derive from inhibition of prostaglandins synthesis, inhibition of increased vascular permeability, which may enhance tissue repair and healing.

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