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Anti-nociceptive and Anti-inflammatory Effects of the Leaf Extract of *Vitex trifolia* Linn. in Experimental Animals

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ABSTRACT

The effect of *Vitex trifolia* Linn. (FamilyVerbenaceae) leaf extract was studied for the anti-nociceptive and anti-inflammatory activity in experimental animals. *V. trifolia*, 25 – 100 mg/kg administered orally for 3 days exhibited graded dose response equivalent to 21.95% - 89.90% protection in the tail flick latent test in rat. The *V. trifolia* leaf extract (50 and 100 mg/kg, p.o X 3 days) was effective in hot plate reaction time (64.05% and 112.97%, $p< 0.01$ and $p< 0.001$), analgesymeter induced mechanical pain (28.17% and 54.42%, $p < 0.01$ and $p < 0.001$) and acetic acid- induced writhing (26.68% and 51.79%, $p < 0.5$ and $p < 0.05$) in mice. The leaf extract of *V. trifolia* potentiated the analgesic activity with pentazocine (10 mg/kg, i.p.) and aspirin (25 mg/kg, i.p.). In the carrageenan- induced paw edema *V. trifolia* leaf extract (50 and 100 mg/kg, p.o X 3 days) decreased paw volume significantly (26.68% and 51.79%) and dose dependent anti-inflammatory activity in 1-3 hour time interval and potentiated with nimesulide (50 mg/kg, p.o.). In summary, this study demonstrates that leaf extract of *V. trifolia* has significant anti-nociceptive and anti-inflammatory activity.

Key words: *Vitex trifolia*, leaf extract, pain, inflammation.

INTRODUCTION

Vitex trifolia (Family Verbenaceae), three leaved chaste tree, a shrub or small tree growing from 1 to 4 meters in height widely prevalent in south India mostly in Tamil Nadu and some parts of Telangana in

Andhra Pradesh, has been used for various medicinal purposes in Ayurvedic and Unani systems of medicine. Almost all parts of the plant are employed, but the leaves and roots are important as drugs. The leaves are traditionally used as anodyne, diuretic and emmenagogue [1]. An application of these leaves 3 to 4 times daily prevents the burning of the feet and showed marked relief. The preliminary phytochemical studies reveal the presence of persicogenin, luteolin, penduletin, vitexicarpin, chrysosplenol and artemetin [2]. But no scientific work has been carried on the leaf [1]. The ethnic tribal communities have been using the *V. trifolia* from many generations and information regarding the efficacy remains primarily anecdotal. There is no previous record and research work available on the traditional medicinal values of *V. trifolia*. Most of the ancient knowledge systems continued to survive by oral communication from generation to generation in rural as well as in tribal communities. Therefore, the present study was undertaken to demonstrate scientifically the antinociceptive and anti-inflammatory activities of the leaf extract of *V. trifolia* in experimental animals.

EXPERIMENTAL

Plant material

V. trifolia leaves were collected from the premises of Annamalai University, Tamil Nadu, South India in the month of September- 2006. The plant material was authenticated by a systemic botanist Dr. Venkatesulu from the Department of Botany, Annamalai University. A voucher specimen of the collected sample was also deposited in the herbarium of department of pharmacy, Annamalai University for the future reference.

Alcoholic extraction of leaf of *V.trifolia*

Alcoholic extract was prepared from a powder of the leaf of *V. trifolia* prepared in electric grinder. The 750 g powder was extracted with alcohol (95% v/v) in soxhelet apparatus. The extract was evaporated to dryness under vacuum desiccator (14.5% w/w).

Test animals

Charles-Foster (CF) albino rats (110-125gm) and Wistar strain mice (16-18 gm) of either sex were obtained from the animal house of B. V. Raju foundation, Bhimavaram. They were kept in the departmental animal house at 25 ± 2^{o}C and relative humidity 45 – 51.5%, light and dark cycles of 10 and 14 h respectively for 1 week before and during the experiments. The animals were provided with standard rodent pellet diet (Hind lever) and water was allowed *ad libitum*. Rearing up of animals in the experimental period and there upkeep during the entire experimental span confirmed to ethical guidelines laid down by Institutional Animal Ethical Committee.

Drug treatment

The essential leaf extract of the leaves *V. trifolia* (suspended in 0.5% carboxy methyl cellulose in distilled water) in doses of 25 - 100 mg/kg was administered once daily for three consecutive days. Nimesulide (Cipla, India) in the dose of 50 mg/kg, p.o was used as the standard anti-inflammatory agent, where as pentazocine (Ranbaxy, India) 10 mg/kg, i.p. and aspirin (Astra – IDL Ltd, India) 25 mg/kg, i.p. were used as standard analgesic agents. All the reference drugs were administered 30 minutes before the experiment. Control group of animals received suspension of 0.5% carboxy methyl cellulose in distilled water. Experiments were conducted on day 3, one hour after last drug or vehicle administration.

PHARMACOLOGICAL TESTS

Anti-nociceptive activity:

- *Tail flick latent period*: The technique described by Davies et al., [3] was adopted, using a techno analgesiometer. The rat was placed in a rat holder with its tail coming out through a slot in the lid. The tail was kept on the bridge of the analgesiometer, called jacket with an electrically heated nichrome wire underneath. The tail received radiant heat from the wire, heated by passing current by 6 mA. The time taken for the withdrawal of the tail after switching on the electric power was taken as a latent period, in seconds of "tail flicking response and was considered as the index of nociception. The cut off time for determination of latent period was taken at 30 seconds to avoid injury to the skin [4]. Three tail flick latencies were measured per rat at each time interval and the means of the tail flick latencies were used for statistical analysis. Pentazocine (10 mg/kg, i.p.) was used as a standard reference.
- ***Hot plate reaction time in mice***: Mice were screened by placing them on a hot plate maintained at 55 ± 1°C and recording the reaction time in seconds for fore paw licking or jumping. Only mice which reacted within 15 seconds and which did not show large variation when tested on four separate occasions, each 15 minutes apart, were taken for the test. Pentazocine (10 mg/kg i.p) was used as reference standard. The time for fore paw licking or jumping on the heated plate of the analgesiometer was taken as a reaction time [5].
- ***Analgesy-metre induced pain***: The analgesic effect of *V. trifolia* was tested in mice of either sex using an Ugo Basile analgesy metre. This method involves the application of force to the paw of the mice using the analgesy-metre which exerts a force that increases at a constant rate. The mice were gently placed between the plinth and plunger. The instrument was switched on and constant motor rate was used to drive the plunger on to the paw of mice. When the mice struggles the instrument is switched off and force at which the animal felt pain was read on a scale calibrated in gram X 10 by pointer [6].

- ***Acetic acid induced writhing response in mice***: Acetic acid solution at a dose of 10ml/kg (0.6%) was injected i.p. and the number of writhes during the following 15 minutes period was observed [7]. Significant reductions in number of writhes by drug treatment as compared to vehicle treatment animals were considered as a positive analgesic response. The percent inhibition of writhing was calculated.

Antiinflammatory activity:

- ***Carrageenan-induced paw edema*:** Rats were injected with 0.1 ml of 1 % λ carrageenan into the subplantar region of the left hind paw [8]. The paw was marked with ink at the level of lateral malleolus and dipped in perspex cell up to the mark. The paw volume was measured with Ugo Basile Plethysmometer (No: 6142, 7140 Comerio-varese, Italy) before and 60, 120 and 180 minute's after injecting the λ carrageenan suspension.

STATISTICAL ANALYSIS

The values are expressed as mean ± SEM. Statistical significance of the differences between control and treated groups was calculated using unpaired Students't test followed by Mann-Whitney U-test (two tailed). A value of $p < 0.05$ was considered to be significant.

RESULTS

Tail flick latent period- The leaf extract of *V. trifolia* at the dose levels of 25, 50, 100 mg/kg exhibited graded dose response equivalent to 21.95% - 89.90% protection. Pretreatment with pentazocine significantly potentiated the antinociceptive effect of *V. trifolia* at the dose of 50 and 100 mg/kg producing 115.21% and 144.48% protection (Table 1).

Hot plate reaction time in mice *V. trifolia* (50 and 100 mg/kg) significantly increased the reaction time and the percent protection is equivalent to 64.05% and 112.97% respectively. Pentazocine increased the reaction time of *V. trifolia* to 238.20% and 257.30% at 50 and 100 mg/kg (Table 2).

Analgesy-meter induced pain- The data (Table 3) indicates that the leaf extract of *V. trifolia* treated mice exhibited resistance against mechanical pain after 30 minutes. The weight that indicates pain after treatment was dose dependent and significantly synergies the activity of aspirin.

Acetic acid induced writhing- The leaf extract of *V. trifolia* showed a significant decrease in writhing response induced by acetic acid and the degree of percent inhibition was 26.68% and 51.79% at 50 and 100 mg/kg. Under the same experimental condition the analgesic effect of *V. trifolia* potentiated the analgesic activity of aspirin as shown by further decrease in the writhing response and prevented the abdominal cramping, when given in combination (Table 4).

Carrageenan induced paw edema- Treatment with different doses of *V. trifolia* leaf extract at 50 and 100 mg/kg showed a significant and dose dependent anti-inflammatory activity with time interval 1-3 hours. The effect was similar to nimesulide and significantly potentiated the activity of *V. trifolia* (Table 5).

DISCUSSION

V. trifolia showed significant anti-nociceptive and anti-inflammatory effects on the experimental animal models. The leaf extract of *V. trifolia* was found to increase significantly the tail flick reaction time. This test is useful for discriminating between centrally acting opiate and non opiate analgesics, giving positive response with the former only. The essential leaf extract of *V. trifolia* exhibited analgesic activity in rats and potentiated with the analgesic activity with pentazocine. Hot plate reaction time in mice method was originally described by Woolfe and MacDonald [4]. This test has been found to be suitable for evaluation of centrally but not of peripherally acting analgesics. The validity of this test has been shown even in the presence of substantial impairment of motor performance [9]. The significant results indicate that *V. trifolia* may be acting centrally. Analgesy-meter induced pain is the force applied to the paw by the plinth increases at a constant rate, being the motor synchronous with mains frequency, its speed (60rpm) is constant, unaffected by friction and wear. The force measured on the scale is in 10 gram steps by a pointer riveted to the slide. *V. trifolia* significantly alleviated the pain threshold. This offers new perspectives in the treatment of pain, as there is evidence that a symptom of vital pain varies in intensity with central and peripheral somato sensory pathways. In the acetic acid induced writhing response, *V. trifolia* significantly inhibited the abdominal constriction and potentiated the activity of aspirin in mice. Acetic acid causes an increase in peritoneal fluids of PGE_2 and $PGF_2\alpha$ involving in part, peritoneal receptors [10-11] and is very sensitive method of screening anti-nociceptive effect of compounds [12].

Similarly leaf leaf extract of *V. trifolia* exhibited significant anti-inflammatory activity in λ carrageenan- induced edema in rats. It is evident that λ carrageenan is a sulphated polysaccharide obtained from sea weed (Rhodophyceae) commonly used to induce acute inflammation and it is believed to be biphasic. The first phase is due to release of histamine and sertonin. The second phase is caused by the release of bradykinin, protease, prostaglandin and lysosome [13]. It has been reported that the second phase of edema is sensitive to the most clinically effective anti-inflammatory drugs, which has been used frequently to access the anti-edematous effect of natural product [14-15]. Prostaglandin plays a major role in the development of second phase of reaction that is measured at three-hour time [16]. Based on these reports it can be inferred that the inhibitory effect *V. trifolia* on carrageenan - induced inflammation in rats might be due to inhibition of mediators responsible for inflammation and pain.

Thus, the present observation indicates the antinociceptive and anti-inflammatory activity of *V. trifolia*.

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Table 1. Effect of *V. trifolia* leaf extract on tail flick latent period in rats.

Treatment	Dose (mg/kg)	Mean latent period of tail flick response (sec)

		Initial	After 30 min
Control	_	5.65 ± 1.01	7.41 ± 1.32
V. trifolia	25	6.42 ± 1.02	7.92 ± 1.31
V. trifolia	50	6.06 ±1.1.62	11.04 ± 1.61[a]
V. trifolia	100	5.64 ± 1.35	14.25 ± 1.92[b]
Pentazocine	10	10.12 ±1.25	15.30 ± 1.25[b]
V. trifolia + Pentazocine	50 + 10	9.81 ± 0.93	18.53 ± 1.71[b]
V. trifolia + Pentazocine	100 + 10	10.10 ± 1.01	23.05 ± 1.25[c]

Values are mean ± SEM for six rats
P: [a]< 0.05, [b]< 0.01 and [c]< 0.001 compared to control group

Table 2. Effect of *V. trifolia* leaf extract on hot plate reaction time in mice.

Treatment	Dose (mg/kg)	Mean latent period (sec)	
		Initial	After 30 min
Control	_	9.96 ± 1.10	10.10 ± 1.12
V. trifolia	50	11.15 ± 1.15	16.21 ± 3.15[a]
V. trifolia	100	11.93 ±1.32	23.31 ± 2.89[b]
Pentazocine	10	11.45 ± 1.39	33.33 ± 4.10[c]
V. trifolia + Pentazocine	50 + 10	10.40 ± 1.05	37.54 ± 3.54[c]
V. trifolia + Pentazocine	100 + 10	10.69 ± 1.19	39.66 ± 3.93[c]

Values are mean ± SEM for six mice
P: [a]< 0.05, [b]< 0.01 and [c]< 0.001 compared to control group

Table 3. Effect of *V. trifolia* leaf extract on force induced pain in mice.

Treatment	Dose (mg/kg)	Weight causing pain (g)	
		Before administration	After administration
V. trifolia	50	85.9 ± 4.72	110.1 ± 5.99[a]
V. trifolia	100	86.0 ± 5.61	133.8 ± 7.05[b]
Aspirin	10	86.2 ±6.20	130.4 ± 7.07[b]
V. trifolia + Aspirin	50 + 25	83.3 ± 4.35	137.0 ± 7.13[b]
V. trifolia + Aspirin	100 + 25	85.1 ± 5.17	148.0 ± 8.32[b]

Values are mean ± SEM for six mice
P: [a]< 0.01 and [b]< 0.001 compared to respective before administrative group

Table 4. Effect of *V. trifolia* leaf extract on acetic acid-induced writhing in mice.

Treatment	Dose (mg/kg)	Number of writhing	% Inhibition
Control	-	25.41 ± 3.11	-
V. trifolia	50	18.63 ± 2.33	29.68
V. trifolia	100	12.25 ± 2.85[a]	53.79
Aspirin	10	10.55 ± 1.94[b]	59.48
V. trifolia + Aspirin	50 + 25	8.07 ± 1.51[c]	64.69
V. trifolia + Aspirin	100 + 25	7.69 ± 1.21[c]	67.74

Values are mean ± SEM for six mice
P: [a]< 0.05, [b]< 0.01 and [c]< 0.001 compared to control group

Table 5. Effect of *V. trifolia* leaf extract on λ carrageenan induced paw edema in rats.

Treatment	Dose (mg/kg)	Paw volume (ml) at

		60 min	120 min	180 min
Control	-	0.89 ± 0.03	1.05 ± 0.04	1.06 ± 0.03
V. trifolia	50	0.85 ± 0.03	0.94 ± 0.03	0.85 ± 0.02[c]
V. trifolia	100	0.83 ± 0.02	0.88 ± 0.02[b]	0.59 ± 0.01[c]
Nimesulide	50	0.75 ± 0.02[b]	0.64 ± 0.02[c]	0.60 ± 0.01[c]
V. trifolia + Nimesulide	50 + 50	0.80 ± 0.01[a]	0.52 ± 0.02[c]	0.43 ± 0.01[c]
V. trifolia + Nimesulide	100 + 50	0.77 ± 0.01[b]	0.47 ± 0.01[c]	0.39 ± 0.01[c]

Values are mean ± SEM for six rats

P: [a]< 0.05, [b]< 0.01 and [c]< 0.001 compared to respective control group