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Research Article

ANALGESIC POTENTIAL OF *VITEX TRIFOLIA* LINN (VERBANEACAE).

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ABSTRACT

Acetic acid induced writhing test in mice and Tail immersion method in rats were employed to study the analgesic potential of *Vitex trifolia* Linn. (Verbaneaceae). The no of writhes were significantly reduced in acetic acid induced writhing test at lowest (200mg) & at the highest (400mg) dose levels whereas increased tail immersion time was observed in tail immersion method in rats at highest dose (400mg) levels. The data concluded that the *Vitex trifolia* Linn. possess the both central and peripheral analgesic potential.

Keywords: Acetic acid, Writhing, Tail immersion, Vitex trifolia Linn, Verbaneaceae

INTRODUCTION

The plant Vitex trifolia Linn (Verbaneaceae) is well known in Hindi as 'Pani-ki-Sanbhalu', 'Sufed-Sanbhalu[**1**]. 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. It is a large coastal shrub or small tree[**2,3**].

Leaves and twig yields 0.11-0.28% of essential oil of spicy odour[**4**]. In addition, leaves also contain aucubin, agnuside, casticin, orientin, Iso-orintin, and α-D-glucoside of tetrahydroxy-monomethyl flavone. Leaves are used medicinally, for rheumatic pain, inflammation[**5, 6**], analgesic, anticonvulsant and sedative, hypnotic[**7**] etc. Leaves also possess insecticidal, cytotoxic, fungicidal properties[**8**].Leaves showed inhibitory action against Mycobacterium tuberculosis[**10**].The roots are antiemetic, expectorant, tonic and beneficial in thirst[5]. However, the scientific data on analgesic potential of the said plant is unavailable.

In present study the plant leaves was evaluated for the analgesic potential in chemical (acetic acid induced writhing) and Thermal/Heat (Tail immersion method) pain models.

Materials and Methods

Animals

The Albino Mice (25-30g) and Wistar rats (150-180g) of either sex were housed in a group of five per cage and were maintained under natural day and night cycle at 25±2°C ambient temperature, 45-55% relative humidity. Animals were allowed to acclimatize one week before the experiment. Animals were allowed with free access to standard pellet and water ad libitum.

Drugs, reagents and chemicals

Acetic acid, Indomethacin, Aqueous extract and alcoholic extracts of Vitex trifolia L. were used in the present study. Acetic acid was administered at (0.7%) at a dose of 0.1 ml/10g

of body weight. Indomethacin (10 mg/kg) and the plant extracts were administered by oral route of administration. A suspension of leaves extract were prepared with tween 80 (1%) and then was suspended in distilled water quantity sufficient to produce a suspension of suitable strength and administered at a dose of 200mg/kg, 400mg/kg b.w. orally.

Preparation of Plant extract

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 leaves were shed-dried,coarse powdered and then used for extraction. The extraction was carried out using Soxhlet Apparatus taking absolute ethanol (99.5%) as the solvent of extraction in the round bottom flask for 40-50 cycles at a temperature of 60°C. Later, the extract was condensed on water bath to remove excess of solvent and then dried using flash evaporator to maximum dryness. The extract was then subjected to qualitative phytochemical investigation.

Acute oral toxicity – Acute toxic class method

There was no mortality amongst the graded dose groups of mice up to a dose of 2000 mg/kg for duration of 72 h. This finding probably suggests that the plant extract is relatively safe or non-toxic at the doses used for this study.

Methodology

Acetic-acid induced writhing test in mice[10,11]

Mice of either sex (18-25gm) were used in Acetic-acid induced writhing test. The pain sensitization in animals was initiated by injecting 0.1ml of 0.6% of solution of acetic acid intraperitoneally. The standard drug, plant extracts were administered at the respective doses 10-15 min prior to acetic acid administration. The animals were then observed for the period of 10 min and number of writhes, indicated by stretching of abdomen with simultaneous stretching of at least one hind limb, for each animal was noted. The percent inhibition in each animal treated group was then calculated to evaluate the analgesic activity.

Tail immersion method in rats[10,11]

Wistar rats (150-180g) of either sex were used in Tail immersion method. The animals were allowed to acclimatize in the animal cage for 30min before testing. The lower 5 cm portion of the tail was marked. The marked portion of the tail was then immersed in a beaker filled with the fresh warm water of exactly 55°C.

The animal reacted by withdrawing the tail within few seconds. The tail immersion time was then noted at definite period of time up to 3 hr with subsequent administration of standard drug and plant extracts. The tail is carefully dried every time before immersion. The cut off time of the immersion was 15 sec.

Statistical Analysis

The data were expressed as MEAN ± SEM. The data were analyzed by One way analysis of variance (ANOVA) followed by Dunnet test. $P<0.05$ were considered significant.

RESULT

In acetic acid induced writhing test in mice, the standard drug and both the plant extracts showed significant inhibition in writhing response and reduced number of writhes as compared to control group. The standard drug Indomethacin has shown highest inhibition (74.53%) at a dose of 10mg/kg b. w. than extract treated groups. Among the extract treated groups, alcoholic extract treated animals has significantly reduced number of writhes and thus showed highest percent of inhibition in writhing response as compared to aqueous extract treated animals. The percent inhibition in writhing response at higher dose (400mg) and lower dose (200mg) in alcoholic extract treated group (68.53% & 39.32% respectively) was higher than aqueous extract (30.71% & 19.10% respectively) treated group. The result of acetic acid induced writhing test is shown in Table 01.

The effect of various treatments in tail immersion method in rats is shown in Table 02. The standard drug Indomethacin and both the plant extracts (400mg) significantly delayed tail immersion time as compared to control group. The statistically significant delay in response in alcoholic extract treated group was higher after 30 min onwards as compared to aqueous extract treated group.

Table1: Effect of various treatments on acetic-acid induced writhing test in mice

Sr No.	Treatment Groups	Number of Abdominal writhes	Percent Inhibition (%)
01	Normal Control (Saline-10ml/kg p.o.)	53.40 ±2.315	--
02	Standard drug treated (Indomethacin 10 mg/kg bw, p.o)	13.60 ±1.503***	74.53%
03	Alcoholic Extract treated (200mg/kg bw, p.o)	32.40 ±2.293***	39.32%
04	Alcoholic Extract treated (400mg/kg bw, p.o)	16.80 ±1.655***	68.53%
05	Aqueous Extract treated (200mg/kg bw, p.o)	43.20 ±2.782**	19.10%
06	Aqueous Extract treated (400mg/kg bw, p.o)	37.00 ±1.732***	30.71%

N=5, Data expressed as MEAN±SEM. One way ANOVA followed by Dunnet test. **P<0.01 Vs Control & ***P<0.001 Vs Control

Table 2: Effect of various treatments in tail immersion method in rats

Groups	Dose (mg/kg p.o.)	Tail Immersion time (in sec)					
		0 min	15 min	30min	60 min	120min	180 min
Normal Control	10ml/ kg	2.4 ±0.5099	3 ±0.0	2.6 ±0.2449	2.8 ±0.2000	3 ±0.0	2.8 ±0.2000
Standard drug (Indomethacin) treated	10	3.6 ±0.2449***	5.2 ±0.3742***	6.4 ±0.2449***	6.8 ±0.2000***	7.4 ±0.2449***	9.2 ±0.3742***
Alcoholic Extract treated	400	3.2 ±0.3742	3.6 ±0.2449 NS	4.8 ±0.2000***	5.4 ±0.2449***	6.2 ±0.3742***	7.4 ±0.2449***
Aqueous Extract treated	400	3.2 ±0.3742	3.8 ±0.4472NS	4.6 ±0.2449***	4.6 ±0.2449***	4.8 ±0.2449***	5.2 ±0.3742***

N=5, Data expressed as MEAN±SEM. One way ANOVA followed by Dunnet test. **P<0.01 Vs Control & ***P<0.001 Vs Control, NS: Not significant

DISCUSSION

The present study was carried out to evaluate the analgesic potential of *Vitex trifolia* Linn leaves extracts. The pain was induced in animals with chemical (Acetic acid) and thermal (tail immersion) pain models.

Acetic acid induced writhing test is a simple screening model to detect central and peripheral Analgesic activity. Acetic acid, an irritating agent, was injected intraperitoneally which irritates the serous membrane to produce stereotype behavior in animals, characterized by abdominal stretching, and reduced motor activity etc. The high levels of prostaglandins

(PGE2α and PGF2α) during 30 min after pain stimulus, generated by intraperitoneal injection of acetic acid was demonstrated in radioimmunoassay[12].

The tail immersion pain model was useful to differentiate between central analgesics to peripheral analgesics.In tail immersion method, it was interpreted that analgesic activity does not have a central origin supported by the fact of consistent lack of extract influence. The number of writhes was reduced in acetic acid induced writhing test, indicating that analgesic activity is mediated mostly peripheral mechanism via interference with the local reaction caused by an irritant and/or by inhibiting pain mediators synthesis or release at the sites[13].

Indomethacin is used as a standard drug because Indomethacin is more potent nonselective COX inhibitor than Aspirin and may have a direct COX independent vasoconstrictor effect.[14]

The data from the present study indicate that the plant *Vitex trifolia* Linn possess both peripheral and central effects as reduction in number of writhes and prolongation of tail withdrawal time was observed.Thus, the plant could be of therapeutic adjuvant in relieving pain stimuli along with the other analgesic agents. Further extensive work is desirable.

CONCLUSION

The results obtained from the preliminary investigation in lab showed that the plant *Vitex trifolia* Linn. Possesses both the central and peripheral analgesic potential which is further supported by reduction in number of writhes and delayed tail withdrawal time.

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