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GENERAL PHARMACOLOGY OF *VITEX LEUCOXYLON* LINN LEAVES

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Abstract : Ethanol extract (ETE) and cold aqueous infusion (CAI) of *Vitex leucoxylon* leaf were evaluated in a battery of tests to define the activity profile of the plant. CAI depressed SMA, antagonised d-amphetamine stereotypy and oxotremorine tremors, shortened the duration of mice immobility in behavioural 'despair' test and lowered serum total cholesterol level. ETE showed significant inhibition of carrageenin paw oedema and granulation tissue formation in rats. Suppression of acetic acid writhing was observed with both ETE and CAI. LD_{50} value of ETE was > 3000 mg kg^{-1} (ip) and that of CAI 1050 (800-1200) $mgkg^{-1}$.

Key words : *Vitex leucoxylon* leaf, anti-inflammatory, anti-tremor, analgesic, hypocholesterolaemic

INTRODUCTION

Plants belonging to genus Vitex (Family : Verbenaceae) are therapeutically important in Ayurvedic Materia Medica. *Vitex leucoxylon* Linn, is a small to large tree with a short thick trunk and a spreading crown found almost throughout Deccan peninsula. The root and the bark are astringent and root is used as a febrifuge. The leaves are smoked for relieving headache and catarrh and are also used for medicinal baths in fevers and anaemia (1). No pharmacological study has been reported on the plant. The present study was undertaken to delineate the pharmacological activity profile of the leaf and to compare its activities with those of root and leaf extracts of *Vitex negundo* Linn (2,3) to assess whether it could be used as a substitute for the latter.

METHODS

Plant material used was collected from the silent valley area in Kerala. Powdered, dried leaf from pharmacognostically identified plant material was successively extracted with petroleum ether 60-80°C, chloroform and 90% ethanol in a soxhlet apparatus, from the residual marc cold aqueous infusion was prepared. Since the yield with petroleum ether and chloroform was low, studies were conducted only with 90% ethanol extract (ETE) and cold aqueous infusion (CAI). ETE was used as a fine suspension in 3% Tween 80. Extract administration was by intraperitoneal injection. Control groups received 3% Tween 80 and distilled water in equal volume. The volume of the drug solution injected was 10 ml kg^{-1} in mice and 5 $mlkg^{-1}$ in rats in all the experiments. Statistical analysis of the data was carried out by applying student's 't' test.

Wistar albino rats (90-150 g) and Swiss albino mice (20-30 g) of either sex bred in the Institute's animal house and maintained on Lipton's Goldmohur laboratory animal feed and water given *ad libitum* were used in the experiments.

Acute toxicity and general behaviour : The extracts

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were administered in graded doses to groups of mice (5 each) and were periodically observed (30 min to 4 h) for behavioural changes according to the method of Morpurgo (4). In the same group of animals apparent toxicity and incidence of mortality were noted for 72 hrs. LD_{50} was calculated (5).

Pentobarbitone sleep : Sodium pentobarbitone (50 $mgkg^{-1}$, ip) was injected to mice 45 min after test extract administration. The lag time from injection to disappearance of righting reflex and duration of sleep were recorded.

Spontaneous motor activity (SMA) in mice : Mice exposed to habituation sessions (daily for five consecutive days) in an Actophotometer were used. Effect of extract administration on locomotor count (5 min), recorded at various time intervals was noted and compared with the counts recorded prior to extracts injection (6).

Forced Locomotor activity (FLA) : The performance of trained mice on a rotarod (MQ. LAB) was assessed following the method of Bansinath et al. (6).

Antipsychotic activity in mice : was evaluated by noting the effect on d-amphetamine (5 $mgkg^{-1}$, ip) induced stereotypy in mice. Stereotyped behaviour was measured by assigning relative score (7).

Antidepressant activity : (a) Antireserpine test was carried out by noting the effect of test extracts on reserpine induced (2.5 $mgkg^{-1}$, ip) ptosis, catatonia, sedation and hypothermia (8).

(b) Mice immobility test was carried out by behavioural 'despair' method (9) by noting the effect of extracts on duration of mice immobility.

Antiparkinsonian activity : was assessed on oxotremorine (500 $\mu g\ kg^{-1}$, ip) induced tremors, head twitches, ataxia, salivation, lachrymation, diarrhoea and hypothermia by assigning relative scores based on severity of symptoms (10).

Anticonvulsant activity in mice :

(a) *Maximal electric shock seizure (MES)* : Each mouse was subjected to 30 mA. current for 0.2 sec duration delivered through corneal electrodes. Number of mice protected and effect on various phases of convulsion were noted (11).

(b) *Pentylenetetrazol and strychnine induced seizures* : Pentylenetetrazol (120 $mgkg^{-1}$, sc) and strychnine (2 $mgkg^{-1}$, ip) were injected 45 min after extract administration. Effect of extracts on incidence of mortality and various phases of seizure was noted (12, 13).

Analgesic activity :

(a) *Evaluation by radiant heat method* : was done by Analgesiometer (INCO). Effect of extracts on latency of tail-flick response at various time intervals was recorded (14).

(b) *Acetic acid writhing test in mice* : Effect of prior administration of extracts on 3% acetic acid (v/v 10 $mlkg^{-1}$, ip) induced writhing was noted by recording number of writhing episodes in each mouse (15).

Anti-inflammatory activity :

(a) *Carrageenin paw oedema test* : Hind paw oedema in rats was induced by subplantar injection of 1% carrageenin in normal saline. The paw volume was plethysmographically determined before and 3 h after carrageenin injection (16, 17).

(b) *Cotton pellet granuloma* : Extracts were administered daily for six days starting on the day of pellet insertion. On seventh day rats were sacrificed by cervical dislocation, pellets removed, dried in a hot air oven for 8 hr at 80°C and weighed (18).

Evaluation for antivenom effect was done by noting effect of extracts on cobra venom (1 $mgkg^{-1}$, sc) induced mortality in mice. Incidence of mortality and survival time were recorded.

Effect on blood sugar and serum cholesterol levels in rats : The extracts were administered daily to rats for seven days, on eighth day after overnight fasting they were sacrificed, blood and serum collected. Blood sugar and serum total cholesterol were estimated (19, 20).

Antimicrobial activity

Antibacterial activity was tested against *Pseudomonas aeruginosa, Escherichia coli* and *Staphylococcus aureus* and for antifungal activity against

Aspergillus fumigatus, Trichophyton rubrum and *Trichophyton violaceum* following the agar dilution (tube) method. Nutrient agar medium was used for antibacterial studies and sabouraud's glucose agar medium for antifungal studies.

Dose : 1 and 10 mg/ml of the culture medium.

RESULTS

ETE did not produce any mortality up to the dose of 3000 $mgkg^{-1}$. The LD_{50} of CAI was 1050.48(800-1200) $mgkg^{-1}$ (ip). Both ETE and CAI did not affect rectal temperature.

The extracts failed to modify pentobarbitone sleep, forced locomotor activity and reserpine syndromes in mice. They also did not protect mice against electro and chemoconvulsions, inhibit test bacteria and fungi and elevate pain threshold in tail-flick test.

Both the extracts suppressed SMA significantly. The suppression noted with lower dose (100 $mgkg^{-1}$) of CAI was not statistically significant. Suppression noted with higher dose (200 $mgkg^{-1}$) of CAI and both the doses of ETE was statistically significant and it lasted for more than four hours (Fig. 1).

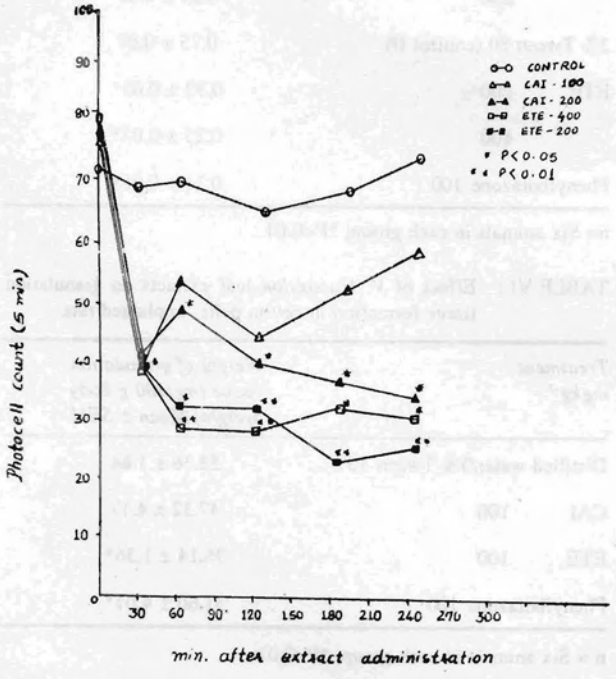

Fig. 1 : Effect of *Vitex leucoxylon* leaf extracts on SMA in mice.

ETE did not antagonise d-amphetamine stereotypy significantly. CAI at higher dose (200 $mgkg^{-1}$) showed moderate but statistically significant antagonism. Peak stereotypy scores (45 min. after d-amphetamine) have been summarised in Table I. CAI at a dose of 200 $mgkg^{-1}$ decreased the duration of mice immobility ($P < 0.05$ Table II).

TABLE I : Effect of *V. leucoxylon* leaf extracts on d-amphetamine stereotypy in mice.

	Treatment $mgkg^{-1}$	*No of animals used*	*Peak stereotypy scores Mean ± SEM*
Control		6	3.00 ± 0.00
ETE	200	6	2.40 ± 0.20
	400	6	2.60 ± 0.25
CAI	100	6	2.80 ± 0.20
	200	6	1.80 ± 0.38*
Chlorpromazine	5	6	0.00 ± 0.00**

*P < 0.05, **P < 0.01

TABLE II : Effect of *V. leucoxylon* leaf extracts on duration of mice immobility.

	Treatment $mgkg^{-1}$	*Duration of immobility (sec) Mean ± SEM*
Distilled water/ 3% Tween 80		188.80 ± 7.08
CAI	100	182.20 ± 11.59
	200	137.40 ± 12.65*
ETE	200	188.80 ± 9.97
	400	191.60 ± 7.06
d-amphetamine	10	76.78 ± 6.78**

*P < 0.05, **P < 0.001

ETE didnot antagonise oxotremorine symptoms. CAI produced significant antagonism of oxotremorine induced tremors, head twitches and ataxia (Table III).

TABLE III : Effect of *V. Leucoxylon* leaf extracts on Oxotremorine symptoms.

Treatment $mgkg^{-1}$		Peak severity score					
		Tremors	*Head twitches*	*Ataxia*	*Lachrymation*	*Salivation*	*Diarrhoea*
Distilled water/ 3% Tween 80		3.00 ± 0.00	2.40 ± 0.20	3.00 ± 0.00	1.20 ± 0.20	1.60 ± 0.25	1.40 ± 0.68
CAI	100	2.00 ± 0.20*	1.00 ± 0.00**	2.45 ± 0.25*	1.60 ± 0.40	1.60 ± 0.40	1.00 ± 0.32
	200	2.00 ± 0.20*	1.40 ± 0.25**	1.60 ± 0.40**	1.80 ± 0.20	1.80 ± 0.20	2.40 ± 0.68
ETE	200	3.00 ± 0.00	3.00 ± 0.00	2.60 ± 0.24	1.24 ± 0.20	2.00 ± 0.32	1.40 ± 0.51
	400	3.00 ± 0.00	3.00 ± 0.00	2.60 ± 0.29	1.00 ± 0.32	1.80 ± 0.20	1.60 ± 0.68

*P < 0.05, **P < 0.01 (n= Six animals in each group)

Acetic acid writing test : As could be observed from the data in Table IV, the extracts, at both the dose levels studied, produced statistically significant decrease in number of writhing episodes.

TABLE IV : Effect of *V. leucoxylon* leaf extracts on acetic acid induced writhing in mice.

	Treatment $mgkg^{-1}$	No of mice protected/total mice	Stretching episodes (30 min count) after acetic acid injection Mean ± SEM
Distilled water/ 3% Tween 80		0/5	73.50 ±7.18
CAI	100	0/5	22.00 ± 5.31*
	200	0/5	19.60 ± 4.30*
ETE	200	0/5	24.00 ± 6.91*
	400	0/5	36.40 ± 4.57*
Phenylbutazone			
	100	2/5	21.08 ± 3.96*

*P < 0.01

CAI did not cause suppression of carrageenin induced paw oedema. However, ETE produced statistically significant (P < 0.01) suppression of oedema (Table V). ETE showed significant suppression of granulation tissue formation while CAI produced only weak effect which was statistically non-significant (P > 0.05, Table VI).

ETE at the dose of 400 $mgkg^{-1}$ protected 2/5 mice while CAI failed to protect mice against cobra venom. Both extract did not influence survival time significantly.

TABLE V : Effect of *V. leucoxylon* leaf extracts on carrageenin paw oedema in rats.

Treatment $mgkg^{-1}$		Volume of paw oedema (ml) Mean ± SEM
Distilled water (control I)		0.62 ± 0.13
CAI	100	0.42 ± 0.08
	200	0.38 ± 0.06
3% Tween 80 (control II)		0.75 ± 0.07
ETE	200	0.33 ± 0.05*
	400	0.25 ± 0.05*
Phenylbutazone 100		0.21 ± 0.06*

n= Six animals in each group, *P<0.01

TABLE VI : Effect of *V. leucoxylon* leaf extracts on granulation tissue formation in cotton pellet implanted rats.

Treatment $mgkg^{-1}$		Weight of granulation tissue (mg/100 g body weight) Mean ± SEM
Distilled water/3% Tween 80		53.36 ± 1.84
CAI	100	47.32 ± 4.17
ETE	100	36.14 ± 1.36*
Phenylbutazone 100		31.60 ± 4.01*

n = Six animals in each group, *P<0.01

ETE did not affect blood sugar and serum total cholesterol levels. CAI produced significant decrease ($P < 0.05$) in serum total cholesterol level but it didnot affect blood sugar level (Table VII).

TABLE VII : Effect of *V. leucoxylon* leaf extracts on blood sugar and serum total cholesterol levels in rats.

Treatmentn $mgkg^{-1}$		*Blood sugar (mg/dL)*	*Serum total cholesterol (mg/dL)*
Distilled water/ 3% Tween 80		117.60 ± 8.62	74.80 ± 3.87
CAI	100	117.00 ± 8.39	61.86 ± 3.25*
ETE	100	119.20 ± 2.58	79.40 ± 6.48

Values : Mean ± SEM of six animals, *P <0.05

DISCUSSION

Perusal of the data presented above indicate that the extracts are devoid of sedative effect as evidenced by the inactivity of the extracts in hypnotic potentiation, rotarod and gross behaviour tests.

The results of the study show that the extracts are devoid of anticonvulsant, antimicrobial and antivenom (against cobra venom) effects.

CAI produced significant antagonism of d-amphetamine stereotypy in mice, shortened the duration of mice immobility in behavioural 'despair' test without affecting reserpine induced ptosis, sedation, hypothermia and catatonia. It also suppressed oxotremorine induced tremors and related symptoms. This clearly indicates that CAI may contain active ingredients affecting CNS and need further elucidation.

The extracts failed to elevate the threshold for tail-flick response in mice indicating that they may not possess central analgesic effect. However, significant antagonism of acetic acid induced writhing was observed. Since ETE produced significant anti-inflammatory activity also its anti-writhing effect may be indicative of peripheral analgesic effect. Unlike ETE, CAI did not possess anti-inflammatory effect. Hence, its anti-writhing effect may not be indicative of analgesic activity. It may be due to counter-irritation or possible local anaesthetic or pseudo local anaesthetic effects. Further testing would be required to arrive at an unequivocal inference.

CAI lowered blood cholesterol level. It would be beneficial to evaluate the effect of CAI on other blood lipid components to elucidate the possible mechanism of activity. ETE had no effect on blood cholesterol level.

In possessing analgesic and anti-inflammatory activities, the activity profile of *Vitex leucoxylon* leaf resembles that of *Vitex negundo*. Clinical trials should be undertaken to assess the possibility of substituting *Vitex leucoxylon* leaf in place of the latter for treating inflammatory disorders. Since, the activity is present in different extracts the observed activity may be due to different active principles. On CNS, CAI of *Vitex leucoxylon* produced important activities which were not observed with CAI of *Vitex negundo* (2). The extracts differ in the magnitude of toxicity also. ETE of *V. leucoxylon* leaf in the present study, did not produce any mortality up to the dose of 3000 $mgkg^{-1}$ while LD_{50} of ETE of *V. negundo* leaf is reported to be 1000 $mgkg^{-1}$ (2). Data from the previous (2) and present study show that the anti-inflammatory activity observed with ETE of *V. negundo* and *V. leucoxylon* is almost similar at the dose of 400 $mgkg^{-1}$. Since ETE of *V. negundo* is more than 3 fold toxic in comparison to ETE of *V. leucoxylon*, the latter has better toxic to effective dose ratio. LD_{50} of CAI of *V. leucoxylon* is 1050 $mgkg^{-1}$, CAI of *V. negundo* is non toxic (2). This indicates that the extracts of the two plants differ in CNS activity and toxicity profiles.

Essential oils, aromatic acid glycosides, alkaloid, β-sitosterol and flavonoids have been reported from the leaf extracts of *V. negundo*. Chemical characterisation of CAI and ETE of *V. leucoxylon* may lead to identification of novel phytochemicals. And it would be useful to evaluate the isolated phytochemical fractions for the pharmacological activities found with the extracts.

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