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ANTINOCICEPTIVE ACTIVITY OF *VITEX-NEGUNDO* LINN LEAF EXTRACT

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Abstract : Tail flick test in rats and acetic acid induced writhing in mice were employed to study the antinociceptive activity of ethanolic leaf extract of *Vitex-negundo* (VN) (100, 250 & 500 mg/kg, p.o). The effect was compared with meperidine (40 mg/kg, sc) in tail flick method and aspirin (50 mg/kg, p.o) in writhing test as a standard control respectively. An interaction with naloxone hydrochloride was also studied in tail flick method for its mechanism of central analgesic action. The test drug showed significant analgesic activity in dose dependant manner in both the experimental models. In comparison to standard drug (meperidine), more than ten times dose of VN extract was required to produce comparable significant antinociceptive activity. The sub-effective dose (5 mg/kg, po) of VN potentiated the analgesic activity of meperidine (4 mg/kg, sc) and aspirin (25 mg/kg, po). Naloxone (1 mg/kg, sc) did not reverse the analgesic effect of VN extract. Our observations suggest that VN possesses both central and peripheral analgesic activity. The central analgesic action does not seem to be mediated through opioid receptors. It, may prove to be a useful adjuvant therapy along with standard analgesic drug.

Key words : antinociceptive, vitex-negundo, writhing, tailflick, naloxone, potentiation

INTRODUCTION

Vitex-negundo (VN) Linn, is a large aromatic shrub with typical five folio late leaf pattern. It belongs to family verbenaceae and is found in the warmer parts of India. While, anodyne, anti-inflammatory, antipyretic and febrifuge properties (1), are claimed, it has also been investigated for an anti-inflammatory (2–4), anticonvulsant (4, 5), hepatoprotective (6) and bronchial relaxant (7) actions. Studies conducted on its analgesic action (2, 4, 5) have suggested both central (2, 5)

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and peripheral (2, 4) activities and an inhibition of prostaglandin synthesis (2) by an alcoholic leaf extract of VN. However, a conflict remains about the central analgesic action of alcoholic leaf extract of VN, since Ravishankar et al. (4) did not notice any central analgesic activity with ethanolic leaf extract of VN. Moreover mechanism of its central analgesic activity, especially involvement of opioid receptor mechanism is yet to be elucidated. Gupta et al. (5) indicated potentiation of antinociceptive activity of morphine/meperidine by VN, but no body has yet studied the potentiation of antinociceptive effect of aspirin to evaluate its potential role as an adjuvant. Therefore, the present work was undertaken in Department of Pharmacology, MGIMS, Sevagram, Wardha (M.S), to investigate antinociceptive acivity and the mechanism of central antinociceptive action of *Vitex-negundo* (VN) as well as potentiation of standard analgesic drugs especially aspirin in sub-effective doses by test drug.

METHODS

The plant material and preparation of extract:

The plant was collected from local area of Sevagram. It was identified and authenticated by an expert (botanist). The fresh leaves of VN were shade dried and powdered. The powder was macerated for 24 hrs. in 70% v/v ethanol. Then it was subjected to percolation by using 70% v/v ethanol as solvent. The men strum collected was again dried in a desiccator. The final yield (9.5%) was then suspended in 1% gum acacia and dissolved in distilled water to prepare suspension in desired concentration just before use.

Animals

Albino rats (wistar strain) and swiss albino mice of either sex, weighing (125–160 gm) and (25–35 gm) were used. They were procured from National Institution of Nutrition, Hyderabad. The clearance for the use of 150 animals for experimental purpose was obtained from Institutional Ethical Committee constituted for the purpose. Animals were housed in polypropylene cages (4 per cage) with dust free rice husk as a bedding material under laboratory condition with control environment of temperature 25° ± 2°C, humidity (60% ± 10%) and 12 h light/dark cycle (16.00–18.00) as per Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India, guidelines. They were provided standard rodent chow/feed and water *ad-libitum*. Before subjecting them to experimentation, the animals were given a weeks time to get acclimatized with laboratory conditions. The animals were fasted for 24 hrs before the experiment.

Drugs

Test drug: ethanolic leaf extract of *Vitex negundo* (VN) in the form of suspension was fed orally in a volume of 10 ml/kg/wt, in doses of 100, 250, and 500 mg/kg of body weight in both the experimental models. Where as, to study the potentiation of analgesic activity of standard drugs, it was used in the dose of 5 mg/kg/wt, p.o. For naloxone antagonism, VN in the dose (500 mg/kg, p.o.), which produced maximum analgesic action was used. Dose selection of test drug was based on previously reported studies (2–5) and preliminary trial in our laboratory.

Meperidine hydrochloride : was dissolved in distilled water and used in the doses of 40 mg/kg, s.c as standard drug and 4 mg/kg, s.c to study its potentiation effect by VN extract.

Acetylsalicylic acid (aspirin) : was used as a 2% aqueous suspension in gum acacia in the doses of 50 mg/kg orally as standard drug and 25 mg/kg, orally to study its potentiation by VN.

Naloxone hydrochloride : It was used in the dose of 1 mg/kg, s.c.

Experimental Design

For antinociceptive activity : The animals were divided into five groups in both the experimental models with each group consisting 6 animals. Group I received distilled water and served as control. Groups II, III and IV were administered three graded doses of VN extract (100, 250 and 500 mg/kg, p.o) in tail flick as well as in acetic acid induced writhing method. Where as, group V received meperidine hydrochloride in tail flick test and acetyl salicylic acid in acetic acid induced writhing method as standard drugs for comparison respectively (Table I and IV).

For naloxone antagonism : The animals were divided into 6 groups of six animals each. Pretreatment drugs were given 30 minutes before inducing pain by tailflick analgesiometer. Groups I, II, III and IV received distilled water, meperidine (40 mg/kg, sc), VN (500 mg/kg, p.o) and naloxone HCl (1 mg/kg, sc) respectively. In group V, naloxone (1 mg/kg, sc) and meperidine HCl (40 mg/kg, sc) were given and group VI was treated with naloxone (1 mg/kg, sc) and VN in the dose of (500 mg/kg, p.o). In group V and VI naloxone was given 30 minutes prior to meperidine or test drug (Table III).

Potentiation of antinociceptive activity of meperidine HCl and acetyl salicylic acid by Vitex negundo (VN) extract : the animals divided into four groups each to investigate potentiation of antinociceptive activity of meperidine HCl as well as acetyl salicylic acid by VN extract, with each group consisting of six animals. Group–I received distilled water and served as control. Group–II received sub-effective doses of meperidine HCl (4 mg/kg, s.c) in tailflick test and acetyl salicylic acid (25 mg/kg, p.o) in acetic acid induced writhing method. Group–Ill received 5 mg/kg, p.o, the dose of test drug which did not produce any antinociceptive activity in both the experimental models. Whereas, Group–IV was given combination of meperidine HCl and VN extract or acetyl salicylic acid and VN extract in above doses in respective experimental models (Table II and V).

Two methods were employed to evaluate the analgesic activity.

(i) Tailflick test in albino rats: tailflick analgesiometer was used to screen the antinociceptive activity of VN as described by D'amour and Smith (8). The rats were subjected to a preliminary screening and those showing variation of more than 2 second between two reaction times at 15 minutes interval or more than three seconds from group mean were discarded. The rats

were kept in rat holder and middle section of the tail was placed on the wire. After some times when animals adapted to new environment, the analgesiometer was switched on (6 mA current). The time when animal withdraws the tail (reaction time) was noted. The increase in reaction time in drug treated group in comparison to control indicates the antinociceptive activity. Thirty seconds was taken as cut off point for recording tail flick response as exposure more than thirty seconds caused tissue damage. The reaction time was noted before and 30, 60, 90 and 120 minutes after drug administration.

(ii) Acetic acid induced writhing in mice (9) : The writhing was induced by administration of 1 ml/100 g of 0.6% acetic acid, i.p. It consisted of a wave of constriction and elongation of abdominal musculature followed by extension of hind limbs. The animals were pretreated with drugs 45 minutes before induction of writhing. The animals were observed for onset and number of writhings within a span of 20 minutes. The animals showing a positive response within the period of 20 minutes on preliminary screening were included in the study. The abolition or inhibition of writhing response in number and frequency were considered to be the criteria for analgesic activity.

Study of interaction of Vitex negundo (VN) extract and naloxone HCl by analgesiometer : The method adopted by Chandra D (10) was followed with slight modification i.e instead of administering naloxone in dose of 5 mg/ kg, i.m we administered 1 mg/kg, s.c. This experiment was done to explore the possible mechanism of central analgesic activity of VN. The dose of naloxone was selected from Gosh MN "Fundamentals of Experimental Pharmacology" (11).

Statistical analysis

Data are expressed as mean ± S.E.M. Statistical analyses were performed by one-way ANOVA followed by Dunnett's test. Whereas, variance ratio test (comparison of two sample variance) was applied for study of interaction of VN extract and naloxone HCl. P values <0.05 were considered significant.

RESULTS

1. Tail flick method : oral administration of VN extract showed a significant analgesic activity ($P<0.05$, 0.01, 0.001) in a dose dependant manner. The analgesia began at 30 minutes, remained for 2 hrs and the peak effect was noted at 1 hr in comparison to control. The maximum analgesic response was observed in 500 mg/kg dose. Meperidine (40 mg/kg, sc) showed significant ($P<.001$) antinociception. In comparison to meperidine, more than ten times dose of VN extract was required to produce comparable significant antinociceptive activity (Table I).

Potentiation of meperidine analgesia by Vitex-negundo (VN) : the sub-effective doses of VN (5 mg/kg, po) and meperidine HCl (4 mg/kg, sc) did not show any analgesic effect. However, their co-administration produced a statistically significant ($P<0.01$) analgesia with a peak activity at 90 minutes (Table II).

TABLE I: Antinociceptive effect of *Vitex-negundo* extract in albino rats (tail flick method).

Group no. (n = 6)	*Drug, dose and route*	*Reaction time (sec) (mean ± S.E.M.)*				
		Time after drug administration				
		0 min	*30 min*	*60 min*	*90 min*	*120 min*
I	D.W. (10 ml/kg, p.o.)	6.33±0.33	6.33±0.21	6.50±0.22	6.66±0.33	6.33±0.21
II	VNE (100 mg/kg, p.o.)	6.00±0.25	8.66±1.33	10.16±1.45*	11.66±1.56*	8.83±1.47
III	VNE (250 mg/kg, p.o.)	6.16±0.40	11.33±1.63*	16.66±2.42**	16.50±2.18**	12.00±1.77**
IV	VNE (500 mg/kg, p.o.)	6.16±0.54	13.66±1.89**	19.50±0.88***	19.16±0.40***	16.00±1.56***
V	Meperidine (40 mg/kg, s.c)	6.66±0.21	>30±0.0***	>30±0.0***	>30±0.0***	>30±0.0***
	One-way ANOVA	NS F=0.47 df=4, 25	P<0.001 F=54.002 df=4, 25	P<0.001 F=47.29 df=4, 25	P<0.001 F=51.95 df=4, 25	P<0.001 F=55.96 df=4, 25

VNE = Vitex-negundo extract; D.W. = Distilled Water; NS = Non-significant; one-way ANOVA followed by Dunnett's test, *P<0.05; **P<0.001; ***P<0.001 in comparison to control.

TABLE II: Potentiation of meperidine analgesia in albino rats by *Vitex-negundo* extract (tail flick method).

Group no. (n = 6)	*Drug, dose and route*	*Reaction time (sec) (mean ± S.E.M.)*				
		Time after drug administration				
		0 min	*30 min*	*60 min*	*90 min*	*120 min*
I	D.W. (10 ml/kg, p.o.)	6.33±0.33	6.33±0.21	6.50±0.22	6.66±0.33	6.33±0.21
II	Meperidine (4 mg/kg, s.c)	7.00±0.25	7.30±0.21	7.0±0.36	7.30±0.33	7.50±0.34
III	VNE (5 mg/kg, p.o.)	6.50±0.42	6.33±0.21	6.00±0.36	6.33±0.34	6.83±0.34
IV	Meperidine (4 mg/kg, s.c) & VNE (5 mg/kg, p.o.)	6.66±0.33	8.66±1.59	13.33±1.50**	15.16±1.50**	9.16±1.08
	One-way ANOVA	NS F=0.54 df=3, 20	NS F=1.85 df=3, 20	P<0.001 F=18.42 df=3, 20	P<0.001 F=24.92 df=3, 20	NS F=0.69 df=3, 20

VNE = Vitex-negundo extract; D.W. = Distilled Water; NS = Non-significant; one-way ANOVA followed by Dunnett's test, *P<0.05; **P<0.001; ***P<0.001 in comparison to control.

Interaction of Vitex-negundo (VN) and naloxone : pretreatment with naloxone (1 mg/kg, s.c) reversed the meperidine analgesia significantly but failed to do so for the VN extract 500 mg/kg, p.o. (Table III).

2. Acetic acid induced writhing method: in the dose of 500 mg/kg, VN delayed the onset (P<0.05) and decreased the number of writhings in 20 minutes (P<0.001). In the doses of 100 and 250 mg/kg, though delayed the onset of writhing, it was not statistically significant, however, it decreased the number of writhings significantly (P<0.05, and P<.001) respectively. The maximum effect was seen in the dose of 500 mg/kg, p.o. Aspirin (50 mg/kg, p.o) showed

TABLE III : Interaction of *Vitex-negundo* extract and naloxone hydrochloride in albino rats (tail flick method).

Group no. (n = 6)	*Drug, dose and route*	*Reaction time (sec) (mean ± S.E.M.)*				
		Time after drug administration				
		0 min	*30 min*	*60 min*	*90 min*	*120 min*
I	D.W. (10 ml/kg, p.o.)	7.00±0.25	7.30±0.21	7.00±0.36	7.33±0.33	7.50±0.34
II	Meperidine (40 mg/kg, s.c)	6.66±0.21	>30±0.0[a]	>30±0.0[a]	28±0.42[a]	28±0.60[a]
III	VNE (500 mg/kg, p.o.)	6.16±0.40	13.66±1.89[b]	19.50±0.88[b]	19.16±0.40[b]	16.00±1.56[b]
IV	Naloxone (1 mg/kg, s.c)	7.16±0.44	6.83±0.47	6.66±0.42	6.83±0.30	6.83±0.56
V	Naloxone (1 mg/kg, s.c) + Meperidine (40 mg/kg, s.c)	6.66±0.21	10.00±0.47[a]	10.00±0.42[a]	8.00±0.38[a]	8.00±0.38[a]
VI	Naloxone (1 mg/kg, s.c) + VNE (500 mg/kg, p.o.)	7.16±0.70	14.33±1.75[b]	19.66±0.42[b]	18.33±0.91[b]	16.66±1.45[b]

VNE = Vitex-negundo extract; D.W. = Distilled Water; NS = Non-significant;

(a) Group II and V shows significant variation ratio with P<0.001.
(b) Group III and VI shows non-significant variation ratio.

Pretreatment with naloxone reversed the meperidine analgesia whereas, it fail to reverse the analgesia produced by *vitex-negundo* extract (500 mg/kg, p.o)

TABLE IV : Antinociceptive effect of *Vitex-negundo* extract in albino mice (acetic acid induced writhing method).

Group no. (n = 6)	*Drug, dose and route*	*Writhing response (mean ± S.E.M.)*	
		Onset of writhing (minutes)	*Number of writhings (in 20 minutes)*
I	D.W. (10 ml/kg, p.o.)	3.0±0.22	33.83±1.37
II	VNE (100 mg/kg, p.o)	3.16±0.33	22.50±2.10**
III	VNE (250 mg/kg, p.o.)	3.66±0.45	19.16±0.79***
IV	VNE (500 mg/kg, p.o)	5.016±0.78*	15.00±0.51***
V	Aspirin (50 mg/kg, p.o)	8.33±1.00***	8.66±0.27***
	One-way ANOVA	NS F=1.69 df=3, 20	P<0.001 F=8.71 df=3, 20

VNE = Vitex-negundo extract; D.W. = Distilled Water; NS = Non-significant; one-way ANOVA followed by Dunnett's test, *P<0.05; **P<0.001; ***P<0.001 in comparison to control.

significant decrease in number of writhings as well as induction time in comparison to control (Table IV).

Potentiation of aspirin analgesia by Vitex-negundo (VN) : aspirin (25 mg/kg, p.o) and VN extract (5 mg/kg, p.o) did not exhibit analgesic activity whereas simultaneous administration of both the drugs in above doses showed a significant (P<0.01) decrease in the number of writhings which indicates potentiation of aspirin analgesia by VN (Table V).

TABLE V: Potentiation of analgesic action of aspirin in albino mice by vitex-negundo extract (acetic acid induced writhing method).

Group no. *(n = 6)*	*Drug, dose and route*	*Writhing response (mean ± S.E.M.)*	
		Onset of writhing (minutes)	*Number of writhings (in 20 minutes)*
I	D.W. (10 ml/kg, p.o.)	3.0±0.22	33.83±1.37
II	Aspirin (25 mg/kg, p.o)	3.08±0.15	33.83±1.33
III	VNE (5 mg/kg, p.o.)	2.91±0.23	33.60±1.23
IV	Aspirin (25 mg/kg, p.o) & VNE (5 mg/kg, p.o)	3.58±0.37	24.00±2.41**
	One-way ANOVA	NS F=1.69 df=3, 20	P<0.001 F=8.71 df=3, 20

VNE = Vitex-negundo extract; D.W. = Distilled Water; NS = Non-significant; one-way ANOVA followed by Dunnett's test, *P<0.05; **P<0.001; ***P<0.001 in comparison to control.

DISCUSSION

In the present study, *Vitex-negundo* (VN) produced a significant antinociception in both the experimental models (tail flick test and acetic acid induced writhing method). The antinociceptive activity of VN as noted in our study support the findings of Telang et al. (2). This is, however, in contrast to Ravishanker et al. (4) who failed to observe the analgesia on intraperitoneal administration of ethanolic extract of VN in tail flick method. Although, they did find a weak analgesic effect of VN against acetic acid induced writhing in mice. This discrepancy might be attributed to different experimental situation as they used mice to observe tail flick response, higher dose of acetic acid (3%) in writhing test, used 90% of ethanol for extraction and used intraperitoneal route to administer test drug in their study. In the present work, a sub-effective dose of VN extract also potentiated the analgesic activity of sub-effective doses of meperidine and aspirin. Potentiation of meperidine analgesia, is also in agreement with Gupta et al (5) who observed a potentiation of analgesia produced by normal doses of morphine/meperidine by VN extract. Whereas, this is a first report to best of our knowledge indicating potentiation of analgesic action of aspirin. These findings therefore are indicative that, it can lower the requirement of meperidine and aspirin and thus may avoid dose related untoward effects of these drugs if used as an adjuvant therapy.

Telang et al. (2) have reported an inhibitory action of VN extract on prostaglandin biosynthesis and thereby indicating NSAID's like activity. Whereas, in our study, while trying to explore the mechanism of antinociceptive action of VN, we observed that naloxone did not antagonise this effect in albino rats. Since naloxone is opioid receptors antagonist (12). This indicates that an opioid mechanism may not be responsible for the central analgesic activity of VN and therefore some other mediators might be involved for its central analgesic activity, which still remain

to be elucidated. In comparison to standard drugs (meperidine and aspirin), more than ten times dose of VN extract was required to produce comparable significant antinociceptive activity.

In conclusion, these observations suggest that *Vitex-negundo* (VN) possesses both central and peripheral analgesic activity. It may be useful in relieving both the visceral and integumental pain. The central antinociception action appears not to be mediated by opioid receptor mechanism. The potentiation of analgesic action of meperidine and aspirin by VN is indicative of its possible use as an adjuvant therapy.

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