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ANALGESIC AND ANTI-INFLAMMATORY ACTIVITIES OF *VITEX NEGUNDO* (LEAVES) EXTRACT

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ABSTRACT: Aims: Analgesic and anti-inflammatory effects of *Vitex negundo* leaves extract (VNE) were evaluated in the animal models. The VNE was evaluated for the phytochemical analysis. **Settings and Design:** Factorial design was used for the experimental models. **Methods and Material:** The analgesic effects at graded doses of VNE (40-320 mg/kg, p.o.) were evaluated in mice against acetic acid-induced writhing (chemically induced pain) and hot-plate method (thermally induced pain). The analgesia produced by VNE was compared with the standard analgesics diclofenac sodium (DIS, 5 mg/kg, p.o.) and pentazocine (PTZ, 5 mg/kg, p.o.). Acute anti-inflammatory activity of VNE was also analyzed using carrageenan-induced rat paw edema model at the doses 40, 80 and 160 mg/kg i.p., using diclofenac sodium (5 mg/kg, i.p.) as standard. **Statistical Analysis Used:** ANOVA was applied followed my Tukey's post hoc test for comparison between the group's **Results:** In comparison to control group VNE showed highly significant, dose-dependent analgesic activity against chemically as well as thermally induced pain models ($P<0.05$). In comparison to control, VNE at the employed doses produced marked acute anti-inflammatory activity in rats ($P<0.05$). **Conclusion:** The results suggest that the aqueous extract of *Vitex negundo* has significant analgesic and anti-inflammatory potential as reflected by the parameters investigated. Further investigations are however necessary to explore mechanism(s) of action involved in these pharmacological activities.

INTRODUCTION: Pain is an unpleasant sensation localized to a part of the body. It is both sensation and emotion. Pain perception is a normal physiologic response mediated by the healthy nervous system [1]. Although, there are many analgesic drugs available, due to their adverse effects, like gastric lesions with NSAIDs [2] and dependence induced by opiates [3]. Therefore, analgesic drugs with minimal or no adverse effects along with high efficacy are being searched all over the world as alternatives to NSAIDs and opiates. According to WHO, about 80% of the world population still rely mainly on plant-based drugs [4].

Vitex negundo Linn. (VN) is a large aromatic shrub with typical five foliate leaf pattern. It belongs to family *Verbenaceae* and is found in the warmer parts of India. Some common names are in Hindi nirgundi and Sanskrit as sindhuvara. VN has been investigated for antipyretic [5] anti-inflammatory [6-8], anti-convulsant [6, 9], hepatoprotective [10] and bronchial relaxant [11].

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However, the extract used in all these studies were alcohol based. Therefore, the present work was performed to explore the presence of an analgesic and anti-inflammatory potential in the aqueous extract of *Vitex negundo* at the low doses.

MATERIALS AND METHODS:

Materials: The plant leaves were collected from a local area of Indore and kindly identified by an expert (botanist) Dr. Sanjay Vyas, (Holkar Science College, Indore). Pentazocine (PTZ) and diclofenac sodium (DIS) injections (Novartis and Ranbaxy, India, respectively) were purchased. Solvents and chemicals used for experimental work were of AR grade.

Preparation of Extract: The fresh leaves of VN were shade dried and powdered. The dried coarsely powdered leaves (500 gm) were extracted with water using Soxhlet apparatus for ten hours. The solvent was removed, and the extract was dried using rotatory evaporator. A dark brownish residue was obtained (30 gm). The dried extract was stored in a desiccator in a cool and dark place. For pharmacological screening, VNE was then dissolved in distilled water to prepare fresh drug solution in the desired concentration just before use.

Phytochemical Investigation: Specific qualitative tests were performed for the presence or absence of phytochemicals *viz*., alkaloids, tannins, flavonoids, saponins and glycosides in aqueous extract of VN to identify the constituents by the methods described by Kokate [12].

Animals: Swiss albino mice (20-25 g) and Wistar rats (150-200 g) of either sex were used for analgesic and anti-inflammatory studies, respectively. They were divided into groups of six animals each. The animals were housed in polypropylene cages in central animal house, facility with food and water freely available *ad libitum*. The rooms were maintained at the temperature of 24 ± 2 ºC with 12 hour light/dark cycles. All the animal experiments were carried out according to the Committee for Control and Supervision of Experiments on Animals (CPCSEA), India, guidelines. The animals were fasted overnight before the experiment and given water *ad libitum*. The Institutional Animal Ethics Committee approved the study (IAEC), M.G.M. Medical College, Indore, India (IAEC-709/05/2010, dated 31/07/2010) and the work was conducted at the Department of Pharmacology.

Acute Toxicity Study: As per OECD guidelines, acute toxicity study was done in female mice[13]. The animals were observed for any gross behavioral changes, sedation, morbidity, and mortality. Based on this preliminary study doses of 40, 80, 160 and 320 mg/kg were selected for further experiments.

Analgesic Studies: Analgesic activity in mice was assessed in chemically as well as thermally induced pain using acetic acid-induced writhing model [14] and hot plate assay [15], respectively.

Acetic Acid Induced Writhing Method: Total of 36 mice was randomly divided into 6 groups (n = 6). The groups were treated as control (distilled water, p. o.) and standard (DIS 5 mg/kg, p.o.) while test - VNE-40, VNE 80, VNE-160 and VNE-320 received VNE (40, 80, 160 and 320 mg/kg, p.o, respectively). Acetic acid solution 0.6% v/v (10 mL/kg) was injected intraperitoneally one hour after treatment and number of writhes (*i.e.,* the index of pain reaction against chemical stimuli characterized by abdominal muscle contraction together with the turning of trunk and extension of hind limbs) were counted throughout 20 min. Analgesic activity was expressed as the percent of reduction of writhes concerning the control group **Table 1**. The percent of reduction of writhes was calculated according to the formula:

% reduction = [1 – (mean of writhes in test or standard group/mean of writhes in control group)] × 100

Hot Plate Method: The hot plate was maintained at 55 ± 1 ºC. Albino mice were divided into six groups (n=6). The animals were placed on the hot plate and the basal reaction time is taken to cause discomfort (licking of paw or jumping response whichever appeared first) was recorded as 0 min. Cut-off period 15 sec. was established to prevent damage to the paws. The treatment and groupings of mice were done similarly to that of the acetic acid-induced writhing model except that the standard group received pentazocine (PTZ, 5 mg/kg, p.o.) instead of diclofenac sodium. The reaction time in seconds was reinvestigated at 30,

60, and 120 min. after the treatment. Changes in the reaction time were noted as described in **Table 2.**

Anti-inflammatory Study: Acute anti-inflammatory potential of VNE was also investigated using carrageenan-induced edema in rats described by Winter *et al*., [16] at 40, 80 and 160 mg/kg doses administered by i.p. route. Five groups of albino rats (n = 6) were randomly distributed in control, standard, and test (VNE-40, VNE-80 and VNE-160) groups. The initial paw volume of each animal was measured using a mercury plethysmometer. The standard group was treated with DIS injection (5 mg/kg, i.p.), while VNE (40, 80 and 160 mg/kg, i.p.) and distilled water (10 mL/kg, i.p.) was given to the test and control groups, respectively. Thirty minutes after treatment 0.1 mL of 1% carrageenan solution was injected in the plantar region of the left hind paw of rats. Paw volumes were again measured one hour after carrageenan injection. The acute difference in edema volume was calculated in each control, test and standard group and compared with the control group for the determination of the percent inhibition of the paw edema **Table 3**. Anti-inflammatory activity was calculated as percent inhibition concerning control according to the formula:

$$\% \text{ Inhibition from control} = [1 - (dt / dc)] \times 100$$

Where, dt = difference in paw volume of a rat before and after administration of treatment in standard or test groups. dc = difference in paw volume of a rat before and after administration of vehicle in the control group

Statistical Analysis: SPSS-20 statistical computer software was used to evaluate the results. One-way analysis of variance (ANOVA) test followed by multiple Tukey's comparison tests was applied, $P<0.05$ was considered statistically significant.

RESULTS:

Phytochemical Investigation: Qualitative phytochemical analysis showed the presence of alkaloids, glycosides, tannins, flavonoids, and saponins in the aqueous extract of VN **Table 1.**

TABLE 1: PRIMARY QUALITATIVE PHYTOCHEMICAL ANALYSIS

Qualitative test for	Test reagent	Result
Alkaloids	Hager's test	+
	Wagner's test	+
Glycosides	Fehling's test	+++
(on acid hydrolysis)	Molisch's test	+++
Tannins	Ferric chloride test	+++
Saponins	Froth test	++
Flavonoids	Ammonia, conc.	+++
Steroids	H_2SO_4 Conc. H_2SO_4	-

+ = mild positive, ++ = moderate positive, +++ = strong positive, - = nil

Preclinical Investigations: Acute toxicity screening of VNE was carried out in mice for 2000 mg/kg, administered orally as well as intra-peritoneally and indicated no gross behavioral changes, sedation, morbidity, and mortality at this dose. Therefore, 2000 mg/kg dose was considered as a safe dose for oral and intra-peritoneal administrations and *in-vivo* activities were evaluated using low doses 40-320 mg/kg. VNE indicated highly significant and dose-dependent analgesic activity against both chemically and thermally induced pain. In acetic acid induced writhing method, standard (DIS 5 mg/kg, *p.o.*) and VNE (40, 80, 160 and 320 mg/kg *p.o.*) treated animals showed significantly reduced number of writhes in 20 min at the rate of 67.91% and 4.69%, 39.81%, 55.26%, 50.92% respectively when compared to that of control group ($P<0.01$). However, analgesia produced by VNE was not found to be higher than that produced by standard, diclofenac sodium at any of the employed doses **Table 2**.

TABLE 2: ANALGESIC EFFECT OF *VITEX NEGUNDO* LEAF EXTRACT IN ACETIC ACID INDUCED WRITHING IN MICE

Treatment	Dose, *p.o.* (mg/kg, body weight)		No. of writhes in 20 min (mean ± SE)	% Reduction
Control (DW)	10 mL/kg		54 ± 0.73	-
DIS	5		17.33 ± 0.80 *	67.91
VNE-40	40		51.83 ± 0.91	4.69
VNE-80	80		32.5 ± 0.92 *	39.81
VNE-160	160		24.16 ± 0.79 *	55.26
VNE-320	320		26.50 ± 0.99 *	50.92
One- Way		F	308.98	
ANOVA		P	< 0.01	

DIS = diclofenac sodium; DW = distilled water; VNE = *Vitex negundo* aqueous leaves extract. One way ANOVA followed by multiple Tukey's comparison test. Values are presented as mean ± SEM (standard error mean); n = 6, df = 5, 30. * $P<0.05$ as compared to control

On the hot-plate test, VNE showed a significant elevation in pain threshold in comparison to control, as represented in **Table 2** and indicated significant analgesic activity (P<0.05) as compared to control at 80, 160 and 320 mg/kg doses. In this pain model, treatment with VNE produced dose-dependent analgesia and increased mean reaction time. The analgesic effect was observed within 30 min. in PTZ and all VNE groups; however, it was maintained for 120 min. in all groups except VNE-40. The results also demonstrated that analgesic activity produced by VNE-160 and 320 mg/kg was comparable to that of standard PTZ **Table 3**.

TABLE 3: ANALGESIC EFFECT OF *VITEX NEGUNDO* LEAF EXTRACT ON HOT PLATE TEST IN MICE

Treatment	Dose, *p.o.* (mg/kg, body weight)	Mean reaction time in seconds			
		0 min	30 min	60 min	120 min
Control (DW)	10 mL/kg	3.91 ± 0.03	3.89 ± 0.02	3.85 ± 0.04	3.86 ± 0.02
PTZ	5	3.94 ± 0.05	7.74±0.20 *	8.66±0.19 *	7.70±0.32 *
VNE-40	40	3.89±0.04	4.13±0.11	4.25±0.14	3.83±0.16
VNE-80	80	3.91±0.06	5.22±0.09 *	6.30±0.16*	5.72±0.17 *
VNE-160	160	3.84±0.05	6.04±0.36 *	7.42±0.16 *	7.25±0.08 *
VNE-320	320	3.94±0.12	6.16±0.14 *	7.94±0.24 *	6.92±0.09 *
One- Way ANOVA	F	0.295	57.53	137.51	99.44
	P	> 0.05	< 0.05	< 0.05	< 0.05

PTZ = pentazocin; DW = distilled water; VNE = *Vitex negundo* aqueous leaves extract. One way ANOVA followed by multiple Tukey's comparison test. Values are presented as mean ± SEM (standard error mean); n = 6, df = 5, 30. * P<0.05 as compared to control

Acute anti-inflammatory potential of VNE was determined at 40, 80 and 160 mg/kg, *i.p*. doses against carrageenan-induced paw edema in rats. It was noted that the standard drug DIS (5 mg/kg, *i.p*.) showed 74.6 % inhibition of edema whereas VNE at 40, 80 and 160 mg/ kg showed 23.81%, 58.73%, and 49.21% inhibition, respectively, exhibiting significant anti-inflammatory activity concerning control (P<0.01). As VNE decreased the size of edema displaying marked anti-inflammatory response in comparison to control, it was found to be weaker in action as compared to the standard DIS at the selected doses. The results are summarized in **Table 4.**

TABLE 4: ANTI-INFLAMMATORY EFFECT OF *VITEX NEGUNDO* LEAF EXTRACT ON CARRAGEENAN INDUCED RAT PAW EDEMA

Treatment	Dose, *i.p.* (mg/kg, body weight)	The difference in paw volume (mL)	% inhibition from control
Control (DW)	10 mL/kg	0.63±0.02	-
DIS	5	0.16±0.02	74.60 *
VNE-40	40	0.48±0.03	23.81 *
VNE-80	80	0.26±0.02	58.73 *
VNE-160	160	0.32±0.01	49.21 *
One –Way ANOVA	F	64.97	
	P	< 0.01	

DIS = diclofenac sodium; DW = distilled water; VNE = *Vitex negundo* aqueous leaves extract. One way ANOVA followed by multiple Tukey's comparison test. Values are presented as mean ± SEM (standard error mean); n = 6, df = 4, 25. * P<0.05 as compared to control

DISCUSSION: Thus, in the present studies, VNE was studied for its analgesic potential in both peripheral (non-narcotic) and central (narcotic) type of pain models along with an exploration of anti-inflammatory activity. Diclofenac sodium (5 mg/kg, *p.o*.) and pentazocine (5 mg/kg, *p.o*.) were used as standard drugs for comparing analgesic effects at peripheral and central levels, respectively. VNE pretreatment markedly reduces the painful response produced by acetic acid, manifested as writhing at the employed doses. Pain is a complex process mediated by many physiological mediators, *e.g.* prostaglandins, bradykinin, substance P, etc. In the acetic acid-induced writhing model the constrictions induced by acetic acid in mice results from an acute inflammatory reaction with production of PGE2 and PGF2α in the peritoneal fluid [17, 18]. Therefore, it is likely that VNE might suppress the formation of these substances or antagonize their action for exerting analgesic activity. The hot-plate test is commonly used to assess narcotic analgesics or other centrally acting

drugs [19], and the present results showed that VNE also significantly elevates the response latency period suggesting a centrally mediated analgesic effect. It also showed moderate anti-inflammatory potential at the employed doses. It has been proposed that inflammatory reaction occurs in two phases *viz*, the release of histamine, serotonin, and bradykinin in the early or first phase, followed by the release of prostaglandins in the late or second phase [17]. The significant acute anti-inflammatory response produced by VNE against carrageenan induced inflammation is suggesting the influence of VNE on synthesis, release or action of the inflammatory mediators.

Gupta and Tandon, reported antinociceptive activity in an ethanolic extract of VN, at doses 100, 250 and 500 mg/kg, orally, using tail flick and acetic acid-induced writhing models [20]. Interestingly, in our study, we observed high analgesic and anti-inflammatory activity at comparatively lower doses in aqueous extract.

The present study support that the potential active principle is a polar compound(s) present in the aqueous extract of VN. Therefore, we suggest that further isolation and purification of aqueous VNE would develop a potential new lead compound with analgesic and anti-inflammatory action from the herbal origin. Also, further exploration is also required to understand its influence on various pain and inflammatory mediators.

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CONFLICT OF INTEREST: Nil

REFERENCES:

1. Fields HL and Martin JB: In: Harrison's Principles of internal medicine. Pain pathophysiology and management, 17th ed., New York, McGraw-Hill, 2008: 81-86.
2. Goodman and Gilman's, et al. The pharmacological basis of therapeutics, 11th ed., McGraw-Hill 2006: 683-685.
3. Goodman and Gilman's: The pharmacological basis of therapeutics, 11th ed., McGraw-Hill, 2006: 560-563.
4. Kumara NK: Identification of strategies to improve research on medicinal plants used in Sri Lanka. In: WHO Symposium, University of Ruhuna, Galle, Sri Lanka 2001.
5. Nair CKN and Mohenan N: Medicinal plants in India with special reference to Ayurveda, NAG Publisher, India 1998.
6. Telang RS, Chatterjee S and Varshneya C: Studies on analgesic and anti-inflammatory activities of *Vitex negundo* Linn. Indian Journal of Pharmacology 1999; 31: 363-366.
7. Jana U, Chattopadhyay RN and Shaw BP: Preliminary studies on anti-inflammatory activity of *Zingiber officinale* Rose, *Vitex negundo* Linn. and *Tinospora cordifolia* (wild) miers in albino rats. Indian Journal of Pharmacology 1999; 31: 232-233.
8. Ravishankar B, Bhaskaran NR and Sasikala CK: Pharmacological evaluation of *Vitex negundo* (Nirgundi) leaves. Bulletin of Medico-Ethno-Botanical Research 1985; VI: 72-92.
9. Gupta M, Mazumder UK and Bhawal SR: CNS activity of *Vitex-negundo* Linn. in mice. Indian Journal of Experimental Biology 1999; 37: 143-146.
10. Avadhoot Y and Rana AC: Hepatoprotective effect of *Vitex negundo* against carbon tetrachloride induced liver damage. Archives of Pharmacal Research 1991; 14: 96-98. http://dx.doi.org/10.1007/BF02857823
11. Nair AM and Saraf MN: Inhibition of antigen and compound 48/80 induced contractions of guinea pig trachea by ethanolic extract of the leaves of *Vitex negundo* Linn. Indian Journal of Pharmacology 1995; 27: 230-233.
12. Kokate CK: Practical Pharmacognosy. India: Vallabh Prakashan 1985: 107-113.
13. OECD. OECD Guidelines for Testing of Chemicals [internet], Section 4, Health effects: Test No. 425: Acute oral toxicity: Up and down procedure, OECD Publishing, France 2006.
14. Ghosh MN: Fundamentals of Experimental Pharmacology, 2nd edn. India: Scientific Book Agency 1984: 144.
15. Eddy NB and Leimbach D: Synthetic analgesics. II. Dithienylbutenyl- and dithienylbutylamines. Journal of Pharmacology and Experimental Therapeutics 1953; 107: 385-393.
16. Winter CA, Risley EA and Nuss GW: Carrageenin-induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. Proceedings of the Society for Experimental Biology and Medicine 1962; 111: 544–547.
17. Deraedt R, Jouquey S, Benzoni J and Peterfalvi M: Inhibition of prostaglandin biosynthesis by non-narcotic analgesic drugs. Archives Internationales de pharmacodynamic et de thérapie 1976; 224: 30-42.
18. Deraedt R, Jouquey S, Delevalee F and Flahaut M: Release of prostaglandins-E and prostaglandins-F in an algogenic reaction and its inhibition. European Journal of Pharmacology 1980; 61: 17-24. http://dx.doi.org/10.1016/0014-2999(80)90377-5
19. Beirith A, Santos ARS, Rodrigues ALS, Creczynski-Pasa TP and Calixto JB: Spinal and supraspinal antinociceptive action of dipyrone in formalin, capsaicin and glutamate tests. Study of the mechanism of action. European Journal of Pharmacology 1998; 233-245. http://dx.doi.org/10.1016/S0014-2999(98)00026-0
20. Gupta RK and Tandon VR: Antinociceptive activity of *Vitex negundo* Linn leaf extract. Indian Journal of Physiology and Pharmacology 2005; 49: 163-170.

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