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Vitex negundo Linn (VN) leaf extract as an adjuvant therapy to standard anti-inflammatory drugs

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***Background & objectives*: Leaves of *Vitex negundo* (VN) have been investigated for their anti-inflammatory activity in past, including its mechanism of action. However, nobody has evaluated its potential role as an adjuvant with standard anti-inflammatory therapy. Therefore, the present study was undertaken to investigate interaction of ethanolic leaf extract of VN Linn with standard anti-inflammatory drugs in sub-effective doses per orally (PO) to evaluate its potential role as an adjuvant therapy.**

***Methods*: Carrageenin induced hind paw oedema and cotton pellet granuloma test in albino rats were employed to study interaction of *Vitex negundo* (VN) leaf extract with standard anti-inflammatory drugs in sub-effective doses per orally to evaluate its potential role as an adjuvant therapy.**

***Results*: The sub-effective dose of VN potentiated anti-inflammatory activity of phenlbutazone and ibuprofen significantly in carrageenin induced hind paw oedema and cotton pellet granuloma models.**

***Interpretation & conclusion*: The potentiation of anti-inflammatory activities phenlbutazone and ibuprofen by VN indicates that it may be useful as an adjuvant therapy along with standard anti-inflammatory drugs.**

Key words Adjuvant therapy - anti-inflammatory - *Vitex negundo*

Vitex negundo (VN) Linn (verbenaceae), a large aromatic shrub with typical five foliolate leave pattern, is found throughout the greater part of India at warmer zones and ascending to an altitude of 1500 m in outer, Western Himalayas. It has been claimed to possess many medicinal properties[1]. Leaves of VN have been investigated for its anti-inflammatory activity in past[2-6], including its mechanism of action[2, 6]. Telang *et al*[2] first noticed non steroidal anti-inflammatory drugs (NSAID) like activity of VN. Similarly, fresh leaves of VN have been suggested to possess anti-inflammatory and pain

suppressing activities possibly mediated via prostaglandin (PG) synthesis inhibition, antihistaminic, membrane stabilizing and antioxidant activities[6].

However, nobody has documented the interaction of standard anti-inflammatory drugs in sub-effective doses with VN for evaluating its potential role as an adjuvant therapy. Therefore, the present work was undertaken to investigate interaction of ethanolic leaf extract of VN Linn with standard anti-inflammatory drugs in sub-effective doses per orally (PO) to evaluate its potential role as an adjuvant therapy.

Material & Methods

This study was conducted in the Department of Pharmacology, Mahatma Gandhi Institute of Medical Sciences, Sevagram, Wardha, Maharashtra in May 2002.

Preparation of extract: The plant was collected from local area of Sevagram, and identified and authenticated by an expert (botanist). The fresh leaves of VN were cleaned of extraneous matter, shade dried and powdered. The powder was macerated for 24 h in 70 per cent v/v ethanol. It was subjected to percolation by using 70 per cent v/v ethanol as solvent. The menstrum collected was again shade dried and viscous extract suspended in 1 per cent gum acacia. Total yield of extract was 11.5 per cent.

Animals: Albino rats of either sex (weight 125-180 g) of Wistar strain, procured from National Institute of Nutrition, Hyderabad, India were used for the present study. The study protocol was approved by the institutional ethical committee. Animals were housed in polypropylene cages (4 per cage) with dust free rice husk as a bedding material under laboratory conditions with control environment of temperature $22^0 \pm 3^0$C, humidity (60%±10%) and 12 light/dark cycle (6.00-18.00). They were fed *ad libitum* with rodents chow and given free access to drinking water. Before subjecting them to experimentation, the animals were given a week time to get acclimatized with laboratory conditions. The animals were fasted overnight before the experiment.

Drugs: Ethanolic leaf extract of VN in the form of suspension was fed orally in a volume of 10 ml/kg/body wt, in the sub effective dose of 50 mg/kg wt, PO. Dose selection of test drug (sub-effective) was based on preliminary trial carried out in our laboratory over a dosage range of VN varying from (5-500 mg/kg, PO) and oral LD_{50} dose of VN leaf extract (7.58 g/kg, b.wt) in one of our previously reported study[7]. The minimal effective anti-inflammatory dose of VN in our preliminary trial was 100 mg/kg, PO.

Phenylbutazone (Ciba Geigy, India) and ibuprofen (Boots Company, India), were used as a 2 per cent suspension in gum acacia in (sub-effective) doses of 20 mg/kg, PO and 50 mg/kg BD.POx7days, respectively (sub effective doses) to study their interaction with VN. The sub-effective dose selection of standard drugs was also based on pilot study over a dosage varying from (10-100 mg/kg, PO) for phenylbutazone and (10-200 mg/kg) for ibuprofen.

Experiment design and drug treatment: The rats were divided into four groups of 10 rats each. Group I received distilled water and served as control. Group II received sub-effective dose of phenylbutazone (20 mg/kg/P.O one hour before experiment in a single dose) in carrageenin induced rat hind paw oedema method[8] and ibuprofen (50 mg/kg BD.POx7days) in cotton pallet granuloma test[9]. Group III, was administered the test drug VN leaf extract (50 mg/kg/orally one hour before experiment in single dose in carrageenin induced rat hind paw oedema method and 50 mg/kg.BD.POx7days in cotton pallet granuloma test), which did not produce anti-inflammatory activity, whereas group IV was given combination of phenylbutazone + VN and ibuprofen + VN in the

above doses in respective anti-inflammatory models.

Carrageenin induced rat hind paw oedema method[8]: The effect of test drug on acute phase of inflammation was studied with the help of plethysmometer Ugo Basile, Italy. Distilled water or drugs were administered 1 h before injecting 0.15 ml of 1 per cent (Sigma,USA) carrageenin in sub-planter tissue of rat hind foot to produce oedema. The paw volume was measured first at half an hour and then at 1, 3, 5 and 6 h after administration of drugs. The reduction in the volume displacement of hind foot in comparison to control was taken as anti-inflammatory effect.

Cotton pellet granuloma test: The method used by Meir R *et al* given in Ghosh MN[9] was followed to screen the effect of drugs on exudative and proliferative phases of inflammation by forming the granuloma pouch. The granuloma was produced by implanting the sterilized cotton (weight 50 mg) pellets sub-cutaneously in the ventral region of the groin under light ether anesthesia. After seven days of consecutive treatment with drugs, the cotton pellets were taken out on 8th day. The wet as well as dry weights of granuloma estimated. The difference in wet and dry weights of granuloma from control group to that of treated group indicated the anti-inflammatory activity.

Statistical analysis: The results were analyzed by one-way analysis of variance (ANOVA) followed by post hoc Dunnett's multiple comparison test, using sigma stat software (version 2.0, jandel scientific Inc. USA). Differences between means were considered to be significant at $P<0.05$.

Results & Discussion

The sub-effective doses of VN, phenylbutazone and ibuprofen did not show any anti-inflammatory activity in their respective models. However, the simultaneous administration of VN with phenylbutazone in carrageenin induced hind paw oedema model showed statistically significant ($P<0.001$) anti-inflammatory activity, which was maximum at 3 h. The anti-inflammatory effects started at 1 h, which continued till 5 h in comparison to control (Table I). Combination of sub-effective doses of VN extract and ibuprofen significantly ($P<0.01$) reduced both wet as well as dry weights in cotton pellet granuloma (Table II).

The findings of our study showed that the sub-effective doses of VN potentiated anti-inflammatory activity of phenylbutazone and ibuprofen in the respective inflammatory models, indicating that VN may possibly be useful as an

Table I. Interaction of *Vitex negundo* leaf extract with phenylbutazone (by carrageenin induced rat hind paw oedema method)

Group (n=10)	Drug	Dose (mg, ml^{-}/kg PO)	Hind paw volume					
			BDA	ADA				
				½ h	1 h	3 h	5 h	6 h
I	DW	10	2.26 ± 0.06	3.88 ± 0.12	4.18 ± 0.14	5.00 ± 0.18	3.95 ± 0.11	3.38 ± 0.10
II	PBZ	20	2.30 ± 0.08	3.88 ± 0.77	4.11 ± 0.11	5.15 ± 0.09	4.03 ± 0.17	3.33 ± 0.14
III	VNE	50	2.16 ± 0.09	3.91 ± 0.13	4.20 ± 0.07	5.03 ± 0.06	3.98 ± 0.18	3.41 ± 0.16
IV	PBZ+ VNE	20+ 50	2.33 ± 0.08	3.76 ± 0.11	3.76* ± 0.11	3.41^{+} ± 0.12	2.93** ± 0.21	2.93 ± 0.11

n, no. of animals; DW, distilled water; PO, per orally; BDA, before drug administration; ADA, after drug administration
VNE, *Vitex negundo* extract; PBZ, phenylbutazone; One-way ANOVA followed by Dunnett's test
*$P<0.05$; **$P<0.01$; $^{+}P<0.001$ in comparison to control (Group I)

Table II. Interaction of *Vitex negundo* extract with ibuprofen (by cotton pellet granuloma method)

Each group (n=10)	Drugs	Dose (mg, ml/kg B.D x 7 days, PO)	Weight of cotton pellets (mg)		
			BDA (one pellet)	ADA	
				Wet weight	Dry weight
I	DW	10	50.00	511.66 ± 30.49	265.00 ± 24.28
II	Ibuprofen	50	50.00	501.66 ± 13.80	243.33 ± 11.78
III	VNE	50	50.00	510.00 ± 12.50	237.50 ± 10.66
IV	Ibuprofen + VNE	50 + 50	50.00	353.33 ± 29.96*	164.16 ± 6.13*

DW, distilled water; PO, per orally; BD, twice daily; BDA, before drug administration; ADA, after drug administration
VNE, *vitex negundo* extract; One-way ANOVA followed by Dunnett's test
*P<0.01; in comparison to control (Group I)

adjuvant along with standard anti-inflammatory drug. It can possibly lower the dose requirement as well as adverse effects of standard anti-inflammatory drugs. Our findings may be of clinical relevance in view of recent controversies[10-12] associated with the NSAIDs use.

In conclusion, VN leaf extract can be used orally as an adjuvant therapy along with standard anti-inflammatory agents.

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