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RESEARCH ARTICLE

Anti-inflammatory effect of petroleum ether extract of *Vitex negundo* leaves in rat models of acute and subacute inflammation

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Abstract

The anti-inflammatory effect of a petroleum ether extract of *Vitex negundo* Linn (Verbenaceae) leaves, (PEVNL) (250 and 500 mg/kg, p.o.), was evaluated in carrageenan-induced hind paw edema and cotton pellet granuloma models. Diclofenac sodium was used as a standard drug. The biochemical parameters were estimated in serum. In carrageenan-induced rat paw edema model PEVNL exhibited significant ($p < 0.01$) inhibition of edema volume at 4 h in a dose-dependent manner. In the cotton pellet granuloma model, dry weight of cotton pellets was significantly inhibited in a dose-dependent manner with maximum effect noticed at 500 mg/kg, p.o. Both doses of PEVNL were found to normalize the increased alkaline phosphatase, acid phosphatase, alanine amino transferase (ALT) and aspartate amino transferase (AST) and reversed the decreased serum albumin. In conclusion, PEVNL exhibited anti-inflammatory activity in acute and subacute models. At a dose of 500 mg/kg, p.o., PEVNL was found to possess maximum activity, and this effect was comparable with reference drug diclofenac sodium (5 mg/kg, p.o.).

Keywords: *Anti-inflammatory; carrageenan; cotton pellet; diclofenac sodium; paw edema;* Vitex negundo

Introduction

Vitex negundo Linn (Verbenaceae) (common names: nirgundi, nochi) is a large aromatic shrub with bluish purple flowers, found throughout India (Chadha, 1976). Although nearly all plant parts are used, the root and leaf extracts are considered medically important and are sold as herbal drugs (Chadha, 1976). The leaf extract is used in Ayurvedic and Unani systems of medicine (Kapur et al., 1994) and has been shown to be useful in acute rheumatism, headache, toothache, snake bite treatment and is also used as a vermifuge (Kirthikar & Basu, 1975). Leaves of *V. negundo* have been investigated for their anti-inflammatory activity (Dharmasiri et al., 2003). Flavonoids, flavone glycosides (Prema & Mishra, 1978) triterpenoids and ursolic acid (Chandramu et al., 2003) have been isolated from the leaves of this plant. Volatile oil of leaves is used as an indigenous drug in different diseases such as rheumatic diseases, headache, catarrhal fever and cervical spondylitis (Nadkarni, 1976). Dried leaf powder of *V. negundo* has been shown to be anti-arthritic in rats (Tamhankar & Saraf, 1994) and hydroalcoholic extract of *V. negundo* has been reported to possess anti-inflammatory activity in subacute inflammation (Telang et al., 1999). Petroleum ether extract of *V. negundo* leaves (PEVNL) has been reported to possess analgesic activity (Gupta et al., 1997).

However, there is no report regarding the anti-inflammatory effect of PEVNL. Therefore, the present work was undertaken to investigate the anti-inflammatory effect of PEVNL and its effect on inflammation-induced biochemical changes.

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Materials and methods

Animals

Wistar rats of either sex (140 ± 10 g) were procured from Laboratory Animal Medicine, Tamilnadu Veterinary and Animal Sciences University, Madhavaram Milk Colony, Chennai, and were used for the study. A simulated dark and light cycle rhythm was maintained. The animals were housed in individual cages and acclimatized to the laboratory conditions. The animals were fed on standard dry pellet feed (Poultry Research Station, Chennai) and drinking water was provided ad libitum. The study was approved by the Institutional Animal Ethical Committee (IAEC).

Chemicals and reagents

Carrageenan was purchased from Sigma USA. Diclofenac sodium was provided by Alved Pharmaceuticals, Chennai, gratis. Other chemicals and drugs were of analytical grade and obtained from local manufacturers.

Plant material and extract preparation

V. negundo leaves were obtained from Tamilnadu Medicinal Plant Farms and Herbal Medicine Corporation Limited (TAMPCOL), Chennai. The identity of the plant was confirmed by D. Narayanappa, Chief Botanist of TAMPCOL. The voucher specimen (ID: 2004:23) was deposited in the herbarium of the Department of Veterinary Pharmacology and Toxicology, Madras Veterinary college, Chennai (India). The dried leaf powder of *V. negundo* (10 g) was subjected to heat extraction using petroleum ether (boiling point of 60°C to 80°C) and a Soxhlet apparatus. The extract was collected at the bottom of the flask and was dried at a temperature of 55°C in a hot air oven. The yield with respect to original dry plant material was 4.5% w/w.

Drug administration

The PEVNL suspended in 10% Tween 80 was orally administered at a dose of 250 or 500 mg/kg body weight (BW) Diclofenac sodium dissolved in distilled water was orally administered at a dose of 5 mg/kg BW. In carrageenan-induced inflammation studies the plant extract or diclofenac was administered orally 30 min before the induction of carrageenan inflammation. In the cotton pellet granuloma studies, the plant extract or diclofenac sodium was administered orally for 7 days daily.

Anti-inflammatory activity

The animals were divided into six groups each containing six rats. Group I served as vehicle control, group II received diclofenac sodium (5 mg/kg, p.o.) as standard drug. Groups III and IV received PEVNL (250 and 500 mg/kg, p.o., respectively). Group V served as normal control (distilled water, p.o.).

Carrageenan-induced inflammation (acute inflammation)

The acute anti-inflammatory activity was evaluated by carrageenan-induced rat paw edema as described by Winter et al. (1992). Inflammation was induced by injection of 0.1 mL of freshly prepared carrageenan (1%) aqueous suspension in normal saline underneath the plantar tissue of the right hind paw. The change in the volume of the hind paw was measured plethysmometrically, immediately, then 2 and 4 h after the administration of carrageenan. Prior to the commencement of the experiment, the initial level of the colored fluid in the plethysmometer was adjusted and set to zero. The hind paw volume was measured by dipping the foot in the mercury bath up to the anatomic hair line, i.e., the lateral malleolus and observing the extent of displacement of mercury as reflected by an increase in the level of the colored fluid.

The difference between the initial and final volumes indicated the edema volume. The percentage inhibition produced by the drugs was calculated by assuming the edema volume in the carrageenan control group as 100%. The anti-inflammatory activity of the drugs was expressed as percentage inhibition, which was calculated as follows:

$$\% \text{ inhibition} = 1 - \frac{\text{Edema volume of treated group}}{\text{Edema volume of carrageenan control group}} \times 100$$

Cotton pellet granuloma (subacute inflammation)

A subacute inflammation model was done as described previously by Swingle and Shideman (1972). Cotton pellets were made by rolling 5 mm sections of cotton so that the initial weight of each pellet was 10 ± 1 mg. Rats were subjected to ether anesthesia. The ventral surface of the rat was shaved clearly, swabbed with 70% (v/v) ethanol and a 1-cm incision was made in the mid ventral region. Using blunt forceps, a small channel was made bilaterally to the axilla and groin region and one sterile cotton pellet of known weight was placed in each axilla and groin region through this channel using blunt forceps. Air was removed from the pouch and the incision was closed with sutures. Drug treatment was instituted from the day of implantation and continued for 7 days. The animals were sacrificed on day 8. The pellets were removed, dried overnight at 60°C and finally, the dry granuloma weight

was determined. The difference between the final weight of the pellet after drying and its initial weight was taken as the granuloma tissue weight. Results were expressed as percentage inhibition of granuloma in drug treated groups compared to the control group.

$$\% \text{ inhibition of granuloma} = 1 - \frac{GT}{GC} \times 100$$

where GT = granuloma tissue weight in treated group, GC = granuloma tissue weight in control group.

Biochemical analyses

Blood was collected and the serum was separated in plain tubes. Activity of phosphatases (acid phosphatase and alkaline phosphatase), transaminases (alanine amino transferase, ALT and aspartate amino transferase, AST) and albumin in blood serum was estimated using kits procured from Span Diagnostics, Surat, Gujarat (India).

Table 1. Effect of PEVNL on edema volume in carrageenan-induced inflammation.

Groups	Group name	Edema volume (mL) at 4 h	% inhibition of edema volume
I	Vehicle control	0.73 ± 0.02^{a}	-
II	Diclofenac	0.27 ± 0.01^{c}	63
III	PEVNL (250 mg/kg)	0.36 ± 0.02^{b}	50
IV	PEVNL (500 mg/kg)	0.29 ± 0.01^{c}	60

Values are mean ± SEM, n = 6 animals in each group. Means bearing different superscripts differed significantly, $p < 0.01$.

Table 2. Effect of PEVNL on granuloma weight in cotton pellet granuloma.

Groups	Group name	Granuloma weight (mg)	% inhibition of granuloma weight
I	Vehicle control	43.10 ± 0.86^{a}	-
II	Diclofenac	17.24 ± 0.78^{c}	60
III	PEVNL (250 mg/kg)	21.74 ± 0.51^{b}	49
IV	PEVNL (500 mg/kg)	14.49 ± 0.64^{d}	66

Values are mean ± SEM, n = 6 animals in each group. Means bearing different superscripts differed significantly, $p < 0.01$.

Statistical analysis

All data was subjected to one-way analysis of variance (Snedecor & Cochran, 1989). Statistical significance between means was determined by the method of the new multiple range of Duncan (1955).

Results

Anti-inflammatory activity

Carrageenan-induced inflammation

Pretreatment of animals with PEVNL (250 and 500 mg/kg, p.o.) had no effect on edema volume at 2 h when compared to Group I (data not shown), but resulted in a significant ($p < 0.01$) and dose-related inhibition of edema volume at 4 h. The standard drug, diclofenac sodium, showed significant ($p < 0.01$) edema volume inhibition. PEVNL, at a dose of 500 mg/kg, BW, exhibited greater anti-inflammatory activity (60%) at 4 h, which was comparable with the standard drug diclofenac (63%) (Table 1).

Cotton pellet granuloma

The PEVNL (250 and 500 mg/kg, BW, p.o.) showed significant ($p < 0.01$) activity in inhibiting dry weight of granuloma, whereas PEVNL at 500 mg/kg possessed greater activity (66%) than 250 mg/kg dose of PEVNL (49%) and standard drug (60%) (Table 2).

Biochemical analyses

The effects of PEVNL on various biochemical parameters in serum of rats injected with carrageenan and exposed to cotton pellet are summarized in Tables 3 and 4, respectively. The levels of serum enzymes in carageenan-injected and cotton pellet-implanted control rats were found to be significantly increased ($p < 0.01$) when compared with the normal group (group V), whereas treatment with PEVNL at the dose level of 250 and 500 mg/kg, p.o., showed a decrease in the activities of serum enzymes. The standard drug diclofenac sodium-treated groups also showed a significant ($p < 0.01$) decrease in enzyme activity. Serum albumin levels were found to be significantly ($p < 0.01$) decreased in both carrageenan-injected and cotton pellet-implanted control rats, when compared with the

Table 3. Effect of PEVNL on serum biochemical parameters in carrageenan-induced inflammation.

Group	Group description	Acid phosphatase (IU/L)	Alkaline phosphatase (IU/L)	ALT (IU/L)	AST (IU/L)	Albumin (g/dL)
I	Vehicle control	169.82 ± 0.16^{a}	210.73 ± 0.32^{a}	54.21 ± 0.50^{a}	192.38 ± 0.97^{a}	3.40 ± 0.13^{c}
II	Diclofenac	166.54 ± 0.10^{c}	199.73 ± 0.16^{d}	41.42 ± 0.54^{c}	156.39 ± 0.84^{c}	4.21 ± 0.49^{b}
III	PEVNL (250 mg/kg)	167.48 ± 0.11^{b}	206.38 ± 0.23^{b}	44.32 ± 0.63^{b}	162.32 ± 0.45^{b}	4.11 ± 0.06^{b}
IV	PEVNL (500 mg/kg)	166.73 ± 0.12^{c}	202.28 ± 0.58^{c}	42.10 ± 0.64^{c}	158.48 ± 0.80^{c}	4.17 ± 0.03^{b}
V	Normal control	165.19 ± 0.26^{d}	198.36 ± 0.24^{e}	37.80 ± 0.36^{d}	126.29 ± 0.70^{d}	5.77 ± 0.10^{a}

Values are mean ± SEM, n = 6 animals in each group. Means bearing different superscripts in a column differed significantly, $p < 0.01$; IU/L, International units per liter; g/dL – gram per 100 mL.

Table 4. Effect of PEVNL on serum biochemical parameters in cotton pellet granuloma.

Group	Group description	Acid phosphatase (IU/L)	Alkalaline phosphatase (IU/L)	ALT (IU/L)	AST (IU/L)	Albumin (g/dL)
I	Vehicle control	178.30 ± 0.42^{a}	236.33 ± 0.55^{a}	95.48 ± 0.43^{a}	230.30 ± 0.41^{a}	$3.15^{c} \pm 0.07^{c}$
II	Diclofenac	169.54 ± 0.28^{d}	209.68 ± 0.27^{d}	70.32 ± 0.80^{bc}	170.47 ± 0.57^{c}	4.22 ± 0.06^{b}
III	PEVNL (250 mg/kg)	173.34 ± 0.30^{b}	215.89 ± 0.40^{b}	72.06 ± 0.73^{b}	172.40 ± 0.63^{b}	4.16 ± 0.06^{b}
IV	PEVNL (500 mg/kg)	171.38 ± 0.35^{c}	212.33 ± 0.31^{c}	69.25 ± 0.56^{c}	166.22 ± 0.57^{d}	4.27 ± 0.05^{b}
V	Normal Control	165.19 ± 0.26^{e}	198.36 ± 0.24^{e}	37.80 ± 0.48^{d}	126.29 ± 0.70^{e}	5.77 ± 0.10^{a}

Values are mean ± SEM, n = 6 animals in each group. Means bearing different superscripts in a column differed significantly, $p < 0.01$; IU/L, International units per liter; g/dL, gram per 100 mL.

normal group (group V). Both PEVNL and the standard drug were able to reverse this effect.

Discussion

The development of edema in the paw of the rat after injection of carrageenan is a biphasic event (Vinegar et al., 1969). Histamine and serotonin are usually responsible for eliciting the immediate response of inflammation in rats (first phase), whereas the kinins and prostaglandins (PG) mediate the more prolonged delayed onset responses (second phase) (Vane & Botting, 1987). PEVNL inhibited edema volume at 4 h after carrageenan injection (second phase), so the inhibitory effect of PEVNL on carrageenan-induced inflammation in rats could be due to inhibition of the enzyme cyclooxygenase leading to an inhibition of PG synthesis. It has been demonstrated that the suppression of carrageenan-induced rat paw edema after 3 h correlates reasonably well with the therapeutic doses of most clinically effective non-steroidal anti-inflammatory agents (DiRosa & Willoughby, 1971). Water extract of *V. negundo* leaves inhibited the carrageenan-induced inflammation at 2 h (first phase) (Dharmasiri et al., 2003), but controversially in the present study PEVNL inhibited this inflammation at 4 h (second phase). This may be due to the presence of different active principles in the petroleum ether extract of *V. negundo* leaves.

The cotton pellet granuloma method has been widely employed to assess the transudative, exudative and proliferative components of subacute inflammation. The fluid absorbed by the pellet greatly influences the wet weight of the granuloma and dry weight correlates well with amount of granulomatous tissue formed (Swingle & Shideman, 1972). The results indicate that PEVNL at both dose levels exhibited significant ($p < 0.01$) antiproliferative effects by inhibiting dry weights of cotton pellets when compared with controls. The antiproliferative effect of *V. negundo* at 500 mg/kg was greater than that of the standard drug (diclofenac sodium).

By stabilizing the lysosomal membrane, anti-inflammatory drugs may cause interference with the *de novo* synthesis of lysosomal enzymes, which have been observed to participate in the common pathway of inflammation. Acid phosphatase is frequently employed as a marker enzyme to assess the lysosomal change both *in vivo* and *in vitro* because it is localized almost exclusively in the particles, and its release parallels that of lysosomal hydrolases (Vimala et al., 1997). In the current study the level of serum phosphatases increased during inflammation, and this effect was reversed by PEVNL. This reversal effect of the test drug on decrease in serum phosphatases activity indicates that drug may have a membrane stabilizing effect.

The activity of blood transaminase is extensively used as an indicator of inflammation-induced hepatic lesion (Fries et al., 1990). Our research reveals increased transaminase activity in inflammation, and this effect was significantly reduced by PEVNL. There is a decrease in serum albumin, which may be due to an increased release of albumin into extracellular fluid during inflammation. The PEVNL normalized the decreased serum albumin level in inflammation. This is consistent with the findings of Ismail et al. (1997).

In the present study, PEVNL demonstrated significant reduction of paw edema volume in carrageenan-induced inflammation, probably due to inhibition of PG synthesis. The efficacy in the subacute model of granuloma may suggest that the anti-inflammatory effect of *V. negundo* might also be due to the inhibition of proinflammatory cells. Both doses of PEVNL showed very good membrane stabilizing effect by reversal of altered phospahatase activity. Thus, in conclusion, inhibition of prostaglandin synthesis and proinflammatory cells combined with membrane stabilization may be contributing factors in the effectiveness of the PEVNL. However, further detailed investigation is required to determine the exact phytoconstituents that are responsible for the observed anti-inflammatory activity.

***Declaration of interest*:** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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