

# *Vitex negundo* inhibits cyclooxygenase-2 inflammatory cytokine-mediated inflammation on carrageenan-induced rat hind paw edema

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## ABSTRACT

**Background:** *Vitex negundo* L. (*Verbenaceae*) is a hardy plant widely distributed in the Indian subcontinent and used for treatment of a wide spectrum of health disorders in traditional and folk medicine, some of which have been experimentally validated. In present study, we aimed to investigate the anti-inflammatory effects of *V. negundo* in carrageenan-induced paw edema in rats, and to investigate the probable mechanism of anti-inflammatory action. **Materials and Methods:** Paw edema was produced by injecting 1% solution of carrageenan, and the paw volume was measured before and after carrageenan injection up to 5 h. *V. negundo* leaf oil was extracted using a *Clevenger* apparatus and administered by a trans-dermal route to Wistar rats and the percentage of inhibition of inflammation was observed using a Plethysmometer by comparing a compound aerosol-based formulation with 1 mg diclofinac diethylamine BP and 7 mg methyl salicylate IP/kg body weight served as a standard drug whereas paraffin oil served as the placebo group. After withdrawing of blood, serum was separated and cyclooxygenase (COX)-1 and COX-2 inhibitory activities were measured by the enzyme immuno assay (EIA) method by using a COX inhibitor screening assay kit. **Results and Discussion:** *V. negundo* leaf oil significantly ( $P < 0.05$ ) reduced the carrageenan-induced paw edema as compared to the placebo group (paraffin oil) and 1 mg diclofinac diethylamine BP and 7 mg methyl salicylate IP showed the maximum inhibition of paw edema as compared to the *V. negundo* leaf oil treated group and the control group. Also in the present study *V. negundo* leaf oil showed significantly ( $P < 0.05$ ) inhibits COX-1 pathways rather than COX-2 pathways as compared to the *V. negundo* leaf oil treated group. **Conclusion:** It is suggested that the *V. negundo* leaf oil is a potent anti-inflammatory agent and acts via inhibition of COX-2 without much interfering COX-1 pathways.

**Key words:** Anti-inflammatory, cyclooxygenase-2 inhibitors, *Vitex negundo*

## INTRODUCTION

*Vitex negundo* Linn (*Verbenaceae*) (VN) is a woody and aromatic shrub. It commonly bears tri- or penta-foliolate leaves on quadrangular branches, which give rise to bluish-purple colored flowers in branched tomentose cymes. It thrives in humid places or along water courses in wastelands and mixed open forests and has been reported to occur in Afghanistan, India, Pakistan, Sri Lanka, Thailand, Malaysia, eastern Africa, and Madagascar. It is grown commercially

as a crop in parts of Asia, Europe, North America, and the West Indies.<sup>[1]</sup> Leaves of *V. negundo* contain hydroxy-3,6,7,3',4'-pentamethoxyflavone,<sup>[2]</sup> 6'-*p*-hydroxybenzoyl mussaenosidic acid,<sup>[3]</sup> 2'-*p*-hydroxybenzoyl mussaenosidic acid,<sup>[4]</sup> 5,3'-dihydroxy-7,8,4'-trimethoxyflavanone,<sup>[5]</sup> 5,3'-dihydroxy-6,7,4'- trimethoxy flavanone,<sup>[6]</sup> etc.

Leaf extracts of *V. negundo* reported as an anti-oxidant<sup>[7]</sup> which decreases the levels of superoxide dismutase, catalase, and glutathione peroxidase in Freund's adjuvant-induced arthritis-rats.<sup>[8]</sup> Roots of *V. negundo* inhibits a number of enzymes actions e.g. lipoxygenase, butyrylcholinesterase,<sup>[9]</sup>  $\alpha$ -chymotrypsin,<sup>[10]</sup> xanthine-oxidase,<sup>[11]</sup> and tyrosinase.<sup>[12]</sup> Administration of *V. negundo* extracts potentiated the effect of commonly used anti-inflammatory

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drugs such as ibuprofen and phenylbutazone;<sup>[13]</sup> analgesics such as meperidine, aspirin, morphine, and pethidine.<sup>[14]</sup> In the view of agonistic activity of *V. negundo*, the present investigation designed to investigate the effects of *V. negundo* leaf oil as an anti-inflammatory agent and to investigate the probable mechanism.

## MATERIALS AND METHODS

### Animals and housing conditions

Male Wistar rats weighing 200–250 g were procured from Laboratory Animal Resources, Division of Pharmaceutical Technology, Defence Research Laboratory, Tezpur, India. The animals were maintained under temperature-controlled rooms at an animal house with 12 h alternating light and dark cycles and given adequate nutrition and water *ad libitum*. All animal experimental protocols were performed according to the “Principles of Laboratory Animal care” (NIH publication 85–23, revised 1985) and approved by Institutional Use and Care Committee.

### Study design

Inflammation was produced as per the method described by Leblanc *et al.*<sup>[15]</sup> Briefly, the male Wistar rats were fasted for 16 h and paw edema was produced by injecting 200 µl of 1% solution of carrageenan in saline into the left hind paw. After 15 min observing the swelling in left hind paw the following treatment were followed at the inflammation site: Group I (*n* = 6) was applied aerosol-based formulation equivalent to 1 mg diclofenac diethylamine BP and 7 mg methyl salicylate IP/kg body weight (control group); Groups II, III, and IV (*n* = 6) were applied *V. negundo* leaf oil on the inflammation site equivalent to 200 µl, 1000 µl, and 2000 µl diluted with paraffin oil (treated group); Group V (*n* = 6), were applied equivalent to 1 ml/kg paraffin oil on the inflammation site (placebo control group). The paw volume was measured before and after carrageenan injection up to 5 h, using a water displacement plethysmometer (Orchid Scientific, Nashik, India). The swelling ratio (% swelling) was expressed as the percentage of the increase in the paw volume before carrageenan injection.<sup>[16]</sup> After 1 h, 3 h, and 5 h blood was withdrawn from the tail vein and separated serum and were stored at –20 °C.

### Cyclooxygenase inhibitory activity

The cyclooxygenase (COX) inhibitor screening assay directly measures PGF2α produced by stannous chloride (SnCl<sub>2</sub>) reduction of COX-derived prostaglandin (PGH<sub>2</sub>) produced in the COX reaction. All procedures were performed as indicated in the assay kit (Uscn Life Science Inc. China).

## RESULTS

### Anti-inflammatory effects of *V. negundo* on carrageenan-induced edema in rat hind paws

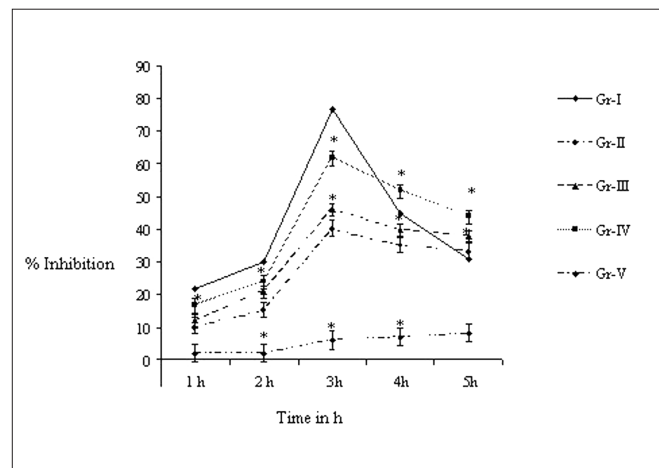
After injecting 500 µl of 1% carrageenan into the hind paw, the paw edema of the control rats was increased along with the time course and the peak edema was observed after 3 h of injecting.

The effectiveness of *V. negundo* was in dose-dependent manner. At the dose of 500 µl/kg, *V. negundo* leaf oil significantly (*P* < 0.05) decreased the edema as compared to the placebo group whereas 1 mg diclofenac diethylamine BP and 7 mg methyl salicylate IP decreased the maximum edema to 29% of swelling as compared to the placebo group [Figure 1].

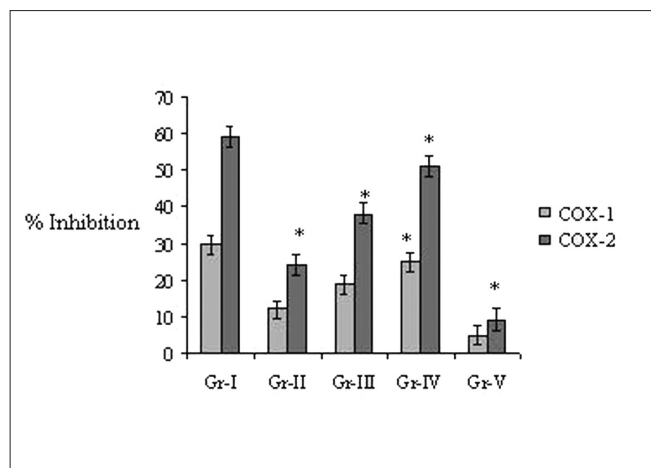
### Effect of *V. negundo* on inhibition of the COX-1 and COX-2 activities

COX inhibitory activities of *V. negundo* leaf oil were measured by using a COX inhibitor screening assay kit. The effectiveness of *V. negundo* on inhibiting of COX-1 and COX-2 oil also showed in a dose-dependent manner.

*V. negundo* leaf oil (500 µl/kg) significantly (*P* < 0.05) reduced COX-2 and COX-1 activities as compared to the placebo group whereas diclofenac spray showed maximum inhibition of COX-1 and COX-2 activity [Figure 2].



**Figure 1:** Effect of *Vitex negundo* leaf oil on paw edema. Results are expressed as mean ± SD (*n* = 6). \*Statistically different (*P* < 0.05) from control rats. Expressed as group (I) (control group, *n* = 6) treated with 1 mg diclofenac diethylamine BP and 7 mg methyl salicylate IP/kg; Groups (II, III, and IV) (treated group, *n* = 6): *V. negundo* leaf oil equivalent to 200 µl, 1000 µl, and 2000 µl diluted with paraffin oil; Group V (placebo control group, *n* = 6) treated with equivalent 1 ml/kg paraffin oil



**Figure 2:** Effect of *Vitex negundo* on inhibition of cyclooxygenase. Results are expressed as mean  $\pm$  SD ( $n = 6$ ). \*Statistically different ( $P < 0.05$ ) from control rats. Expressed as COX-1: Cyclooxygenase-1; COX-2: Cyclooxygenase-2; Group (I) (control group,  $n = 6$ ) treated with 1 mg diclofinac diethylamine BP and 7 mg methyl salicylate IP/kg; Groups (II, III, and IV) (treated group,  $n = 6$ ): *V. negundo* leaf oil equivalent to 200  $\mu$ l, 1000  $\mu$ l, and 2000  $\mu$ l diluted with paraffin oil; Group V (placebo control group,  $n = 6$ ) treated with equivalent 1 ml/kg paraffin oil

## DISCUSSION

Carrageenan-induced inflammation in the rat paw represents a classical model of edema formation and hyperalgesia, which has been extensively used in the development of nonsteroidal anti-inflammatory drugs and selective COX-2 inhibitors.<sup>[17,18]</sup>

COX is involved in the regulation of day-to-day cellular and metabolic activities such as maintaining stomach lining integrity, regulating blood flow within the kidneys, and balancing platelet function,<sup>[19]</sup> whereas COX-2 triggers by response to a variety of pro-inflammatory stimulation.<sup>[20]</sup> COX-2 regulates prostaglandin production by regulating arachidonic acid pathway in inflammatory cells for healing and repairing.<sup>[21]</sup> Therefore, inhibition of COX, and inhibiting the release of prostaglandins, is an important way to suppress inflammatory response.

In the present investigation, *V. negundo* reduced inflammation by inhibiting COX-2 receptor activity. Hong et al. reported that a number of medicinal plants showed anti-inflammatory activity via inhibition of the COX-2 receptor namely *Aristolochia debilis*, *Cinnamomum cassia*, *Cinnamomum loureirii*, *Curcuma zedoaria*, *Eugenia caryophyllata*, *Pterocarpus santalinus*, *Rebmania glutinosa*, and *Tribulus terrestris*<sup>[22]</sup> but the activity on COX-1 receptor of these plants are remain unclear. *V. negundo* inhibits the COX-2 receptor without significant interfering to the COX-1 receptor. Selective COX-2 inhibitors can also inhibit peripheral pain responses when given intrathecally,<sup>[23]</sup> whereas a selective

COX-1 inhibitor has no effect.<sup>[24]</sup> COX-1 inhibition leads to varying degrees of gastric ulcerations, perforations, or obstructions. Therefore, ideal anti-inflammatory drugs should inhibit COX-2 without interfering COX-1. The major drawback for analgesic/anti-inflammatory traditional drugs that provides optimum therapeutic efficacy without the gastro-toxicity which arises mainly COX-1 inhibition along with COX-2 receptors.

The study showed that oil of *V. negundo* prevented carrageenan-induced inflammation via COX-2 inhibition. Further studies are required to elucidate the molecular mechanism of the action *V. negundo*.

## CONCLUSION

This finding indicates that *V. negundo* leaf oil is a potent anti-inflammatory agent and its acts via the inhibition of COX-2 receptor without interfering COX-1 inhibition.

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