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*

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INTRODUCTION

Vitex negundo Linn (Verbenaceae) (VN) is a woody and aromatic shrub. It commonly bears tri- or penta-foliate 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.[1] Leaves of *V. negundo* contain hydroxy-3,6,7,3',4'-pentamethoxyflavone,[2] 6'-*p*-hydroxybenzoyl mussaenosidic acid,[3] 2'-*p*-hydroxybenzoyl mussaenosidic acid,[4] 5,3'-dihydroxy-7,8,4'-trimethoxyflavanone,[5] 5,3'-dihydroxy-6,7,4'- trimethoxy flavanone,[6] etc.

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

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drugs such as ibuprofen and phenylbutazone;[13] analgesics such as meperidine, aspirin, morphine, and pethidine.[14] 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.*[15] 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 diclofinac 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.[16] 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 ($SnCl_2$) reduction of COX-derived prostaglandin (PGH_2) 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 diclofinac 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].

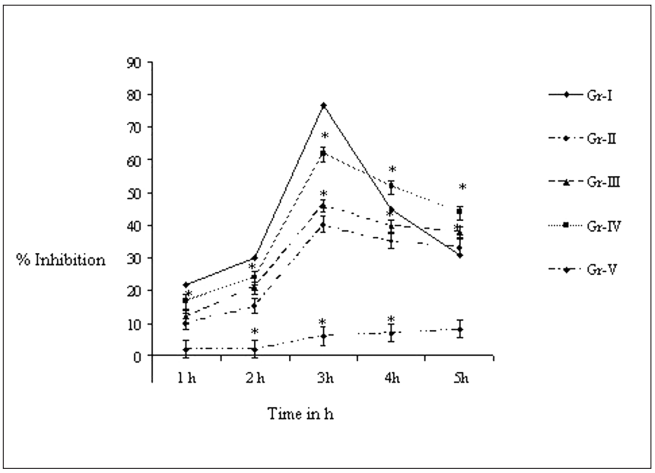

Figure 1: Effect of *Vitex negundo* leaf oil on paw edema. Results are expressed as mean ± SD ($n = 6$). *Statistically different ($P < 0.05$) from control rats. Expressed as group (I) (control group, $n = 6$) treated with 1 mg diclofinac diethylamine BP and 7 mg methyl salicylate IP/kg; Groups (II,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

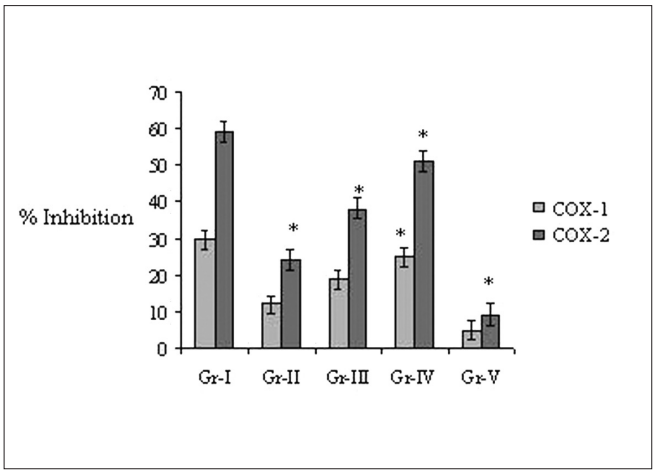

Figure 2: Effect of *Vitex negundo* on inhibition of cyclooxygenase. Results are expressed as mean ± SD (n = 6). *Statistically different (P < 0.05) from control rats. Expressed as COX-1: Cyclooxygenase-1; COX-2: Cyclooxygenase-2; Group (I) (control group, n = 6) treated with 1 mg diclofinac diethylamine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

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.[17,18]

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,[19] whereas COX-2 triggers by response to a variety of pro-inflammatory stimulation.[20] COX-2 regulates prostaglandin production by regulating arachidonic acid pathway in inflammatory cells for healing and repairing.[21] 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 santalius, Rehmania glutinosa*, and *Tribulus terrestris*[22] 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,[23] whereas a selective COX-1 inhibitor has no effect.[24] 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 carrageen an-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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REFERENCES

1. Jabeen A, Khan M, Ahmad M, Zafar M, Ahmad F. Indigenous uses of economically important flora of Margallah Hills National Park Islamabad Pakistan. Afr J Biotech 1999;8:763-84.
2. Banerji A, Chadha MS, Malshet VG. Isolation of 5-hydroxy-36-73'4'-pentamethoxyflavone from *Vitex negundo*. Phytochemistry 1969;8:511-2.
3. Sehgal CK, Taneja SC, Dhar KL, Atal CK. '2'-p-hydroxybenzoyl mussaenosidic acid a new iridoid glucoside from *Vitex negundo*. Phytochemistry 1982;21:363-6.
4. Sehgal CK, Taneja SC, Dhar KL, Atal CK. '6'-p-hydroxybenzoyl mussaenosidic acid an iridoid glucoside from *Vitex negundo*. Phytochemistry 1983;22:1036-8.
5. Achari B, Chowdhuri US, Dutta PK, Pakrashi SC. Two isomeric flavones from *Vitex negundo*. Phytochemistry 1984;23:703-4.
6. Singh V, Dayal R, Bartley J. Volatile constituents of *Vitex negundo* leaves. Planta Med 1999;65:580-5.
7. Tiwari OP, Tripathi YB. Antioxidant properties of different fractions of *Vitex negundo* Linn. Food Chem 2007;100:1170-6.
8. Devi PR, Kumari SK, Kokilavani C. Effect of *Vitex negundo* leaf extract on the free radicals scavengers in complete Freund's adjuvant induced arthritic rats. Indian J Clin Biochem 2007;22:143-7.
9. Azhar-Ul-Haq, Malik A, Anis I, Khan SB, Ahmed E, Ahmed Z, *et al*. Enzyme inhibiting lignans from *Vitex negundo*. Chem Pharm Bull 2004;52:1269-72.
10. Lodhi A, Choudhary I, Malik A, Ahmad S. 'a-Chymotrypsin inhibition studies on the lignans from *Vitex negundo* Linn. J Enzyme Inhib Med Chem 2008;23:400-5.
11. Umamaheswari M, Asok Kumar K, Somasundaram A,

Sivashanmugam T, Subhadradevi V, Ravi TK. Xanthine oxidase inhibitory activity of some Indian medical plants. J Ethnopharmacol 2007;109:547-51.

12. Azhar UH, Malik A, Khan MT, Khan SB, Anwar-Ul-Haq, Ahmad A, *et al*. Tyrosinase inhibitory lignans from the methanol extract of the roots of *Vitex negundo* Linn. and their structure–activity relationship. Phytomedicine 2006;13:255-60.
13. Tandon VR, Gupta RK. Anti-inflammatory activity and mechanism of action of *Vitex negundo* Linn. Int J Pharmacol 2006;2:303-8.
14. Gupta RK, Tandon VR. An experimental evaluation of anticonvulsant activity of *Vitex negundo*. Indian J Physiol Pharmacol 2005;49:163-72.
15. Leblanc Y, Roy P, Boyce S, Brideau C, Chan CC, Charleson S, *et al*. SAR in the alkoxy lactone series: The discovery of DFP, a potent and orally active COX-2 inhibitor. Bioorg Med Chem Lett 1999;9:2207-12.
16. Tan-no K, Nakajima T, Shoji T, Nakagawasai O, Niijima F, Ishikawa M, *et al*. Anti-inflammatory effect of property through inhibition of nitric oxide production on carrageenan-induced mouse paw edema**.** Biol Pharm Bull 2006;29:96-9.
17. Oku H, Ishiguro K. Cyclooxygenase-2 inhibitory 14-naphthoquinones from *Impatiens balsamina* L**.** Biol Pharm Bull 2002;25:658-60.
18. Jocelyne G, Kevin B, Robert G, Joseph M, Denis R. Carrageenan-induced paw edema in rat elicits a predominant prostaglandin E_2 (PGE_2) response in the central nervous system associated with the induction of microsomal PGE_2 Synthase-1. J Biol Chem 2004;279:24866-75.
19. Xie WL, Chipman JG, Robertson DL, Erikson RL, Simmons DL. Expression of a mitogen-responsive gene encoding prostaglandin syntheses is regulated by mRNA splicing. Proc Natl Acad Sci U S A 1991;88:2692-6.
20. Feldman M, McMahon AT. Do cyclooxygenase-2 inhibitors provide benefits similar to those of traditional non-steroidal anti-inflammatory drugs with less gastrointestinal toxicity? Ann Intern Med 2000;132:134-43.
21. Flower RJ. Drugs which inhibit prostaglandin biosynthesis. Pharmacol Rev 1974;26:33-67.
22. Hong CH, Hur SK, Oh OJ, Kim SS, Nam KA, Lee SK. Evaluation of natural products on inhibition of inducible cyclooxygenase (COX-2) and nitric oxide synthase (iNOS) in cultured mouse macrophage cells. J Ethnopharmacol 2002;83:153-9.
23. Samad TA, Moore KA, Sapirstein A, Billet S, Allchorne A, Poole S, *et al*. Interleukin-1beta-mediated induction of Cox-2 in the CNS contributes to inflammatory pain hypersensitivity. Nature 2001;410:471-5.
24. Yaksh TL, Dirig DM, Conway CM, Svensson C, Luo ZD, Isakson PC. The acute antihyperalgesic action of nonsteroidal, anti-inflammatory drugs and release of spinal prostaglandin E2 is mediated by the inhibition of constitutive spinal cyclooxygenase-2 (COX-2) but not COX-1. J Neurol Sci 2001;21:5847-53.

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