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ORIGINAL ARTICLE

Insight into the bronchodilator activity of *Vitex negundo*

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Abstract

Context: Vitex negundo Linn. (Verbenaceae) is traditionally used in hyperactive respiratory disorders.

Objective: This study explored the mechanisms underlying the effectiveness of *Vitex negundo* in hyperactive respiratory disorders.

Materials and methods: Crude extract of *V. negundo* leaves was obtained. For *in vivo* bronchodilatory activity in anesthetized rats, different doses (1, 3, 10, 30, and 50 mg/kg) of the crude extract of *V. negundo* (Vn.Cr) were tested. The underlying mechanisms were studied in isolated guinea pig tracheal strips, suspended in organ baths at 37 °C.

Results: Intravenous doses of the crude extract of Vn.Cr showed dose-dependent bronchodilatory effect (9–50%) against carbachol (CCh; 100 μg/kg)-induced bronchoconstriction, similar to aminophylline. In isolated guinea-pig tracheal strips, Vn.Crrelaxed CCh (1 μM) and high K^+ pre-contractions with respective EC_{50} values of 0.72 (0.48–1.10; $n = 5$) and 3.38 mg/mL (1.84–6.21; $n = 4$), similar to papaverine. Diltiazem also relaxed both contractions with more potency against high K^+ pre-contraction ($p < 0.05$). Pre-incubation of the tracheal strips with Vn.Cr potentiated the isoprenaline inhibitory concentration response curves (CRCs), similar to papaverine.

Discussion: The inhibitory effect against CCh and high K^+ suggests involvement of phosphodiesterase (PDE) inhibitory pathway(s), in addition to an inhibitory effect on Ca^{++} entry. This finding was further strengthened when pre-treatment of the tracheal strips potentiated the isoprenaline CRCs.

Conclusion: Results suggest Vn.Cr possesses a combination of papaverine-like PDE inhibitor and diltiazem-like Ca^{++} entry blocking constituents, which partly explain its bronchodilatory effect, thus validating its medicinal importance in asthma.

Keywords

Aminophylline, asthma, bronchodilatation, carbachol bronchoconstriction, crude extract, diltiazem, guinea pig trachea, papaverine, PDE inhibition

History

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Introduction

Vitex negundo Linn. (Verbenaceae) occurs in different parts of Pakistan, such as Rawalpindi hills, Murree, Muzaffarabad, Mirpur, Lower Hazara, Kurram, and Swat (Baquar, 1989). It is locally known as ''Nirgandi'' and commonly as ''Lagundi''. It is a small tree with an irregular trunk and branches covered with thin grey bark.

Asthma is a chronic respiratory disorder affecting large population of the world. Most asthmatic patients are diagnosed by a triad of episodic symptoms: cough, wheezing, and dyspnea (Nair & Saraf, 2005). Asthma incidence has climbed markedly in the past two decades despite an increased use of medications that suppress airway inflammation and repress contraction of smooth muscle that encircles the airways. Asthmatics exhibit episodes of airway inflammation that potentiates reversible airway smooth muscle spasm (Halayko et al., 2006). Alternative medicine use has increased at a remarkable pace all over the world in recent years. Interest exists in complementary therapies, particularly in herbal remedies for asthma treatment, currently with inconclusive evidence of efficacy. In the past, medicinal plants have contributed a lot in combating various refractory diseases including asthma. Nirgandi is one of such plants having medicinal importance in the treatment of asthma. Preparations of Nirgandi have also been used for a variety of other complaints including rheumatic pain, inflammatory conditions, and as an astringent (Apaya & Chichioco-Hernandez, 2011; Au et al., 2008).

Phytochemical analysis of the plant revealed the presence of casticin, isoorientin, chrysophenol D, luteolin, *p*-hydroxybenzoic acid, and fructose (Gautam et al., 2008). Additionally, alkaloids, glycosides, flavonoids, sterols, resins, tannins, dimethyl ethers of delphinidin and leucocyanidin, rhumano-glucosides, and flavone glycosides have also been

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reported (Puchpangadan et al., 2006). A new diterpenoid, negundol was reported from the seeds of *V. negundo* (Zheng et al., 2012). Pharmacologically, the plant has been reported as hepatoprotective (Avadhoot & Rana, 1991), antibacterial (Perumal-Samy et al., 1998), hypnotic (Gupta et al., 1999), antioxidant (Munasinghe et al., 2001), analgesic and anti-histaminic (Dharmasiri et al., 2003), anticancer (Diaz et al., 2003), antiandrogenic (Das et al., 2004), and anti-inflammatory (Tandon & Gupta, 2006). Nair and Saraf (2005) investigated the extract of *V. negundo* in guinea-pig tracheal smooth muscle and found that it inhibits the release of histamine and products of arachidonic acid metabolism, which are responsible for tracheal smooth muscle contraction. Patel et al. (2010) showed that alcoholic extract of *V. negundo* possesses a bronchodilator effect against histamine and acetylcholine induced bronchospasm. Recently, we have investigated the functional nature of the antidiarrheal and antispasmodic activities of the extract of *V. negundo* (Khan et al., 2013).

Despite the fact that the plant has been studied extensively phytochemically, research on its pharmacological activities in hyperactive respiratory disorders is lacking. Therefore, this investigation was carried out to probe the possible pharmacological basis in support of the medicinal use of *V. negundo* in hyperactive airway disorders, such as asthma.

Materials and methods

Leaves of *Vitex negundo* were collected in District Swat, Khyber Pukhtunkhwa, Pakistan, in 2008, and authenticated by Mr. IlyasIqbal, Assistant Professor, Department of Botany, University of Malakand, Chakdara, Dir Lower, Pakistan. A voucher specimen (UOM/BGH/149) was deposited in the herbarium of the same Department. The plant materials were shade dried, cleaned up of adulterants, and approximately 1 kg of the ground material was soaked in methanol at room temperature ($25 \pm 2.0\,^{\circ}C$) for 3 d with occasional shaking. First, it was filtered through a muslin cloth and then through a qualitative grad filter paper. The process of soaking and filtration was repeated twice more. All the filtrates were combined and evaporated on a rotary evaporator under reduced pressure (-760 mmHg) at 35–40 °C to a thick, dark brown material, the crude extract of *V. negundo* (Vn.Cr). The approximate yield was 21.27%. Vn.Cr was soluble in normal saline (0.9% w/v) and distilled water.

Drugs and standards

The following reference chemicals were obtained from the sources specified: carbamycholine chloride (CCh), diltiazem hydrochloride, potassium chloride, isoproterenol, aminophylline and papaverine hydrochloride (Sigma-Aldrich, St. Louis, MO). All chemicals used were of the highest purity grade. Stock solutions of all the chemicals were prepared in distilled water and the dilutions were made fresh in normal saline on the day of the experiment.

Animals

Experiments performed complied with the rulings of the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council (National Research Council, 1996). Sprague–Dawley rats (200–250 g) and Guinea pigs (450–500 g) of local breed and either sex was used in the study. The animals were bred and housed in the animal house of Aga Khan University under a controlled environment (23–25 °C). The animals were given tap water *ad libitum* and a standard diet.

Bronchodilator activity

As described previously (Khan & Gilani, 2009), Sprague–Dawley rats (200–250 g) were anesthetized with thiopental sodium (80–100 mg/kg, i.p.). A cannula was inserted in the trachea through which the animals were mechanically ventilated by a Miniature Ideal Pump (Bioscience, Lincoln, NE). The ventilator was adjusted to deliver 7–8 mL/kg of carbogen gas per stroke at a rate of 70–75/min. Changes in airway resistance were measured by a pressure transducer (MLT1199) and recorded by a PowerLab 4/25 via Bridge amplifier (Quad Bridge Amp, ML112) and chart software (ADInstrument, Bella Vista, Australia). Drugs were injected through a cannula inserted into the jugular vein followed by a saline flush. Bronchoconstriction was induced with carbachol (CCh 100 μg/kg). The extract of *V. negundo* and standards was administered intravenously 6–8 min prior to CCh-induced bronchoconstriction.

Phosphodiesterase inhibitory activity

As described previously (Khan et al., 2011; Moran et al., 1989; Shah & Gilani, 2010), the trachea was dissected out of the guinea-pig and kept in normal Kreb's solution. The tracheal tube was cut into rings, 2–3 mm wide, each with two cartilages. Each ring was opened by a longitudinal cut on the ventral side, opposite to the smooth muscle, thus tracheal strips were made (Gilani et al., 2008; Khan et al., 2012). The tissues were suspended in 15 mL tissue baths containing Kreb's solution (pH 7.4) at 37 °C and aerated with carbogen. The composition of Kreb's solution was (mM): NaCl: 118.2, $NaHCO_3$: 25.0, $CaCl_2$: 2.5, KCl: 4.7, KH_2PO_4: 1.2, $MgSO_4{\cdot}7H_2O$: 1.2, and glucose: 11.7. A pre-tension of 1 g was applied to tracheal strips continuously throughout the experiment. The tissues were allowed to equilibrate for 1 h before the addition of any drug and were stabilized with a submaximal concentration of CCh (1 μM). Tissues were pre-contracted with CCh and high K^+, after sustained contractions were obtained, different doses (5–9) of the plant extract and standards were added cumulatively and relaxation was expressed as a percent of CCh and/or K^+ pre-contractions (Dar & Channa, 1997; Gilani et al., 2008). The period of dose administration was dependent on the time required for maximum relaxation achieved. For determination of the mechanism of tracheal relaxation, control isoprenaline inhibitory CRCs were constructed against the CCh-induced contractions. When the control CRCs of isoprenaline were found super-imposable (usually after two cycles), the tissue was pre-treated with the test substances for 30 min to test the possible potentiating effect. The CRCs of isoprenaline were reconstructed in the presence of different concentrations of the test material and compared with papaverine, a phosphodiesterase inhibitor (PDEI). Isometric responses were recorded on a Grass model 7 Polygraph (Grass Instrument Company, Quincy, MA).

Statistics

All the data are expressed as mean ± standard error of the mean (SEM), and the median effective concentrations (EC_{50} values) are given with 95% confidence intervals (CI). The statistical parameter applied is Student's *t*-test with $p < 0.05$ noted as significantly different (GraphPad Prism, GraphPad Software, San Diego, CA).

Results and discussion

Based on the medicinal use of *V. negundo* in hyperactive respiratory disorders, such as asthma, cough, and bronchitis, extracts from the leaves of *V. negundo* were found effective in various experimental models of asthma (Patel et al., 2009; Tandon, 2005). The extracts stabilized mast cells showed inhibitory effects on immediate hypersensitivity reactions and exhibited antieosinophilic activity, which were considered underlying mechanisms involved in the antiasthamtic effect of *V. negundo* (Patel et al., 2009). We used direct approaches and tested the extract of *V. negundo* for possible bronchodilatory effect in normotensive anesthetized rats.

Vn.Cr produced a dose-dependent bronchodilatory effect of the CCh-induced bronchospasm (Figure 1), similar to aminophylline, a known bronchodilator (Evans, 2006). The bronchodilatory effect was more significant ($p > 0.05$) at doses of 10, 30, and 50 mg/kg. To investigate the nature of bronchodilatory effect induced by *V. negundo*, further a systematic study was carried out on the tracheal smooth muscles. Tracheal strips from guinea pig were pre-contracted with CCh (1 μM) and high K^+. The crude extract was then added cumulatively to the induced contractions and was found comparatively more potent against CCh than high K^+ pre-contractions, similar to papaverine (Figure 2), a dual inhibitor of phosphodiesterase (PDE) and Ca^{++} channels (Boselli et al., 1998; Boswell-Smith et al., 2006). Diltiazem, unlike the crude extract and papaverine, was more potent against high K^+ than CCh pre-contractions (Figure 2), a characteristic of a typical Ca^{++} channel blockers (Godfraind et al., 1986). This finding suggests that the crude extract of *V. negundo* possesses possible papaverine-like PDE inhibitory constituents. The PDE inhibitory effect of the crude extract was further probed when pre-incubation of the tracheal strips with different concentrations of Vn.Cr shifted the isoprenaline-induced inhibitory CRCs to the left (Figure 2), similar to that caused by papaverine, as PDE inhibitors are known to potentiate the isoprenaline effect (Lorenz & Wells, 1983). However, diltiazem was without potentiating effect (Figure 2), as expected from a Ca^{++} channel blocker (CCB).

The contractions induced by high K^+(>30 mM) are dependent on the entry of Ca^{++} into the cells through voltage-dependent calcium channels (VDCs) (Bolton, 1979) and a substance which can inhibit high K^+-induced contractions is therefore considered to be a CCB (Godfraind et al., 1986). Thus, the inhibition of high K^+ (80 mM)-induced contraction by Vn.Cr also reflects restricted Ca^{++} entry via VDCs (Khan et al., 2013).

Relaxation of airways is associated with a net increase in the concentration of cyclic nucleotides as a consequence of inhibition of PDEs (Abdel-Latif, 2001) and Ca^{++} movements (Yan & Michael, 2006). The PDE inhibitors alone are considered very effective bronchodilators but have limited therapeutic uses due to cardiac stimulation (tachycardia), as a side effect (Raeburn et al., 1993). The co-existence of CCB constituents with PDE inhibitor is likely to offset the cardiac stimulation associated with PDE inhibitors when used alone. Our previous work (Khan et al., 2013) on the preliminary phytochemical analyses on the crude extract of *V. negundo*, revealed the presence of flavonoids and tannins. Flavonoids (Ko et al., 2004) and tannins (Kai et al., 1998) are known to possess PDE inhibitory and calcium channel blocking activities, respectively. The presence of flavonoids and tannins may be responsible for the PDE and calcium channel blocking activities of the extract of *V. negundo*. In the past, medicinal plants have shown therapeutic potential in the respiratory disorders, such as asthma (Edwards et al., 2012; Khan & Gilani, 2009), thus the presence of inhibitors of PDE

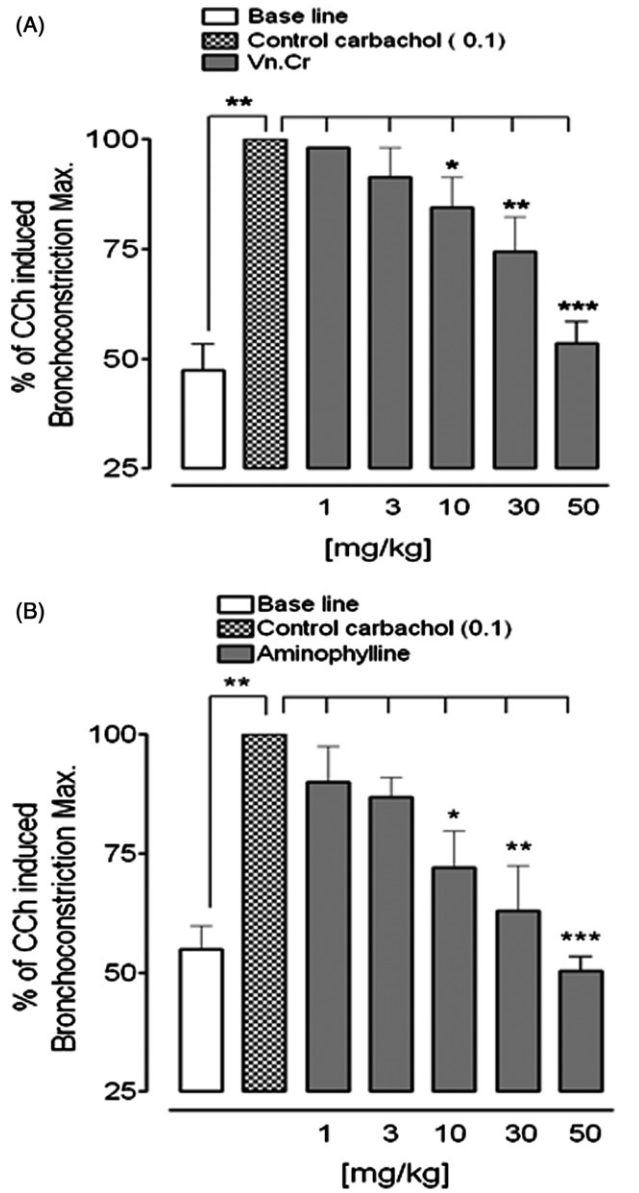

Figure 1. Dose-dependent effects of (A) *Vitex negundo* crude extract (Vn.Cr) and (B) aminophylline on the carbachol (CCh)-mediated bronchoconstriction in anaesthetized rats. Values shown are mean ± SEM, $n = 4$, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ versus carbachol (ANOVA followed by Dennett's test).

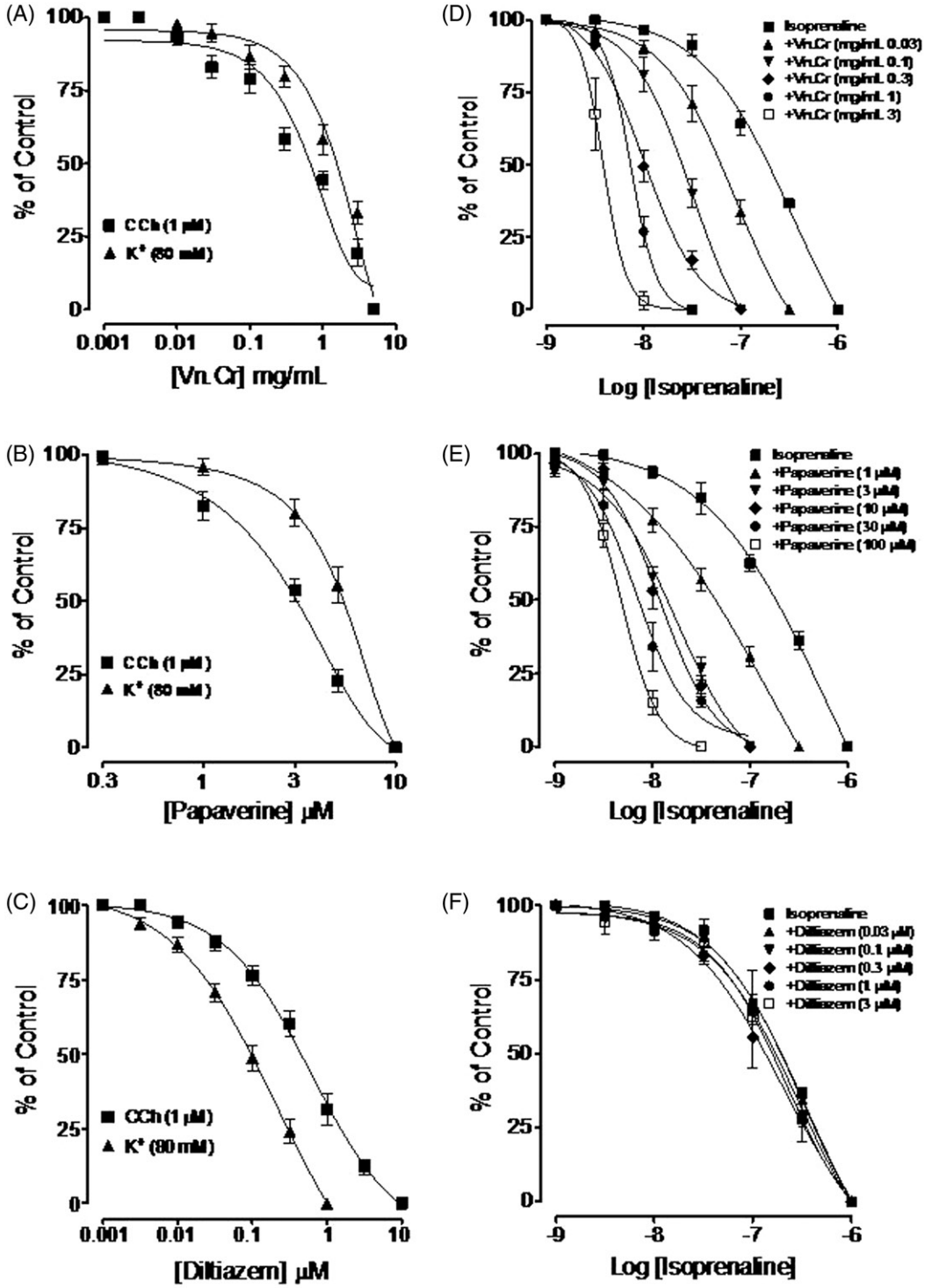

Figure 2. Concentration–response curves showing comparison of (A) *Vitex negundo* crude extract (Vn.Cr), (B) papaverine, and (C) diltiazem against carbachol (CCh) and K^+-induced contractions in isolated guinea-pig tracheal preparations. (D), (E), and (F) show inhibitory concentration–response curves of isoprenaline against carbachol (CCh)-induced contractions in the absence and presence of different concentrations of *Vitex negundo* extract (Vn.Cr), papaverine, and diltiazem in isolated guinea-pig tracheal preparations. Values shown are mean ± SEM, $n = 4$–5.

and Ca^{++} channels explains the underlying mechanisms responsible for the bronchodilatory effect of *V. negundo*.

In summary, these data indicate that the crude extract of *V. negundo* possesses a bronchodilator effect which is mediated through dual inhibition of phoshodiesterase and Ca^{++} channels. This study provides pharmacological rationale to the medicinal use of *V. negundo* in hyperactive respiratory disorders, such as asthma. Further studies of interest might be to explore the chemical constituents and molecular nature of its effect.

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Declaration of interest

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