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Study on the mechanism of the bronchodilatory effects of *Cynodon dactylon* (Linn.) and identification of the active ingredient

Maulik R. Patel, Yagnik S. Bhalodia *, Nimish L. Pathak, Maulik S. Patel, Kunal Suthar, Nilesh Patel, Dharmesh K. Golwala, Nurudin P. Jivani

C. U. Shah College of Pharmacy and Research, Faculty of Pharmaceutical Sciences, C. U. Shah University, Opp. I.B.P. Petrol Pump, Surendranagar–Ahmedabad Highway, Wadhwan 363030, Surendranagar, Gujarat, India

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ABSTRACT

Ethnopharmacological relevance: In the traditional medicine, *Cynodon dactylon* (Linn.) is used in asthma, but scientific studies to provide evidence for medicinal uses are sparse. Thus this study was undertaken to provide evidence for medicinal use in asthma as a bronchodilator, and to identify active ingredient(s).
Materials and methods: In vivo, acetylcholine (Ach)-induced bronchospasm was conducted in guinea pig while isolated rat tracheal strip was suspended in organ bath to measure the concentration response curve using multichannel data acquisition system.
Results: The chloroform extract of *Cynodon dactylon* (CECD) protected against Ach-induced bronchospasm in guinea pigs, similar to atropine. In the in vitro studies, CECD relaxed carbachol (CCh) and high K^+-induced contraction of rat tracheal strip, similar to atropine and verapamil respectively, suggesting antimuscarinic and calcium channel blocking (CCB) activities, which were confirmed by right ward shifting of CCh and Ca^{+2} concentration response curve (CRC). The phosphodiestrase (PDE) inhibitory activity was confirmed by potentiation of isoprenaline-induced inhibitory response, similar to papaverine. Densitometry analyses led to the identification of scopoletin as an active ingredient. Effectively, it significantly inhibited high K^+, and Ca^{+2} induced contractile response, similar to verapamil. The phosphodiestrase (PDE) inhibitory activity was confirmed by direct evidence of potentiation of isoprenaline-induced inhibitory response, similar to papaverine.
Conclusions: These results suggest that the bronchodilator activity of CECD is partly due to presence of scopoletin, and mediated possibly through CCB and PDE inhibition.

1. Introduction

Asthma is a chronic airway inflammatory disease characterized by airway obstruction, inflammation, hyperresponsiveness and remodeling (Barnes, 1996), associated with nonspecific infiltration of various inflammatory cells such as eosinophils, T-lymphocytes, macrophages, neutrophils, and epithelial cells (Brightling et al., 2002). In addition, symptoms are mediated by a wide range of compounds such as histamine, prostaglandins and leukotrienes, which subsequently lead to bronchoconstriction (Holgate and Polosa, 2008; Tattersfield et al., 2002).

Cynodon dactylon (L.) (Family: Poaceae) is a perennial grass, commonly known as "Doob" in India, possess various medicinal properties such as antimicrobial and antiviral activity (Dhar et al., 1968). Furthermore, the aqueous extract of this plant has anti-inflammatory (Sindhu et al., 2009), diuretic (Sadki et al., 2010), and anti-emetic (Ahmed et al., 1994) activity. It has been reported to possess anti-diabetic (Singh et al., 2007), anti-arrhythmic (Najafi et al., 2008), cardioprotective (Garjani et al., 2009), antioxidant (Sindhu et al., 2009), immunomodulatory (Mangathayaru et al., 2009), anti-anaphylactic and mast cell stabilizing (Savali et al., 2010) activity. *Cynodon dactylon* has been used over the centuries as an Indian traditional medicine for asthma (Kirtikar and Basu, 1980; Nadkarni and Nadkarni, 1976) no scientific data have been published, so far, supporting the ethnopharmacological claimed. The phytochemical studies on *Cynodon dactylon* revealed the presence of flavonoids, glycosides, saponins, tannins, carbohydrates and essential oil containing agropyrene, arunodin, furfural, furfural alcohol, 3-methoxy-4-hydroxy benzoic acid, phytol, β-sitosterol-D-glucoside, stigmasterol acetate, and phagostimulant phytone (6,10-14-trimethyl pentadecane-2-one) (Evans et al., 2002; Council of Scientific and Industrial Research, 1948).

Thus, this study was aimed to investigate bronchodilator activity and possible mechanisms responsible behind this effect, and to identify the active principle(s).

* Corresponding author. Tel.: +91 275 229 4003; fax. +91 275 224 0591.
E-mail address: yagnikbhalodia@gmail.com (M.R. Patel).

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2. Materials and methods

2.1. Reagents

Acetylcholine (Ach), carbachol (CCh), histamine, ketotifen, atropine (antimuscarinic agent), papaverine (phosphodiestrase enzyme inhibitor, PDE) and verapamil (calcium channel blocker, CCB) were purchased from Sigma, St. Louis, USA. All other chemicals used for making physiological salt solutions and phytochemical analyses were of analytical grade.

2.2. Animals

Hartley strain guinea-pigs (500–550 g) and Wistar albino rats (200–250 g) of either sex were housed at animal house of C. U. Shah College of Pharmacy and Research, maintained at 23–25 °C. Animals were given tap water ad libitum and a standard diet. Experiments performed complied with rulings of Committee for Purpose of Control and Supervision on Experimental Animal (CPCSEA) (Reg. no. 985/ac/06/CPCSEA/dated 10th Oct. 2006) and were approved by Institutional Animal Ethical Committee (IAEC) of the C. U. Shah College of Pharmacy and Research (CCPR/IAEC/16/Jan. 2013).

2.3. Plant material and extraction

Cynodon dactylon (L.) were collected from local area of Wadhwan, Gujarat, India and authenticated by Mr. Sachin G. Gadhiya, a botanist at the Agriculture department, Surendranagar and voucher specimen was submitted to the herbarium at Department of Agriculture, Surendranagar, Gujarat, India.

The whole plant was cleaned and washed with 2% $KMnO_4$ in distilled water and then dried under shade, until it was free from moisture. The grass was subjected to get coarse powder and then passed through sieve no. 44. The sieved powder was stored in air-tight container before extraction. The powder was soaked in the chloroform for 3 days and filtered through Whatmann (Maidstone, UK) no.1 filter paper. The filtrates so collected were pooled and evaporated on rotary evaporator (Yamato Rotary Evaporators, RE801) under reduced pressure to obtain thick paste like mass; the chloroform extract of *Cynodon dactylon* (CECD), having yield of 26.6% (w/w).

2.4. In vivo studies

2.4.1. Ach and histamine-induced bronchospasm in Guinea pig

Fourty five hartley strain guinea pigs were selected and randomly divided into nine groups ($n=5$). Each individual guinea pig was placed in an aerosol chamber ($24 \times 14 \times 24\ cm^3$) made of Perspex glass and challenged with Ach (0.5% v/v) aerosol using an electronic nebulizer. The pre-convulsion time (PCT) i.e. the duration of aerosol exposure to the onset of respiratory distress leading to the appearance of convulsion, was noted (Herxheimer, 1952). Guinea-pig was removed from the Perspex container at this time and allowed to recover from respiratory distress for 24 h. After 24 h the animals of Group I received vehicle (0.9% W/V NaCl solution, p.o), Group II-V received 5, 10, 50, and 100 mg/kg oral dose of CECD, respectively, and Group VI-IX received 5, 10, 50, and 100 mg/kg oral dose of atropine, respectively. Following an administration of vehicle, CECD, and atropine for 2 h, the PCT was reassessed and % increase in PCT was calculated as per prescribed formula (Sheth et al., 1972). Following the fifteen days of washout period, the same experiment was performed using histamine (0.5% v/v) aerosol. Ketotifen was used as a standard drug in place of atropine on histamine induced bronchospasm in guinea pig.

2.5. In vitro studies

2.5.1. Preparation of isolated rat tracheal strip

Fifty five wistar albino rats (200–250 g) of either sex were sacrificed by cervical dislocation and exsanguination. The thoracic content was placed in a 200 ml oxygenated (5% CO_2+95% O_2) dissection bath filled with normal Krebs solution (mM: KCl 4.7, NaCl 118.2, $NaHCO_3$ 25.0, $CaCl_2$ 2.5, KH_2PO_4 1.3, $MgSO_4$ 1.2 and glucose 11.7 (pH 7.4)) at room temperature. The cervical trachea was dissected free and 3–4 airway rings were then cut transversely, each containing two cartilaginous rings. The airway rings were opened longitudinally along the anterior, cartilaginous part and connected to steel hook as isolated strip. This preparation was mounted in organ bath containing normal Krebs solution, which was maintained at 37 ± 2 °C and continuously aerated with carbogen (5% CO_2+95% O_2). One gm of tension was applied, and kept constant throughout the experiment. At the end of equilibration period of 1 h, the tracheal strip was tested for contractile responses to carbachol (CCh, 1 μM), calcium (Ca^{+2}, 10 mM) and potassium (K^+, 80 mM) repeatedly. The plant materials and standards were then added in cumulative fashion to obtain concentration response curves (CRCs), which was measured with isometric force transducer, connected to multichannel data acquisition system (physiopac, Medicaid system, Chandigarh) (Rehman et al., 2012).

2.5.2. CCh-induced contraction of isolated rat tracheal strip

Isolated rat tracheal strip was incubated in normal Krebs solution and CCh-induced control CRC was constructed. Different concentration of the plant material was pre-incubated to tracheal strip for 45 min, and CCh-induced CRC was reconstructed. The result was compared with atropine (Rehman et al., 2012).

2.5.3. High K^+-induced contraction of isolated rat tracheal strip

To assess CCB activity, bath solution (normal Krebs solution) was replaced by high K^+- Krebs solution (mM: KCl 80, NaCl 118.2, $NaHCO_3$ 25.0, $CaCl_2$ 2.5, KH_2PO_4 1.3, $MgSO_4$ 1.2 and glucose 11.7), to depolarize isolated trachea which produced a sustained contraction. Concentration-dependent inhibitory response was then constructed with the pre-incubation of test material (Rehman et al., 2012).

2.5.4. Ca^{+2}-induced contraction of isolated rat tracheal strip

To confirm the Ca^{2+} antagonist effect of the test substance, normal Krebs solution was replaced by Ca^{2+} free Krebs solution containing EDTA (0.1 mM). The tissue was allowed to rest for 30 min, in order to remove Ca^{2+} from the tissue. The tissue was further exposed to EDTA containing K^+-rich and Ca^{2+}-free Krebs solution (mM: KCl 50, NaCl 91.04, $MgCl_2$ 1.05, $NaHCO_3$ 11.90, NaH_2PO_4 0.42, glucose 5.55 and EDTA 0.1). Following an incubation period of 45 min, control CRC of Ca^{2+} was obtained. When the control CRC was found super imposable, the tissue was pre-incubated with the different concentrations of plant extract for 60 min in order to test the CCB activity. The CRC of plant extract was compared with verapamil (Rehman et al., 2012).

2.5.5. Isoprenaline-induced inhibitory contraction of isolated rat tracheal strip

In order to assess phosphodiestrase (PDE) inhibition activity, rat trachea was suspended in bath solution (normal Krebs solution), and control isoprenaline-induced inhibitory CRC was constructed against the CCh-induced contraction. When the control CRC of isoprenaline was found superimposable, the tissue was pre-incubated with the plant extract and standard (papaverine) for