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# Effects of umbelliferone in a murine model of allergic airway inflammation

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ABSTRACT

The therapeutic effects of umbelliferone (30, 60 and 90 mg/kg), a coumarin isolated from *Typha domingensis* (Typhaceae) were investigated in a mouse model of bronchial asthma. BALB/c mice were immunized and challenged by nasal administration of ovalbumin. Treatment with umbelliferone (60 and 90 mg/kg) caused a marked reduction of cellularity and eosinophil numbers in bronchoalveolar lavage fluids from asthmatic mice. In addition, a decrease in mucus production and lung inflammation were observed in mice treated with umbelliferone. A reduction of IL-4, IL-5, and IL-13, but not of IFN-γ, was found in bronchoalveolar lavage fluids of mice treated with umbelliferone, similar to that observed with dexamethasone. The levels of ovalbumin-specific IgE were not significantly altered after treatment with umbelliferone. In conclusion, our results demonstrate that umbelliferone attenuates the alteration characteristics of allergic airway inflammation. The investigation of the mechanisms of action of this molecule may contribute for the development of new drugs for the treatment of asthma.
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## 1. Introduction

Coumarins constitute a very large class of compounds present in several species belonging to different botanical families, which are widespread in the world. They are secondary metabolites naturally occurring in different parts of the plants, such as roots, flowers and fruits (Leal et al., 2000; Ribeiro and Kaplan, 2002; Razavi et al., 2008). The search for useful pharmaceutical has led to a resurgence of interest in coumarins because these substances display potent and relevant pharmacological activities that are structure-dependent, while at the same time appearing to lack toxicity in mammalian systems (Hoult and Payá, 1996).

Among the biological effects of coumarins are anti-microbian (Thati et al., 2007), anti-viral (Mazzei et al., 2008), anti-thrombotic, vasodilatory (Casley-Smith et al., 1993), antitumoral, antineoplasic, antiinflammatory (Hoult and Payá, 1996; Lino et al., 1997), and antimetastatic properties (Jiménez-Orozco et al., 2001; Finn et al., 2004; Finn et al., 2005; Elinos-Báez et al., 2005), and inhibition of acetylcholinesterase and angiotensin converting enzyme (Barbosa-Filho et al., 2006a,b). In humans, coumarins have a short half-life, and its major biotransformation product (75%) is umbelliferone (Hoult and Payá, 1996; Egan et al., 1997).

Umbelliferone or 7-hydroxycoumarin is a widespread natural product of the coumarin family (Fig. 1). It occurs in many familiar plants from the Apiaceae (Umbelliferae) family such as carrot, coriander and garden angelica, as well as plants from other families such as the mouse-ear hawkweed. It is a yellowish-white crystalline solid which has a slight solubility in hot water, but high solubility in ethanol (Dean, 1963).

Asthma is a chronic inflammatory disorder of the airways in which many cells and elements play a role, associated with increased airway hyperresponsiveness, leading to recurrent episodes of wheezing, breathlessness, chest tightness, and coughing (Barnes, 2001). This chronic inflammation is characterized by infiltration of eosinophils, neutrophils, mast cells and T cells (Casale et al., 1987; Wenzel, 2003), and is promoted by Th2 type immune responses (Barnes, 2001; El Biaze et al., 2003; Reed, 2006). In the current study we aimed to evaluate the effects of umbelliferone, a coumarin purified from *Typha domingensis* (Typhaceae), in a mouse model of asthma, as a potential anti-asthmatic agent.

## 2. Materials and methods

### 2.1. Plant material

The aerial parts of *T. domingensis* Pers. were collected for first extracts in March, 2002 in Bravo, State of Bahia, in temporary shallow lake in Dryland "Caatinga" Bioma and after in December, 2005 near the city of Santa Rita, State of Paraíba, Brazil. The plant was identified by Ana Maria Giulietti and Maria de Fátima Agra and the voucher specimens (Giulietti 2035 et al. and Agra 5520 *et* Góis — JPB) were deposited in the Herbaria HUEFS and JPB.

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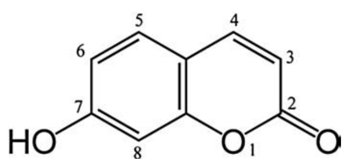


**Fig. 1.** Chemical structure of umbelliferone.

### 2.2. Extraction and isolation of umbelliferone

The aerial parts (2000 g) of *T. domingensis* were extracted with 95% ethanol at room temperature. The extract (150 g) was evaporated under vacuum to yield a brown residue which was suspended in methanol: $H_2O$ (3:7 v/v) and fractionated with chloroform and ethyl acetate. The chloroform phase (25 g) was subjected to column chromatography over silica gel, eluted with hexane, chloroform and methanol mixtures in an increasing order of polarity, yielding three coumarins and one cinnamic acid derivative. The ethyl acetate phase (25 g) was subjected to chromatography column over Sephadex LH-20 using methanol yielding two flavonoids. The isolated compounds were identified as coumarin (0.0044%), scopoletin (0.0014%) (Brateoeff and Perez-Amador, 1994), umbelliferone (0.007%) (Cussans and Huckerby, 1975), *p*-coumaric acid (0.0016%), (Pouchert, 1992), quercetin (0.0028%) (Williams et al., 1971) and isorhamnetin-3-*O*-glucoside (0.0039%) (Bilia et al., 1994) based on $^{1}$H NMR, $^{13}$C NMR, COSY, HMQC and HMBC spectroscopic data and comparison with those reported in the literature.

### 2.3. Animals

Male BALB/c mice, 4–6 weeks old, were used in the experiments. All animals were raised and maintained at the animal facilities of the Gonçalo Moniz Research Center, FIOCRUZ/BA, in rooms with controlled temperature (22 ± 2 °C) and humidity (55 ± 10%) and continuous air renovation. Animals were housed in a 12 h light/12 h dark cycle (6 am–6 pm) and provided with rodent diet and water *ad libitum*. Animals were handled according to the NIH guidelines for animal experimentation. All procedures described here had prior approval from the local animal ethics committee.

### 2.4. Sensitization and challenge with ovalbumin and treatment

Allergic airway inflammation was induced as described before (Vasconcelos et al., 2008). Groups of seven mice received systemic immunization by subcutaneous injection of 10 μg of chicken egg ovalbumin (Grade V, >98% pure; Sigma, St Louis, MO) diluted in 2 mg/ml alum (AlumImject; Pierce, Rockford, IL) followed by a booster injection at day 14. A nasal challenge was performed starting at day 28, by inhalational exposure to aerosolised ovalbumin for 15 min/day, on five consecutive days. Exposures were carried out in an acrylic box. A solution of 1% ovalbumin in saline was aerosolised by delivery of compressed air to a sidestream jet nebuliser (RespiraMax, NS, Brazil). Two hours before each aerosol delivery, mice were treated orally with umbelliferone (30, 60, and 90 mg/kg), dexamethasone (30 mg/kg) or vehicle (10% DMSO in saline).

### 2.5. Collection of blood and bronchoalveolar lavage

Twenty four hours after the last inhalational exposure, mice were anesthetized and bled via the brachial plexus for collection of blood samples used to estimate the IgE production. Bronchoalveolar lavage (BAL) was performed twice by intratracheal instillation of 1 ml of PBS. The first lavage fluid was centrifuged, and aliquots of the supernatant were kept at 70 °C until use for cytokine measurements. The second lavage fluid was centrifuged and the two cell pellets were resuspended in a PBS final volume of 1 ml. The number of total leukocytes in bronchoalveolar lavage fluid was estimated in a Neubauer chamber. Differential counts were obtained using panotic-stained cytospin preparations. A differential count of 200 cells was made in a blinded fashion and according to standard morphologic criteria.

### 2.6. Histopathological and morphometric analysis

The right lobe of the lungs from each animal was removed for histological analysis. The lung was inflated via the tracheal cannula with 4% buffered formalin, fixed in the same solution, and embedded in paraffin. Sections were stained with hematoxylin and eosin for quantification of inflammatory cells by optical microscopy. For each lung 10 fields (400×) were analyzed per section, and the data used to calculate the mean number of cells per $mm^2$. Mucus production was

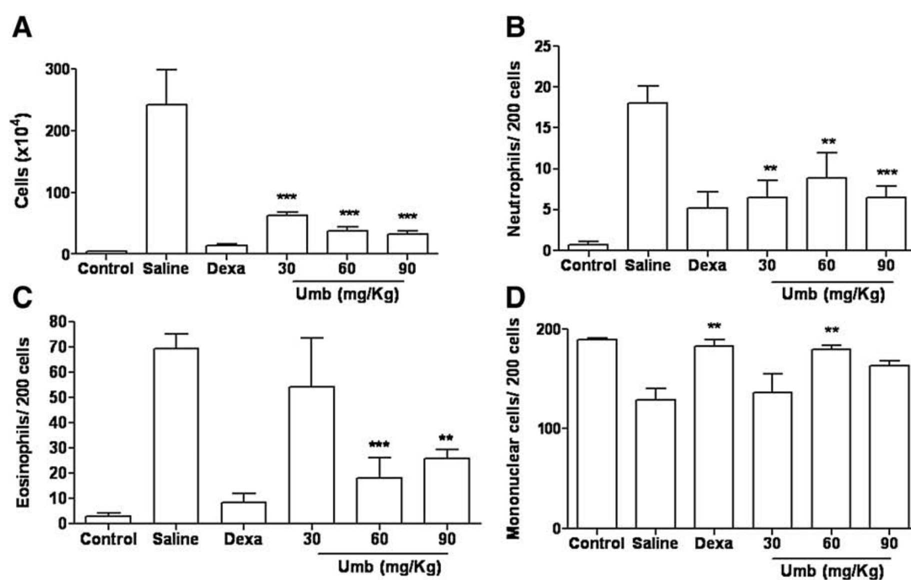


**Fig. 2.** Total cell counts and leukocyte quantification in BAL samples obtained from mice submitted to different treatments. Mice were sacrificed 24 h after the last challenge with ovalbumin. The cellularity in BAL fluid from control or ovalbumin-challenged mice treated with saline, dexamethasone (dexa) or umbelliferone (umb) was evaluated. A, Total cell counts. B, Number of neutrophils in 200 cells. C, Number of eosinophils in 200 cells. D, Number of mononuclear cells in 200 cells. Values are expressed as means ± S.E.M. of 6–7 mice per group, in one of two experiments performed. ***$P<0.001$.

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