A multicomponent nutraceutical (*Perilla frutescens*, quercetin, and vitamin D3) as add-on therapy in patients with grass pollen-induced mild persistent asthma and rhinitis

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Abstract

A multicomponent nutraceutical containing *Perilla frutescens*, quercetin, and vitamin D3 significantly prevented AR exacerbations in children. Thus, the current study explored the add-on use in adult patients with mild persistent asthma and AR due to grass pollen allergy. The treatment lasted three months. Asthma and AR symptoms, asthma control test, spirometry, nasal eosinophils, and use of rescue medications (SABA and NC) were evaluated in the previous grass season and throughout the treatment. All patients were treated with ciclesonide (320 µg/day) and cetirizine (10 mg/day). Patients were randomly stratified into Group A, taking the nutraceutical, and Group B using the predetermined therapy. In this study, 90 patients (13-59 years old) were enrolled, and 84 completed the trial. Group A significantly improved all outcomes ($p<0.001$). Group B did not achieve an improvement in AR symptoms, nasal eosinophils, and nasal steroid use. The intergroup analysis showed that Group A patients experienced less severe bronchial symptoms (- 32 %), AR symptoms (- 39 %), better asthma control (+ 38 %), higher FEV_1 (+ 10 %), lower SABA (- 30 %) and NC use (- 41 %), and nasal eosinophils count (- 35 %) than Group B ($p<0.0001$ for all). No clinically relevant adverse events occurred. A multicomponent nutraceutical containing *Perilla frutescens*, quercetin, and vitamin D3, as an add-on treatment to inhaled ciclesonide and cetirizine, provided a clinically relevant benefit in patients with mild persistent asthma and AR due to grass pollen uncontrolled by standard therapy. Therefore, this compound could be fruitful in clinical practice.

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Conflict of interest: the authors declare no conflict of interest.

Ethics approval and consent to participate: the Ethics Committee of Cuasso al Monte Hospital approved this study (019/2021). The study is conformed with the Helsinki Declaration of 1964, as revised in 2013, concerning human and animal rights.

Informed consent: all patients participating in this study signed a written informed consent form for participating in this study.

Patient consent for publication: written informed consent was obtained from a legally authorized representative(s) for anonymized patient information to be published in this article.

Availability of data and materials: all data generated or analyzed during this study are included in this published article.

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Introduction

Asthma is an inflammatory disease characterized by episodic symptoms, airway hyperresponsiveness, and reversible bronchial obstruction.[1] The asthma definition includes several phenotypes and endotypes.[2] Allergic asthma is a frequent endotype, mainly in young people, and subtends a type 2 immune response, characterized by a T cell helper 2 polarization and eosinophilic infiltrate of airways.[3] Asthma frequently associates with Allergic Rhinitis (AR), the most common immunoglobulin E (IgE)-mediated condition.[4] Asthma and AR share type 2 inflammation and airflow limitation.[5] Indeed, eosinophilic infiltration into airways is a peculiar feature in allergic patients when exposed to the causal allergen.[6] In addition, allergic inflammation causes symptom occurrence.

Consequently, allergen exposure is the *conditio sine qua non* to start and maintain allergic inflammation. Allergic inflammation in the airways essentially results in airflow obstruction, particularly eosinophil count positively correlates with airflow.[7-9] Consistently, eosinophil count obtained by nasal cytology is a reliable biomarker of allergic inflammation severity and predicts symptom relevance.[8,9]

In addition, two seminal studies using the allergen provocation model provided an elegant demonstration of the concept of united airways: the nasal challenge also drives bronchial inflam-

mation, and the bronchial challenge contextually leads to nasal inflammation.[10-11]

The practical translation of this evidence is to treat patients with asthma and AR holistically, thus targeting type 2 inflammation. There is evidence that dampened inflammation may carry forward asthma control, the main target in the management of patients with asthma.[12] However, optimal asthma control is achieved by not even half of the patients.[13] Uncontrolled and partially controlled asthma significantly favor the evolution towards severe asthma and fixed obstruction over time.[14] Many factors influence asthma control, including poor adherence to treatments, comorbidity, and exposure to high allergens.[15] Therefore, a thorough workup and periodic clinical reassessment are mandatory in managing patients with asthma.[1] In this context, standard therapies are not always sufficient in achieving adequate asthma control, even with high dosages. As a result, it could be an attractive option to use add-on nutraceuticals to improve asthma and AR management.[16]

A multicomponent nutraceutical containing *Perilla frutescens*, quercetin, and vitamin D3, has been positioned as an add-on therapy in pediatric AR.[17] *Perilla frutescens* is an aromatic annual plant belonging to the Lamiaceae family also known as aegoma or shiso; it is the most important species belonging to the *Perilla* genus and is widely cultivated in India, Vietnam, Korea, China and Japan. There is evidence that this nutraceutical significantly prevented the risk of AR exacerbations,[18,19] reduced the incidence of respiratory infections and use of symptomatic medications, and improved lung function in children.[20-22] Therefore, the present study aimed to investigate the add-on role of this nutraceutical in adults with asthma and AR due to pollen allergy.

Materials and Methods

This study included a group of patients with grass pollen-induced mild persistent asthma and AR. The study design was randomized, controlled, and open. The local Ethic Committee of the Cuasso al Monte Hospital approved the procedure; patients signed an informed consent.

The inclusion criteria were documented diagnosis of mild persistent asthma and AR, allergy only to grass pollens and adulthood. Exclusion criteria were perennial AR, allergy to other allergens, uncontrolled and intrinsic asthma, and the presence of any comorbidity and/or concomitant medication able to interfere with the interpretation of the results.

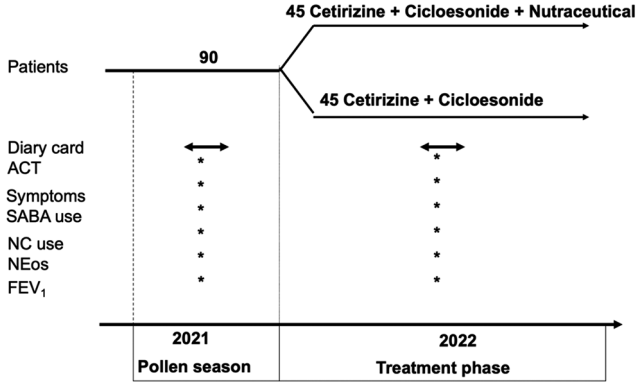

Figure 1. Study design of the trial.

The diagnosis of asthma was performed according to validated criteria recommended by the Global Initiative for Asthma (GINA) guidelines.[1] It included a history of respiratory symptoms compatible with asthma and documentation of bronchial obstruction reversibility after bronchodilation testing or bronchial airflow limitation after the methacholine challenge. The asthma work-up excluded confounding diseases, such as chronic bronchitis, bronchiectasis, cystic fibrosis, primary ciliary dyskinesia, aspergillosis, and eosinophilic pneumonia.

AR to grass pollen diagnosis was considered if there was a documented sensitization to grass pollens, assessed by skin prick test and/or measurement of serum allergen-specific IgE, and a demonstrated cause/effect relationship between exposure to grass pollen allergen and symptom occurrence.[16]

The study protocol was approved by the local Ethics Committee; the study was conducted in accordance with the ethical standards established by the Declaration of Helsinki (revised in 2000). An informed consent was signed by all participants before enrolment in the study.

All patients took inhaled ciclesonide (320 µg/day) and cetirizine tablet (10 mg/day) from April 2022 to June 2022, as well as in the previous grass pollen season 2021. The investigator (MM) instructed all patients about the correct technique of taking the inhaled drug.

Patients were randomly (ratio 1:1) assigned into two groups: Group A (active group) and Group B (control group). Patients assigned to Group A also took the multicomponent nutraceutical two tablets/day for 90 days.

The multicomponent nutraceutical (Quertal®) contains *Perilla frutescens* 80 mg (as dry extract), quercetin 150 mg, and vitamin D3 5 µg (200 IU).

Patients were evaluated throughout the grass pollen season in 2021 and throughout the treatment period, as schematically reported in Figure 1. Two visits were performed: at baseline and at the end of treatment; thorough history and complete physical examination were performed at both visits.

The considered parameters included assessment of respiratory (upper and lower airways) symptoms, symptomatic use of rescue medications (SABA and NC), nasal eosinophils, lung function, and asthma control test.

Upper Airway Symptoms (UAS) included nasal symptoms (nasal itching, sneezing, watery rhinorrhea, and congestion) plus ocular symptoms (conjunctival itching, lacrimation, and eye redness). Symptoms were scored using a four-point scale (0=absent; 1=mild; 2=moderate; 3=severe). A global symptom score was calculated as the sum of daily scores throughout the entire pollen season.

Lower Airway Symptoms (LAS) were cough, wheezing, and breathlessness measured as well as upper respiratory symptoms.

Patients recorded symptoms using a diary card collected during the final visit at the end of the treatment (July 2022).

All patients were allergic to grass pollens present along the spring in the considered geographic area (https://www.regione.lombardia.it). Consequently, patients were just symptomatic at the beginning of the treatment (April).

The Asthma Control Test (ACT) questionnaire was administered to all patients. ACT questionnaire consists of 5 questions with five possible responses, exploring the patient's perception of their asthma control.[23] The result could range between 0 and 25, where 25 was the optimal asthma control.

Nasal cytology is a well-defined methodology.[24] The procedure included sampling, processing, and microscope reading. Sampling required collecting cells from the surface of the middle portion of the inferior turbinate by a sterile disposable curette. The

procedure was performed under anterior rhinoscopy with an appropriate light source, and it was painless. The sample obtained was immediately smeared on a glass slide, air-dried, and stained with May-Grünwald-Giemsa (MGG) for 30 minutes. Next, the stained sample was read at optical microscopy with a 1000x objective with oil immersion. Slide reading was conducted blindly by an experienced cytologist, thus ensuring methodological correctness. The count of eosinophils, neutrophils, and mast cells was expressed as a mean of 10 microscopic fields.

A pulmonary function test was performed by spirometry. The results were interpreted using American Thoracic Society and European Respiratory Society recommendations.[25] Forced expiratory volume in one second (FEV_1) was expressed as the percent of the predicted value.

Symptomatic use (such as on-demand) of intranasal corticosteroid (budesonide 100 µg, one puff per nostril, twice a day) and inhaled albuterol (100 µg, one puff) were recorded and considered as monthly days of use.

The adherence to prescribed drugs was assessed at the end of treatment.

The safety was evaluated by recording the occurrence of adverse events reported in the diary card.

The statistical analysis was performed using the Wilcoxon test (Kruskal-Wallis) for intra-group comparisons and the T-test (Mann-Whitney) for inter-group comparisons; differences were calculated and analyzed between the two pollen seasons.

Results

The study population included 90 patients: 45 patients (22 females and 23 males, mean age 25±10 years, age range 13-58) in Group A and 45 (20 females and 25 males, mean age 27±13 years, age range 13-57) in Group B.

Six patients dropped out, so 84 patients were analyzed.

Intragroup analysis

The comparison considered the scores collected through the two grass pollen seasons (2021 and 2022).

Group A

UAS significantly decreased after treatment: Δ score – 89.1 ($p<0.001$), as reported in Figure 2.

LAS significantly decreased after treatment: Δ score – 154.2 ($p<0.0001$), as reported in Figure 3.

FEV_1 significantly ($p<0.001$) increased (+ 15.6) as well as ACT score significantly ($p<0.001$) improved (+ 11), as reported in Figures 4 and 5.

The number of nasal eosinophils significantly ($p<0.01$) decreased (-10%) as reported in Figure 6.

The symptomatic use of inhaled albuterol and intranasal budesonide significantly ($p<0.001$ and <0.01, respectively) decreased (-12.1 and -7.1, respectively), as reported in Figures 7 and 8.

Group B

UAS slightly decreased after treatment: D score – 12.2 ($p=0.53$ as reported in Figure 2.

LAS significantly decreased after treatment: D score – 98.1 ($p<0.01$), as reported in Figure 3.

FEV_1 significantly ($p<0.05$) increased (+ 5.7) as well as ACT score (+ 7.2) ($p<0.05$) as reported in Figures 4 and 5.

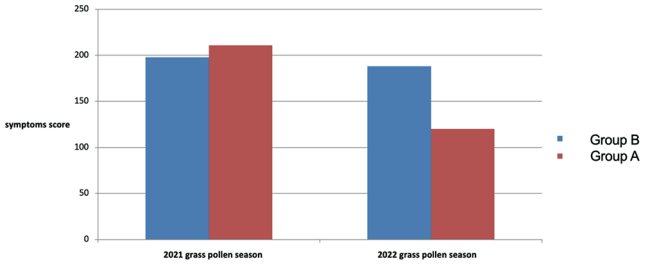

Figure 2. Mean scores of upper airways symptoms (UAS) recorded during the 2021 and 2022 grass pollen seasons in Group A and Group B.

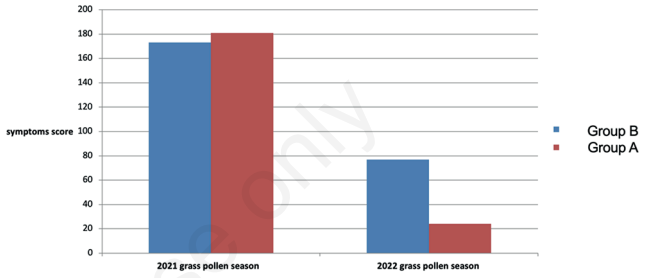

Figure 3. Mean scores of lower airways symptoms (LAS) recorded during the 2021 and 2022 grass pollen seasons in Group A and Group B.

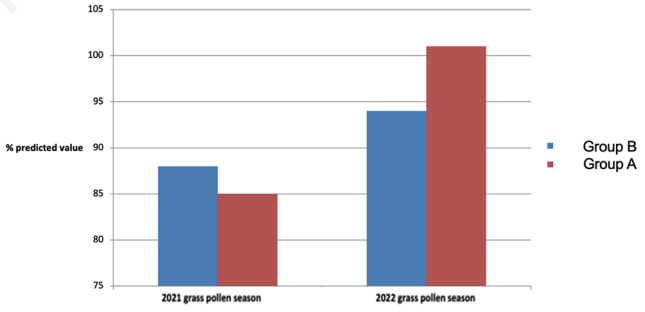

Figure 4. Mean FEV_1 values recorded during the 2021 and 2022 grass pollen seasons in Group A and Group B.

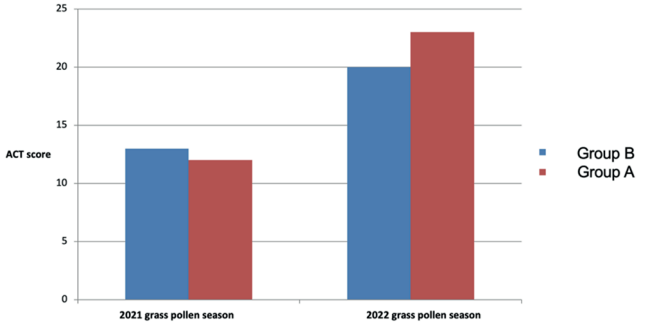

Figure 5. Mean asthma control test (ACT) scores recorded during the 2021 and 2022 grass pollen seasons in Group A and Group B.

The number of nasal eosinophils slightly (p=0.44 decreased (-2.5) as reported in Figure 6.

The symptomatic use of inhaled salbutamol significantly (p<0.01) decreased (-7), whereas the use of intranasal budesonide, was substantially unchanged (-0.9), as reported in Figures 7 and 8.

Intergroup analysis

The comparison considered the scores collected through the 2022 pollen seasons, such as during the treatment period.

UAS decreased more significantly in Group A than in Group B (p<0.0001), with an improvement of + 39% in Group A.

LAS decreased more significantly in Group A than in Group B (p<0.0001), with an improvement of + 32% in Group A.

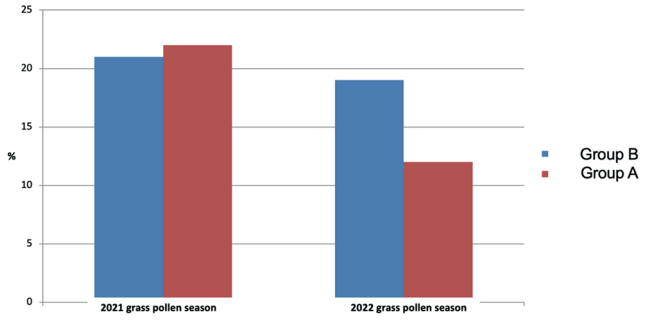

Figure 6. Mean nasal eosinophil counts recorded during the 2021 and 2022 grass pollen seasons in Group A and Group B.

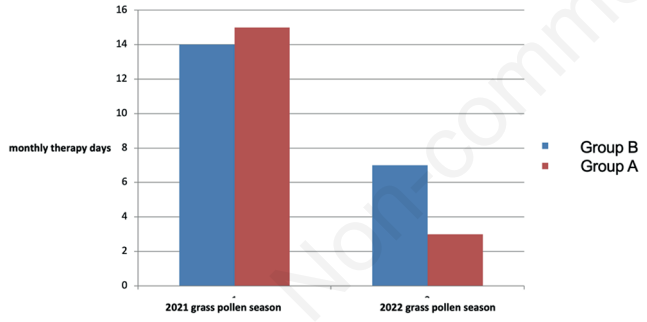

Figure 7. Mean monthly therapy days with albuterol on demand recorded during the 2021 and 2022 grass pollen seasons in Group A and Group B.

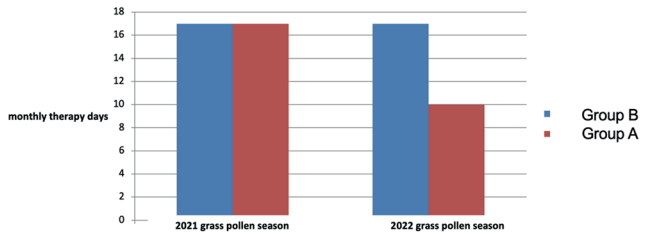

Figure 8. Mean monthly therapy days with intranasal budesonide on demand recorded during the 2021 and 2022 grass pollen seasons in Group A and Group B.

Mean FEV_1 values were significantly (p<0.0001) higher in Group A, with an improvement of + 10% in Group A.

The mean ACT score was significantly (<0.0001) higher in Group A, with an improvement of + 38% in Group A.

Nasal eosinophils were significantly (p<0.0001) lower in Group A, with an improvement of + 35% in Group A.

The symptomatic use of intranasal budesonide was significantly (p<0.0001) lower in Group A, with a reduction of - 41% in Group A.

The symptomatic use of inhaled albuterol was significantly (p<0.0001) lower in Group A, with an improvement of - 30% in Group A.

Adherence to prescribed drugs

The adherence rate was high as it ranged between 81% and 98%.

Safety

The treatments were safe and well tolerated in all patients.

Discussion

Pollen allergy represents a typically seasonal condition. Namely, allergic patients experience symptoms only during the pollen season; consistently, allergic inflammation persists throughout the pollination period.[26] As a result, the treatment period should be limited to the period of allergic exposure.

Patients with grass allergy frequently have an involvement of the entire respiratory tract. It follows that the management of these patients has to be integrated and therefore target both the upper and lower airways. Inhaled corticosteroids and oral antihistamines are first-line drugs in treating respiratory allergies. However, this combination does not always achieve optimal relief of allergic symptoms and control of allergic inflammation.[27] Therefore, there is a growing interest in using nutraceuticals as add-on remedies to conventional therapy. In this regard, a multicomponent nutraceutical containing *Perilla*, quercetin, and vitamin D3, has been successfully used in children with AR.[18-22] Therefore, the present study explored its potential role also in adults with a grass allergy.

This study demonstrated that a three-month course of this nutraceutical, used as an add-on to the standard therapy for asthma and allergic rhinitis, provided a significant improvement of the investigated parameters. Firstly, the ancillary use of this nutraceutical significantly reduced the severity of respiratory symptoms, concerning both nasal and bronchial complaints. In addition, the add-on treatment with this nutraceutical significantly improved the lung function, as demonstrated by the increased values of the FEV_1. Consistently, the nutraceutical allowed to achieve a better asthma control in comparison with standard therapy alone, as showed by the higher ACT scores in active group. In addition, the nutraceutical provided a more marked dampening of type 2 inflammation as demonstrated by a more pronounced reduction of infiltrating eosinophils in active group. The control of inflammation and improved lung function resulted in a reduced use of symptomatic drugs, such as albuterol and intranasal corticosteroids.

These outcomes could depend on the multifaceted activities exerted by the components of this nutraceutical.

The dry extract of *Perilla frutescens* seed contains rosmarinic acid and other flavonoids, such as luteolin, apigenin, and crhysoeriol; all of them have a well-documented *in vivo* and *in vitro* anti-allergic activity.[28]

Curiously, the leaves of *Perilla frutescens* are a popular garnish in Japan, as they are employed to antagonize fish and crab meat allergy, and also are used as a food colorant. Other medical indications include sedation and treatment of indigestion and food poisoning. About the anti-allergic activity of rosmarinic acid, a murine model demonstrated the significant suppression of passive cutaneous anaphylaxis reaction.[29] A *Perilla*-derived methoxyflavanone also inhibited the *in vitro* IgE-mediated histamine release from a basophilic cell culture and *in vivo* prevented allergic rhinitis-like nasal symptoms in a murine model of Japanese cedar pollinosis.[30] *Perilla* also has significant inhibitory activity against both 5-lipoxygenase and 12-lipoxygenase, key enzymes in one of the pathways of allergy and inflammation.[31] Luteolin has been recognized as an antioxidant scavenger of damaging free radicals and inhibits protein kinase C, *i.e.*, key regulator of inflammatory events and smooth muscle constriction.[32] Luteolin is also a potent inhibitor of mast cell activation as could completely block the release of histamine and pro-inflammatory cytokines.[33] Luteolin also reduced interleukin (IL)-4 and IL-5 and increased interferon (IFN)-γ at bronchial level in an asthma model.[34] Apigenin is another flavonoid able to suppress IgE and IL-4 production.[35]

The name quercetin comes from the Latin word *quercetum* meaning oaks' forest. Namely, *Quercus* is a genus of plants belonging to the Fagaceae family, and several oak's species exist. All of them are source of quercetin. Quercetin is a bioflavonoid, belonging to the class of polyphenols, found also in red wine, grapefruit, onions, apples, black tea, and, in lesser amounts, in leafy green vegetables and beans.[36] However, the caper, *Shopora japonica,* and St. John's Wort (*Hypericum*) are the plants that contain the most by weight.

Quercetin has a strong affinity for mast cells and basophils and tends to stabilize their cell membranes, so blocking degranulation, and inhibiting the release of pro-inflammatory mediators and cytokines implicated in allergic inflammation.[37] In particular, a placebo-controlled study showed that 8-week quercetin course significantly reduced ocular symptoms in patients with Japanese cedar allergy.[38] Using the same clinical model, a preventive activity was also documented on conjunctival symptoms.[39] Therefore, all these outcomes confirm and underline its anti-allergic activity.

Vitamin D3 exerts relevant effects on the immune system.[40] Consequently, vitamin D3 is useful in managing AR and asthma as it exerts anti-inflammatory and anti-allergic effects.[41] Consistently, vitamin D3 serum level negatively correlates with inflammatory biomarkers, such as IL-6 and IL-10.[42] Notably, vitamin D3 modulates IgE-mediated mast cell activation.[43]

Interestingly, the pharmaceutical formulation of this nutraceutical is a bilayer tablet, composed of a fast-release layer that allows the rapid antihistamine activity of *Perilla* and a slow-release layer that enhances Quercetin and Vitamin D3 bioavailability and anti-allergy activity spread over time. Thus, this nutraceutical could be considered a fast-slow-release compound.

Moreover, these results confirmed the previous trial conducted on children and expanded the knowledge also on asthma. Previous studies conducted in children showed that this nutraceutical significantly reduced the risk of AR worsening during standard therapy or after its discontinuation.[18,19] In addition, a previous study showed that a nutraceutical course significantly affected lung function, mainly concerning the small airways.[2] However, this is the first study that explored the effects provided by this nutraceutical in an adult population. In fact, the current study demonstrated the positive activity exerted by this multicomponent nutraceutical in patients with mild persistent asthma and AR due to grass allergy. The strength of this study was the simultaneous evaluation of clinical, functional, and biological outcomes through grass pollen exposure. Of particular interest was the further improvement of the results obtained with standard therapy with the addition of Quertal®, on the various parameters examined.

However, the present study had some limitations, including the open design, the absence of a placebo administration, the lack of mediator assessment, and the limited number of recruited patients. Consequently, these findings should be cautiously interpreted and inevitably need to be confirmed by studies conducted with rigorous methodology.

In conclusion, this clinical experience suggested that this multicomponent nutraceutical could relieve upper and lower airway symptoms, dampen eosinophilic inflammation, improve asthma control and lung function, and reduce the use of rescue medications also in adult patients with asthma and rhinitis due to grass allergy.

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