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Efficiency of Propolis and N-acetylcisteine on Reduction in Symptom Severity of Respiratory Infection in Children with Adenoid Hypertrophy

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Abstract: Introduction. Children with adenoid hypertrophy show a tendency towards recurrent or chronic middle ear infection, acute rhinitis or other infections of the upper airway. The purpose of this study was to examine efficacy and safety of combination of N-acetylcysteine and propolis oral suspension and nasal spray in children with adenoid hypertrophy. **Method**. The clinical trial included children with adenoid hypertrophy. Children in the experimental group were given oral suspension with combination of N-acetylcysteine and propolis, at the first and the second appointment with otorhinolaryngologist, parents of the children were evaluating the intensity of 6 different symptoms, data on otoscopic status were collected, nose and pharynx were examined, audilogical analysis was carried out and nasal swab was used to carry out a microbiological analysis. The control group included children with enlarged adenoids who used hypertonic nasal spray. **Key findings**. There was a statistically significant reduce of a nasal discharge intensity ($p < 0.001$), nasal congestion ($p = 0.010$), sneezing ($p = 0.013$), post-nasal drip ($p = 0.034$), cough ($p < 0.001$) as well as hearing loss ($p = 0.002$) in experimental group after treatment. Positive effect on nasal congestion, post-nasal drip and cough, recorded during the second appointment, was significantly higher in the experimental group than in the control one (respectively $p = 0.04$; $p = 0.034$; $p < 0.001$), while in the case of a nasal discharge, sneezing and hearing loss there was no difference in effect between the experimental and the control group. **Conclusion**. The combination of N-acetylcysteine and propolis has justified use for reduction in symptom severity of respiratory infections in children with adenoid hypertrophy.

Key words: Adenoids, respiratory tract infections, N-acetylcysteine, propolis.

1. Introduction

All lymphatic components of the Waldeyer's ring are strategically located in the place where antigens are entering the pharynx in order to develop regional immune function of the body. Antigenic stimulation through mouth and nose has proven to be an important part of naturally acquired immunity, since adenoids appears to be important for the development of an "immunological memory" in younger children [1]. Although recurrent infections of the palatine and the pharyngeal tonsils contribute to their faster involution where the differentiation of B cells into plasma cells is decreased, even in chronic tonsillitis there is significant immune response [2-4]. Opinions on adenoidectomy effects on the immune system of children are not conclusive, however, multiple authors reported that this surgery (with or without tonsillectomy), when done in early childhood, can produce potentially negative effects on immune system [5]. Authors of this study consider that a conservative approach to pediatric patients with adenoid hypertrophy is more desirable from the immunological point of view, especially in children under the age of 3.

On the other hand, children with adenoid hypertrophy show a tendency towards recurrent infection of the middle ear (recurrent acute otitis media), chronic secretory otitis media, acute rhinitis or other infections of the upper airway. Given that these infections have a

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viral etiology in most cases, the specific treatment is usually not required. Initial treatment should be focused on the symptoms reduction and such treatment mainly includes decongestants, antitussives, expectorants or secretolytic agents.

The existence of bacterial biofilm formations is proven within adenoids. Such persistent infections show high resistance to the immune system of the host and reduced sensitivity to antimicrobial agents, which makes it more difficult when it comes to the conservative treatments. This is why phytotherapy, as an alternative to conventional treatment methods, is lately becoming more and more important. In vitro medical studies have shown that N-acetylcysteine is efficient in inhibition of biofilm formation [6]. With its mucolytic action it also reduces mucus viscosity and positively impacts mucociliary clearance. Propolis has clinically and experimentally proven antimicrobial activity and also shows both anti-inflammatory and antioxidant properties [7]. Due to these characteristics, numerous authors recommend use of propolis as an optimal therapy for respiratory infections, without need for any supplementary treatment [8]. The purpose of this study was to examine efficacy and safety of combination of N-acetylcysteine (NAC) and propolis oral suspension and nasal spray in children with adenoid hypertrophy, which consequently leads to recurrent respiratory infections.

2. Methods

A prospective, comparative and controlled study was conducted in the Clinic for Otorhinolaryngology and Maxillofacial Surgery, Clinical Centre of Serbia, during the period November, 2016-April, 2017. Total of 55 children with adenoid hypertrophy, who had symptoms of secretory and recurrent acute otitis media, as well as nasal mucosa inflammation along with pharynx secretion, participated in the study. During the first appointment with otorhinolaryngologist, parents of the children were evaluating the intensity of 6 various symptoms (nasal discharge, nasal congestion, sneezing, post-nasal drip, cough, as well as hearing loss) by using the visual analogue scale (VAS); data on otoscopic status were collected, nose and pharynx were examined, audilogical analysis (audiometry, tympanometry) was carried out and nasal swab was used to carry out a microbiological analysis. The character of the nasal secretion and the appearance of the nasal mucosa were determined by endoscopic examination of nose during the first appointment and later compared with the results at the second appointment.

Children in the experimental group were given PropoMucil® oral suspension for kids (a mixture of purified propolis extract standardized on 12% of total polyphenols in order to increase its antimicrobial efficiency, N-acetylcysteine, honey, marshmallow and rosehip extract; Abela Pharm, Belgrade, Serbia). After a one-month use, above mentioned parameters were compared during the second appointment. Besides the standard therapy, children with allergic rhinitis (AR) were also given PropoMucil® nasal spray (a mixture of purified propolis extract standardised on 12% of total polyphenols and N-acetylcysteine, additionally enriched with quercetin, thyme and eucalyptus essential oils and vitamins E and D3; Abela Pharm, Belgrade, Serbia). The control group included 55 children with adenoid hypertrophy, who used hypertonic 2.2 % saline nasal irrigation for symptoms reduction. Control group corresponded to the experimental group by age, sex and AR distribution. In case where bacteria were isolated from nasal swab, local antibiotic therapy was used according to the antibiogram, regardless of whether the children were from the experimental or the control group.

At the second appointment parents of the children were given the possibility to make a comment on a drug that their child used between the two appointments. Efficiency, taste, side effects of the drug, as well as the rating that child gave were stated.

SPSS software package, version 18, was used for the statistical analysis of the data. Percentages were used to describe attributive features and measures of

central tendencies were used to describe numerical features. Student's t-test was used to determine the difference between symptom severity in the experimental group, at the first and the second appointment. For determining the difference between experimental and control group a paired t-test was used. To compare the appearance of nasal mucosa and the type of nasal secretion as well as to determine the impact of prescribed therapy on the frequency of particular types of tympanometry a chi-square test ($\chi 2$) for contingency tables was used. The results are presented in both graph and tabular forms. Study was conducted in accordance with the ethical principles of the Helsinki Declaration.

3. Results

In the experimental group of pediatric patients there were slightly more girls (50.9%), aged between 2 and 9 years, with an average age of 5.2 years. Only 14.5% of the children were under 3 years old and about two-third of the children were preschoolers, aged between 3 and 7 years (Figure 1).

About 43% of the children from the experimental group were absent from the day care center for 3 or less days during one-month-period before the first appointment with otorhinolaryngologist, while the average number of absence for the rest of the children was 7.5 days. There was no statistically significant difference in demographic characteristics of the experimental and the control group.

The mean of the main symptoms intensity in children from the experimental group, during the first and control examination is shown in Figure 2.

In the experimental group, during control examination there was statistically significant reduce of nasal discharge intensity ($p < 0.001$), nasal congestion ($p = 0.010$), sneezing ($p = 0.013$), post-nasal drip ($p = 0.034$), cough ($p < 0.001$) as well as hearing loss ($p = 0.002$), comparing to the first appointment. The best results were shown in cough reduction, where as much as 96% of the subjects stated reduction of the symptom intensity at the second appointment and the mean of the intensity decreased by 62% (Table 1).

The effect on nasal congestion, post-nasal drip and cough, recorded during the control examination, was significantly better in the experimental group than in the control one (respectively $p = 0.04$; $p = 0.034$; $p < 0.001$), while in the case of nasal discharge, sneezing and hearing loss there was no difference in effect between experimental and control group.

At the first appointment with otorhinolaryngologist, about 11% of the patients from the experimental group had bilateral type A tympanogram, while the same number of patients had unilateral type A tympanogram (combination of types A and C or combination of types A and B). Secretion in the middle ear was present on both sides in 40% of pediatric patients

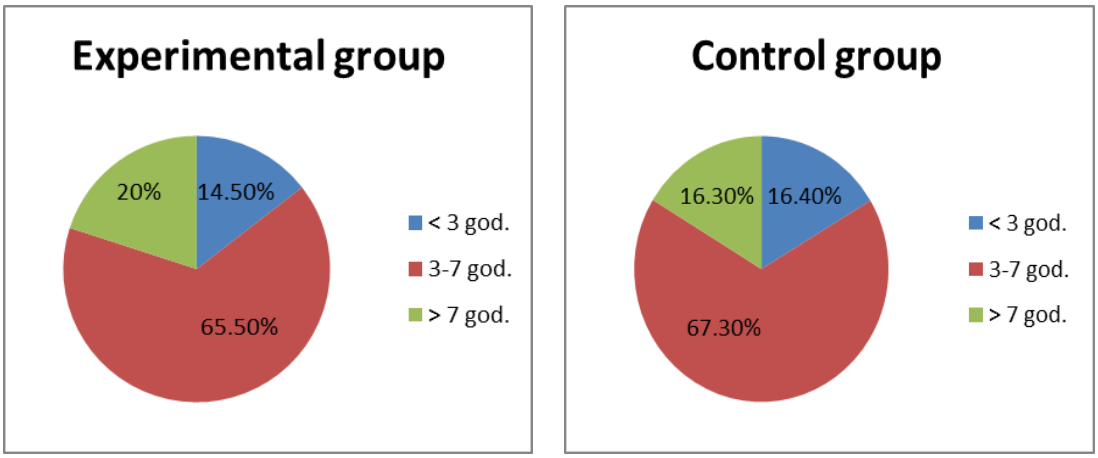

Fig. 1 Age distribution in experimental and control group.

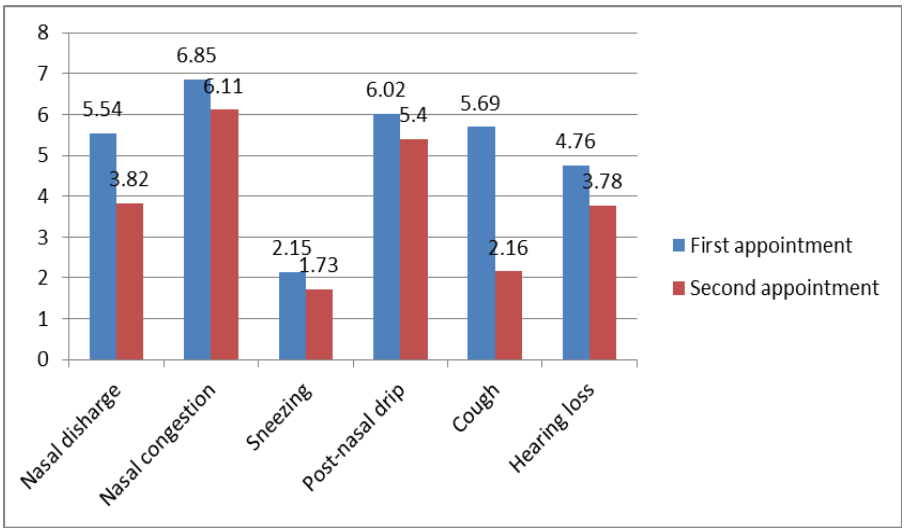

Fig. 2 **The mean of the main symptoms intensity in the experimental group evaluated by the visual-analogue scale.**

Table 1 **Assesment of the treatment effect at the control examination expressed as the percentage of subjects with reduced symptoms intensity or percentage of the symptom intensity reduction.**

Symptom	Patients with reduction of the symptoms' intensity (%)	Decrease of the symptoms' intensity value (%)
Nasal discharge	74.5 %	31.0 %
Nasal congestion	61.8 %	10.8 %
Sneezing	38.1 %	19.5 %
Post-nasal drip	54.5 %	10.3 %
Cough	96.0 %	62.0 %
Hearing loss	54.5 %	20.6 %

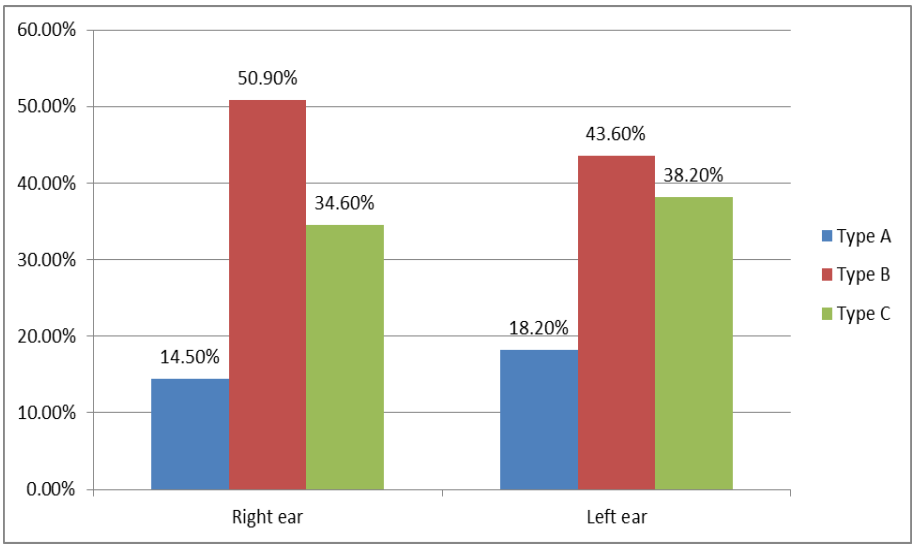

Fig. 3 **Tympanometric results in the experimental group at the first appointment.**

(bilateral type B tympanogram), while 8 patients had it unilaterally. A quarter of the patients had bilateral negative pressure in the middle ear, while just over a fifth of the patients had unilateral negative pressure (Figure 3).

The tympanometry type was changed unilaterally or bilaterally in 67.3% of patients. Compared to the first appointment, the frequency of bilateral B type tympanogram was decreased by 31.8% at the control appointment, while the frequency of bilateral A type

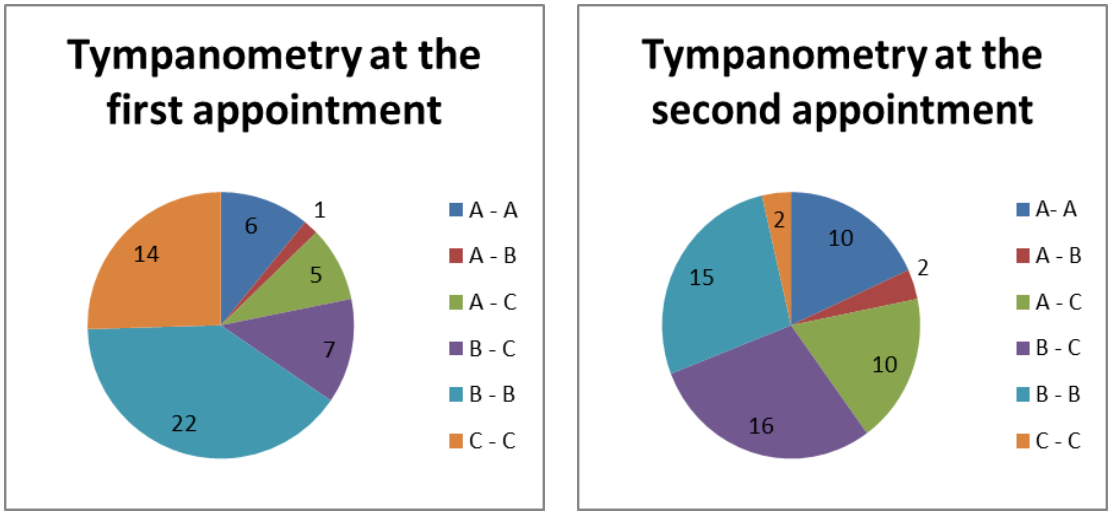

Fig. 4 Tympanometry test results of the subjects at the first and the second appointment.

tympanometry was increased by 66.7%. Unilateral improvement of tympanometry test results at the control examination was noted in 36.7% of the patients in the experimental group and bilateral improvement in approximately 14% of the patients. Despite these results, the use of chi-square test ($\chi 2$) for contingency tables didn't show any significant effect of the treatment implemented in the experimental group on the particular tympanometry types frequency ($p = 0.064$) (Figure 4).

One third of the patients in the experimental group had allergic rhinitis (18 of 55 patients) and similar was in the control group (15 of 55 patients). All of these patients had mild symptoms of allergic rhinitis and main symptoms were nasal secretion and congestion. In the experimental group, 61% of these patients had intermittent allergic rhinitis and used oral antihistamine drugs to control symptoms of rhinitis.

By comparing the values of the symptoms scores at the first and control appointment, in patients with allergic rhinitis, a statistically significant difference in nasal congestion between patients in the experimental and the control group was determined ($p = 0.007$), while there was no statistically significant difference in the nasal mucus and sneezing (respectively $p = 0.407$ i $p = 0.350$). One of the limitations of this study is the fact that the patients with allergic rhinitis were treated with standard treatment according to ARIA guidelines, which should be taken into account when interpreting these results.

By comparing the appearance of the nasal mucosa and the type of the nasal secretion at the control examination, with use of a chi-square test ($\chi 2$) for contingency tables, a statistically significant difference was found compared to the first examination. In a significant number of the patients the appearance of the nasal mucosa and the type of the nasal secretion were normalized. As a result, nasal mucosa in more than half of the patients was of pink color and more than three-quarters of the patients had clear mucus fluid in nasal cavity at the control examination (Table 2).

At the first appointment, microbiological analysis of the nasal mucus detected and isolated bacteria in 22 subjects. The most commonly isolated bacteria was Staphylococcus aureus (41% of the cases). Streptococcus pneumoniae was isolated in 5 subjects, Moraxella catarrhalis in 4, Haemophilus influenzae in 3 and Streptococcus pyogenes in only 1 subject. At the control appointment, a positive microbiological result of the nasal mucus was found in 5 children and, besides, two of those children didn't have a positive microbiological result at the first appointment. These results should be interpreted by having in mind

Table 2 The appearance of the nasal mucosa and the mucus of the patients at the first and the control appointment.

	First appointment n (%)	Second appointment n (%)	Significance, p
Nasal mucosa			
Hyperemic	22 (40.0 %)	10 (18.2 %)	
Swollen	14 (25.5 %)	12 (21.8 %)	
Hyperemic and swollen	8 (14.5 %)	3 (5.5 %)	
Pink	11 (20.0 %)	30 (54.5 %)	0.001
Nasal mucus			
Clear	20 (36.4 %)	43 (78.2 %)	
Mucoid	25 (45.5 %)	11 (20.0 %)	<0.001
Purulent	10 (18.1 %)	1 (1.8 %)	

Table 3 The experimental group: Comments of the parents on PropoMucil® oral suspension.

	Parents'rating	n (%)
Syrup effect		
	Positive	36 (65.5 %)
	Without change	19 (34.5 %)
	Negative	0 (0.0 %)
Syrup taste		
	Good	44 (80.0 %)
	Neutral	10 (18.2 %)
	Bad	1 (1.8 %)
Side effects		
	Yes	2 (3.6 %)
	No	53 (96.4 %)

Table 4 The experimental group: Comments of the parents on the PropoMucil® nasal spray.

	Parents' rating	n (%)
Spray effect		
	Positive	13 (72.2%)
	Without change	5 (27.8%)
	Negative	0 (0.0%)
Spray rating		
	Likes to use	10 (55.5%)
	Neutral	5 (27.8%)
	Doesn't like to use	3 (6.7%)
Side effect		
	Yes	1 (5.5%)
	No	17 (94.5%)

that the children with isolated bacteria from the nasal mucus swab were also treated with local antibiotic therapy according to the antibiogram results.

The parent's comments regarding the effect, rating and side effects of the oral suspension and the nasal spray are presented in Tables 3 and 4.

4. Discussion

It is estimated that biofilms are involved in about two-thirds of bacterial infections in the human body, especially in chronic or recurrent infections [9]. Treatment of acute or chronic infections, partially caused by biofilms, is a real challenge for the clinician. Preschoolers are at increased risk and more likely to develop a upper respiratory tract infection (URTI), due to increased exposure to infectious agents in daycare center, as well as due to immature immune system which has reduced ability to produce certain antibodies. In pediatric patients with adenoid hypertrophy, the most common reasons for visiting pediatrician or otorhinolaryngologist are recurrent or chronic otitis media, Eustachian tube dysfunction, rhinosinusitis and bronchitis. Although systemic antibiotic therapy is prescribed in accordance with treatment protocols or medical doctrines for majority of children, it is often unable to destroy biofilm formations. This is why the professional attention is directed to other, non-antibiotic methods of treatment.

In this study about one-third of pediatric patients had a middle ear infection once a month and 25% of the patients were sent to a tertiary institution for a surgery consideration (adenoidectomy and/or insertion of aeration tubes). In about 50% of the cases, parents stated that they were often concerned about hearing since the children wouldn't react to their calling. About 29% of the children from the experimental group had post-nasal drip episodes several times a day, followed by productive cough, once every two months. All of these indicate that these children had chronic or recurrent infection, which requires surgical treatment in case of the failure of conservative treatment.

It is considered that NAC has multiple inhibitory effects on biofilm formation; it has an effect on bacteria adhesion to the surface, matrix production

and architecture as well as on the pre-formed biofilms spreading. After Peres-Giraldo et al. first described antibiofilm activity, by examining the effect that NAC has on Staphylococcus epidermidis, a number of studies followed, proving such activity in a number of gram-positive and gram-negative bacteria [10-12]. Although in-vitro studies provide strong arguments that NAC can disrupt biofilm, clinical data are still being collected.

There are no strict guidelines on the method of use, dose and duration of the therapy that would be effective with biofilm infections. Peroral use of NAC is common and consequently brings to its metabolism in liver and intestine. On the other hand, topical application provides a high concentration of NAC right at the place where its action is needed. By using it in a form of a nasal spray, not only mucolytic effect is achieved, but also possible prevention of respiratory infections, since NAC has the inhibitory effect on bacterial adherence. Various authors have also reported about remarkable in-vitro activity of the combination of NAC and vancomycin or NAC and ciprofloxacin against bacterial biofilms and proposed them as a possible therapeutic strategy for eradication of biofilm-caused infections or reduction of resistance. Although this combination, in form of a commercial product, doesn't exist in Serbia, the combined clinical use of NAC and local antibiotic therapy has led to the reduction of positive microbiological analysis results and the control of symptoms.

N-acetylcysteine is amino acid L-cysteine derivate that has antioxidant activity. Also, by disrupting disulfide bonds that participate in the formation of gel layer, it reduces viscosity of bronchial secretion (mucolytic effect). Mucolytic action is especially important for understanding the reduced intensity of nasal congestion, cough, post-nasal drip or other symptoms in subjects at the control examination. Bajec-Opancina and associates have shown that the oral use of standardized propolis and N-acetylcysteine combination in children results in a reduction of nasal obstruction, cough and hearing loss [14]. Despite favorable therapeutic properties, no significant impact on tympanometry results is proven in our study, since the problem of the middle ear ventilation is of a more complex nature. However, type A tympanogram was more frequent tympanometry result at the control examination, primarily because of the successful secretion evacuation and anti-inflammatory effect. In order to better understand these results it should be taken into account that one part of the subjects had allergic rhinitis and thus has a greater predisposition to secretory or recurrent otitis media. Future researches should examine whether the therapy has led to decrease in the number of new acute episodes of otitis media or rhinosinusitis.

NAC has a very low rate of side effects and it is considered as a safe drug with very positive risk-benefit ratio. Side effects noted during examinations were mild and rare, thus didn't affect subjects to stop with the therapy. The vast majority of the subjects had positive comment on therapy effect and the product properties (primarily the syrup taste).

Due to its proven antimicrobial, antioxidant and immunomodulatory properties, propolis is used in products intended to produce symptom relief of respiratory infections. Standardized propolis with an addition of polyphenol has proven antibacterial and biofilm eradication activities. Marchisio et al. showed that propolis combined with zinc significantly reduces the risk of a new episode of acute otitis media in children with recurrent middle ear infections [15] and authors from Spain have confirmed the efficacy of propolis on faster recovery and improvement of life quality in patients with acute infective rhinitis [8]. Because of these characteristics, combination of propolis and N-acetylcysteine is recommended as a phytotherapy treatment option to control symptoms in children with adenoid hypertrophy.

5. Conclusions

Based on our results, a combination of propolis

N-acetylcysteine has justified use for reduction of URTI symptoms in children with adenoid hypertrophy. This form of phytotherapy has proven to be effective and safe alternative or complement to conventional treatment. Further research on longer-term use effects are needed in children with recurrent respiratory infections and adenoid hypertrophy.

Conflict of Interest

Author Arsovic, N. has received speaker fees form Abela Pharm. The remaining authors have no conflicts of interest to declare pertaining to this article.

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