

A randomized, double-blind, placebo-controlled study evaluating the efficacy of propolis and N-acetylcysteine in exacerbations of chronic obstructive pulmonary disease

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Abstract. – **OBJECTIVE:** Acute exacerbations of chronic obstructive pulmonary disease (AECOPDs) accelerate the progressive impairment of lung function and general health. Together with maintenance therapy for chronic obstructive pulmonary disease (COPD), N-acetylcysteine (NAC) and natural propolis have demonstrated pharmacological properties that address crucial pathophysiological processes underlying COPD and may prevent AECOPDs.

This study aims at responding to dose-dependent efficacy and safety concerns regarding a propolis-NAC combination for the reduction of COPD exacerbation rates.

PATIENTS AND METHODS: This was a single-center, randomized, double-blind, phase IV trial with three treatment arms: Placebo and two active substance groups, one (AS-600) received 600 mg of NAC + 80 mg of propolis while the other (AS-1,200) received 1,200 mg of NAC + 160 mg of propolis. Following an AECOPD, frequent-exacerbation phenotype patients (n=46) were assigned a once-daily three-month therapy with the study drug and one year follow-up. The primary endpoint was the COPD exacerbation incidence rate during the follow-up period as a measure of dose-dependent efficacy of NAC-propolis combination compared to placebo.

RESULTS: There was a statistically significant difference in the AECOPD incidence rate: 52.6% in patients that received placebo, 15.4% that received AS-600 and only 7.1% that received AS-1,200 (Fisher's exact test, $p = 0.013$). Compared to placebo, AECOPD frequency was significantly lower only in AS-1,200 ($p=0.009$). Compared to placebo, the relative risk for exacerbation was 0.29 in AS-600 and 0.13 in AS-1,200. No adverse events related to the treatment were reported.

CONCLUSIONS: Oral combination of natural propolis with NAC confirmed formulation efficien-

cy with a favorable safety profile. Our results need to be confirmed by larger clinical trials.

Key Words:

COPD, Exacerbation, NAC, Dose-dependent, Propolis, Supplement, Efficacy.

Introduction

Chronic obstructive pulmonary disease (COPD), both preventable and treatable, is one of the leading causes of death¹. COPD is a progressive disease with substantial morbidity, characterized by acute exacerbations (AECOPDs). Each episode of AECOPD contributes to accelerated decline in lung function, physical activity intolerance, overall health deterioration and increase of social and economic burden leading to high rates of outpatients and hospital treatments^{2,3}. It is difficult for Support Vector Machine (SVM) consequently, the prevention of exacerbations is a main strategic point of COPD management and a critical outcome in COPD trials.

Global Initiative for Chronic Obstructive Pulmonary Disease (GOLD) Guidelines⁴ from 2021 state that “regular therapy with mucolytics, such as N-acetylcysteine (NAC), may reduce exacerbations and modestly improve health status” in patients with viscous sputum not receiving inhaled corticosteroids. Some studies^{5,6} found that patients who continue to exacerbate despite standard treatment may benefit from receiving a high dose of NAC (≥ 1200 mg). Moreover, the pharmacological application of NAC addresses all crucial

pathophysiological processes underlying COPD and triggering AECOPD⁷. Another hypothetically highly potent dietary supplement, still not incorporated into guidelines, is natural propolis extract. In addition to the documented mucolytic effect of NAC, both N-acetylcysteine and natural propolis extract exhibit antioxidant, anti-inflammatory and antimicrobial properties⁷⁻¹⁸.

This study aims at responding to efficacy and safety concerns on propolis and NAC combination in the reduction of COPD exacerbation rates. PropoMucil® (AbelaPharm) is the first such formulation (a diet supplement) in Serbia containing both propolis (80 mg) and NAC (600 mg).

Patients and Methods

Study Population

Patients diagnosed with COPD acute exacerbation were recruited (n=46) at the Clinic for Pulmonology during the period between November 2017 and October 2018. Inclusion criteria were: (i) an episode of acute exacerbation of COPD defined as an acute event, characterized by increased intensity of patient's respiratory symptoms outside of normal daily variations that required change in therapy¹⁹; (ii) increased production of thick sputum; (iii) frequent-exacerbation phenotype, defined as COPD patients who presented with ≥ 2 exacerbations per year (classified as group C or D)¹⁹; (iv) acute exacerbation confirmed by spirometry. If the timeframe between the treatment of successive exacerbations was ≥ 4 weeks, exacerbation events were considered as 2 separate events²⁰. COPD diagnosis preceded the episode of exacerbation and was defined by GOLD definition (post-bronchodilator forced expiratory volume in the first second (FEV1)/forced vital capacity (FVC) < 0.7)¹⁹. Spirometry was performed according to ERS standards²¹. Exclusion criteria were: (i) presence of an acute asthma attack; (ii) presence of gastric or duodenal ulcer; (iii) age > 80 years; (iv) concomitant use of nitroglycerine; (v) allergy to any compound of product of propolis with NAC; (vi) evidence of pregnancy or lactation.

Study Design

The study was a randomized, double blind, placebo-controlled phase IV trial with an active treatment group that received a formulation containing 600 mg of N-acetylcysteine (NAC) and 80 mg of propolis (PropoMucil® 600, sachets, manufacturer AbelaPharm, Serbia) and placebo

group. This was an investigator-initiated, interventional prospective clinical trial. An independent clinician conducted randomization and blinding. We recorded individual patients' data on the first day of the study (enrollment day). Complete medical records included: medical history, demographic data, questionnaires for subjective assessment of cough and sputum production, quality and microbiological analysis of sputum, biochemical results (level of serum C reactive protein - CRP, fibrinogen, complete blood count and leukocyte formula), spirometry and chest X-ray. Patients were divided in two groups: (i) study and (ii) placebo group. Furthermore, we divided the study group into two subgroups depending on the active substance (AS) daily dosage: (AS-600) 600 mg of NAC + 80 mg of propolis extract and (AS-1,200) 1,200 mg of NAC + 160 mg of propolis extract. The patients' allocation in the treatment groups was unknown both to investigators and to patients. Placebo and propolis with NAC formulation were identical in size, color, shape (a white powder in sachets that dispersed in water, coded by an eight-digit number) and were prescribed once daily. The duration of the therapy in all the examined groups was 3 months. Only in the case of an adverse event (i.e., allergic reaction) emergency un-blinding was performed, and investigators revealed the sachet content. A follow-up visit was scheduled after 30 days, and all data obtained on the enrollment day were obtained once more. Two months after the start of the treatment all patients were interviewed by phone call and information regarding therapy changes were recorded (including corticosteroid and/or antibiotic use), as well as adherence and compliance, presence of cough and expectoration, amount of sputum production and its color. The third study visit took place 120 days after the enrollment day. Compliance was achieved if each individual patient took at least 80% of the prescribed sachets. The study was time-limited, and it was not possible to include the planned number of patients until its completion. There were no dropouts and withdrawals among patients. Through electronic medical records (hospital admissions, discharge summaries and outpatient specialist reports), we followed COPD exacerbation incidence for each patient for further 12 months from the end of the treatment period. In the case of worsening of symptoms, an attending specialist at the clinic and the study physician examined the patient and confirmed diagnosis of acute COPD exacerbation.

Table I. Baseline clinical and socio-epidemiological characteristics of patients.

Variables	Groups			p
	AS-600 (n=13)	AS-1,200 (n=14)	Placebo (n=19)	
Age (yrs) (mean±SD)	64.46 ± 7.01	65.43 ± 8.03	67.32 ± 5.91	0.733 ^a
Gender (male)	10 (76.9%)	8 (57.1%)	11 (57.9%)	0.508 ^b
Smoking	7 (53.8%)	4 (30.8%)	8 (42.1%)	0.555
Alcohol consumption	1 (7.7%)	2 (15.4%)	1 (5.3%)	0.806
Comorbidities				
Bronchiectasis	1 (7.7%)	0 (0.0%)	4 (21.1%)	0.164
Emphysema	1 (7.7%)	1 (7.7%)	1 (5.3%)	1.000
HTA	2 (15.4%)	2 (14.3%)	1 (5.3%)	0.588
PAH	0 (0.0%)	0 (0.0%)	1 (5.3%)	1.000
Diabetes mellitus	0 (0.0%)	0 (0.0%)	2 (10.5%)	0.329
Cardiomyopathy	1 (7.7%)	0 (0.0%)	1 (5.3%)	0.743
Arrhythmia	1 (7.7%)	0 (0.0%)	0 (0.0%)	0.283
Tachycardia	0 (0.0%)	0 (0.0%)	1 (5.3%)	1.000
Coronary disease	2 (15.4%)	0 (0.0%)	0 (0.0%)	0.075
Disease duration (yrs) (mean±SD)	4.8 ± 6.2	8.3 ± 4.9	6.9 ± 6.7	0.042 ^c
Baseline FEV1 (%) (mean±SD)	49.18 ± 23.87	34.62 ± 13.97	54.56 ± 21.88	0.019 ^a
Baseline TIF index (mean±SD)	46.40 ± 13.99	39.25 ± 10.18	52.00 ± 12.35	0.040 ^a
Inhaled therapy				
Dual	7 (53.8%)	3 (21.4%)	9 (50.0%)	0.166 ^b
Triple	6 (46.2%)	11 (78.6%)	9 (50.0%)	
Cough				
Occasional	13 (100.0%)	11 (78.57%)	10 (52.63%)	0.007 ^b
Persistent	0 (0.0%)	3 (21.43%)	9 (47.37%)	
Sputum				
White	9 (69.2%)	10 (76.9%)	8 (44.4%)	0.192 ^b
Purulent	4 (30.8%)	3 (23.1%)	10 (55.6%)	

^aOne Way ANOVA, ^bFisher's exact test, ^cKruskal-Wallis' test.

Abbreviations: AS-600, study group which received active substance formulation 600 mg of N-acetylcysteine + 80 mg of propolis extract; AS-1,200, study group which received active substance formulation 1,200 mg of N-acetylcysteine + 160 mg of propolis extract; yrs, years; PAH, pulmonary arterial hypertension; HTA, arterial hypertension; FEV1, forced expiratory volume in the first minute; TIF index, Tiffneau index, TIF=FEV1/FVC; FVC, forced vital capacity.

Outcome Measure

The primary endpoint was the COPD exacerbation incidence rate during the follow-up period, as the measure of dose-dependent efficacy of NAC-propolis combination compared to placebo. Efficacy of the treatment was measured by calculating absolute risk and relative risk reduction.

Statistical Analysis

Descriptive and analytical statistics were used. Results are presented as count (%), means ± standard deviation or median (25-75%), depending on data type and distribution. We compared groups using parametric (ANOVA) and non-parametric (Chi-square, Fisher's exact test and Kruskal-Wallis' test) tests. No *p*-value adjustments were performed. *p*-values < 0.05 were considered as significant. All data were analyzed using SPSS 20.0 (SPSS Statistics for Windows, Version 20.0, IBM Corp., Armonk, NY, USA).

Results

Clinical and Socio-Epidemiological Data of Patients

The study population consisted of 46 patients (63% male), 52-83 years old and 42.2% were smokers. All the patients had a frequent-exacerbation phenotype with moderate to very severe air-flow obstruction (GOLD grade 2-4). Demographic characteristics, comorbidities and clinical data of patients at the time of enrollment are summarized in Table I. Comparing patients treated with placebo, 600 mg and 1,200 mg of active substance, there was no statistically significant difference in age, gender, registered comorbidities, regular inhaled therapy and reported cigarettes and alcohol consumption among the groups. Disease duration at the time of enrollment was significantly longer in patients from the AS-1,200 group compared to

the AS-600 group (Kruskal-Wallis test, $p = 0.011$). Patients who received the placebo treatment more frequently reported persistent cough in anamnesis compared to the AS-600 group (Fisher's exact test, $p = 0.004$). However, all patient groups were similar in terms of sputum characteristics (white or purulent) (Fisher's exact test, $p = 0.192$). Based on baseline spirometry measurements, our treatment groups were significantly different in FEV1 ($F = 4.787$, $p = 0.019$), but post-hoc tests revealed only a significant difference between the AS-1,200 and the placebo group ($p = 0.019$). Correspondingly, the Tiffeneau index measured at the time of enrollment was significantly different among the groups (ANOVA $F = 3.550$, $p = 0.040$) and was larger in the placebo group than in the AS-1,200 group ($p = 0.012$).

Adverse Events and Drug Compliance

From the start of the treatment until the end of the follow-up period, all patients reported no adverse effects regardless of the assigned treatment. Compliance was satisfactory, as patients described taking the study drug "as if they were drinking juice" (granules for oral solution) and stated that the taste was either "moderately good" or "good". Considering the drug taste (placebo vs. AS-600 vs. AS-1,200), there were no differences recorded by patients (Fisher's test, $p = 0.733$).

Comparison of Exacerbation Rates Between Groups During Follow-Up

During three months of treatment and one year of follow-up, there was a statistically significant difference in the exacerbation incidence rate between all three study groups (Fisher's exact test, $p = 0.013$) (Figure 1). More than 50% of patients (10/19) from the placebo group had AECOPD during the observed period, while 15.4% (2/13) from the AS-600 group and only 7.1% patients (1/14) from the AS-1,200 group developed AECOPD. Compared to placebo, AECOPD frequency was significantly lower in the AS-1,200 group ($p = 0.009$), while statistical significance was not achieved for the AS-600 group ($p = 0.062$). Relative risk (RR) for exacerbation was 0.29 in the AS-600 group and 0.13 in the AS-1,200 group, compared to the placebo group. Absolute risk reduction (ARR) was 0.372 for the AS-600 group and 0.455 for the AS-1,200 group comparing to the placebo group, meaning that three patients needed to be treated with AS-600 or AS-1,200, respectively, to avoid one exacerbation per year. Median time to exacerbation for the patients treated with active

substance was 6 months (IQR 6.0-6.0) for the AS-600 group and the AS-1,200 group, vs. 8 months (IQR 6.0-9.0) for the placebo group.

Discussion

We found that: (i) NAC combined with propolis extract reduced AECOPD incidence rate; (ii) the efficacy was dose-dependent, the greatest exacerbation rate reduction was in patients treated with 1,200 mg of NAC + 160 mg of propolis extract; (iii) there were no adverse events reported.

Several randomized placebo-controlled trials^{6,9,11,22,23} have investigated the efficacy of NAC in AECOPD rate reduction. Decramer et al¹¹ recruited 523 patients with at least two AECOPDs/year and found a reduction in the exacerbation rate exclusively in the subgroup of patients not receiving ICS (approximately 30% of the study population). However, their BRONCUS (Bronchitis Randomized on NAC Cost-Utility Study) trial assessed a 3-year treatment only with low-dose NAC (600 mg/day) vs. placebo. Additionally, most of the patients receiving placebo (70%) received ICS as part of their standard therapy and most of them (70%) were GOLD stage II (FEV1=30-79% predicted). Our study included a smaller proportion of participants (56.5%) regularly taking inhaled corticosteroids (as a part of triple therapy) and there was no difference in their maintenance therapy (double or triple inhaled combination) between the study groups. When compared to the AS-1,200 group, placebo-treated patients had shorter mean disease duration and better spirometry values of FEV1 and a FEV1/FVC ratio at baseline, indicating a lower grade of the disease. Schermer et al²² investigated two active substances, low-dose NAC (600 mg/day) vs. inhaled fluticasone (1,000 µg/day) during a 3-year treatment and found no beneficial effect on the exacerbation rate compared to placebo in COPD and chronic bronchitis patients. As an explanation, the authors stated that "a small number of patients experienced very frequent exacerbations". The HIACE (The Effect of High Dose N-acetylcysteine on Air Trapping and Airway Resistance of Chronic Obstructive Pulmonary Disease – a Double-blinded, Randomized, Placebo-controlled Trial) study⁹ enrolled 120 Chinese patients with stable COPD four weeks after remission of their exacerbation. They found that one year treatment with high-dose NAC (1,200 mg/day) reduced the exacerbation frequency, increased the time to first exacerbation and the likelihood of being exacer-

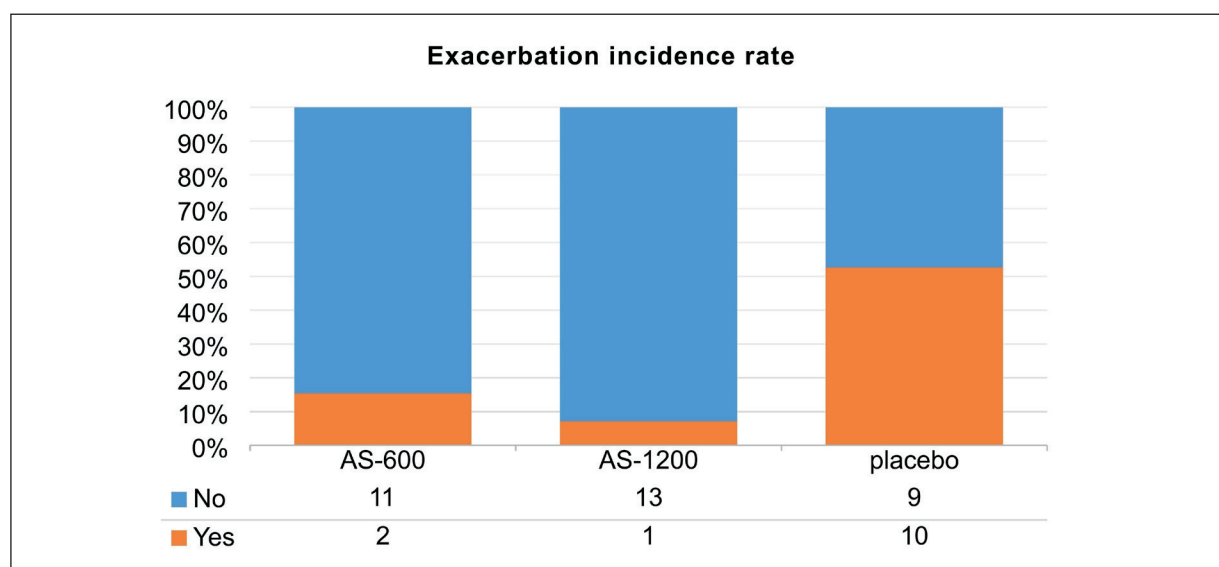


Figure 1. The total number of COPD exacerbations in each treatment group at the end of the follow up.

Abbreviations: AS-600, study group which received substance formulation 600 mg of N-acetylcysteine + 80 mg of propolis extract; AS-1200, study group which received active substance formulation 1200 mg of N-acetylcysteine + 160 mg of propolis extract. *Fisher's exact test, $p = 0.013$.

bation-free at one year compared with placebo in patients with a high risk of exacerbation. The PANTHEON (Placebo-controlled study on efficacy and safety of N-acetylcysteine High dose in Exacerbations of chronic obstructive pulmonary disease) study⁶ examined the effect of one year treatment with high dose NAC (1,200 mg/day) in 1,006 Chinese patients with moderate-to-severe COPD and a history of 2 or more AECOPDs in the previous 2 years, clinically stable for at least 4 weeks before enrollment. They observed a lower incidence rate and shorter duration of AECOPDs with NAC vs. placebo, while time to first AECOPD was prolonged in moderate, but not in severe COPD patients treated with NAC. The treatment effect was independent of ICS. There may be a substantial difference between these four studies linked to ethnicity (Chinese vs. Caucasian) in terms of genetics, environmental and dietary risk factors²³. Thus, the benefits of high-dose NAC in AECOPD prevention may not extend to the global population.

None of the aforementioned studies with NAC considered both a low and a high dose for treatment. Analyzing pooled data on high-dose and low-dose regimens, Wedzicha et al²⁴ found that mucolytic therapy decreased the likelihood of hospitalization, calculating that “25 patients needed to be treated with a mucolytic to prevent one hospitalization”²⁴. Similarly, to previously conducted meta-analysis by Cazzola et al²⁵, they concluded that

“mucolytic therapy reduced the number of COPD exacerbations per patient-year (an effect largely attributable to high-dose therapy)” and “there was no evidence that mucolytic therapy increased adverse events”.

As in our study, no drug-related adverse events were registered in the BRONCUS and HIACE trials^{9,11}. The PANTHEON trial recorded no difference in adverse events between placebo and NAC treatment – 9% of patients had adverse events regarded by the investigators as possibly related to study products, as did 7% of patients who received placebo ($p = 0.29$).

A crucial difference in our study design may be that we assessed the therapeutic effect of combined agents, NAC and propolis. The absolute risk reduction achieved may have been due to their synergistic effect. Both NAC and propolis target the majority of contributing factors for the development of AECOPD. There is evidence that NAC has antiviral action against influenza and respiratory syncytial virus⁷. Moreover, *in vitro* studies on NAC have reported its antimicrobial activity against yeasts, Gram-positive and Gram-negative bacteria and demonstrated its action against bacterial biofilm formation⁸. Additionally, there is some evidence that NAC also improves lung function by reducing air trapping⁹⁻¹¹. Propolis extracts (bees glue) have also shown anti-inflammatory, immunomodulatory, antioxidative, anti-infectious and antiproliferative

properties in multiple *in vitro* studies¹²⁻¹⁷. As a natural antimicrobial, depending on the extract (ethanolic or non-ethanolic), it may act efficiently against bacterial infections caused by Gram-positive and Gram-negative strains¹³⁻¹⁷. Furthermore, the variable proportion of active substances in its composition avoids development of bacterial resistance¹⁷. Antiviral, antifungal and antiparasitic activities of propolis constituents have also been documented^{13,16}. For example, flavonoidal components of Egyptian propolis show *in vitro* inhibitory effects on COVID-19 virus replication, comparable with remdesivir¹⁸. Except for a few small single-center studies²⁶⁻²⁸, there is a lack of laboratory evidence to prove either additive or synergistic effect of these two, propolis and NAC active substances in chronic respiratory inflammation.

Limitations

There are some limitations in our study. First, heterogeneous and a small number of patients in the treatment arms resulted from the inability to recruit eligible patients that met all the inclusion and exclusion criteria in the designed study time frame. Second, due to the small group sizes we did not classify patients based on severity of exacerbations. Finally, mild exacerbations could have been easily missed and not recorded. Turner and Bothamley²⁹ suggested that recognizing exacerbation based only on worsening symptoms is insufficient due to doubts regarding the willingness of participants to regularly record and report their daily symptoms over a longer period.

Conclusions

To our knowledge, this is the first double-blind, placebo-controlled, randomized clinical trial conducted with the aim to investigate the efficacy of high- or low-dose combined formulation of NAC and propolis extract for the prevention of AE-COPD. The significant reduction in exacerbation incidence rate observed in patients treated with oral combination of natural propolis and NAC (600 mg or 1,200 mg daily) confirmed the formulation efficiency with favorable safety profile. Our results need to be confirmed by larger clinical trials.

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Conflict of Interest

The authors declare no conflicts of interest.

Ethical Approval

The Ethical Committee of the Clinical Centre of Serbia approved the protocol No. 1822/3 on January 5, 2018.

Informed Consent

Patients who met the inclusion criteria signed a written informed consent form to participate.

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