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ORIGINAL RESEARCH

Initial Safety and Physiological Impacts of Propolis Inhalation as a Key Component of Apiair Therapy

Serkan Orhan[1], Zeynep Egri Kansu[2], Necdet Ozcelik[3], Ali Timucin Atayoglu[4*]

[1] Department of Physical Therapy and Rehabilitation, Graduate School of Health Sciences, Istanbul Medipol University, Istanbul, Türkiye
[2] Department of Respiratory Diseases, Medipol University Esenler Hospital, İstanbul, Türkiye
[3]Department of Ear, Nose & Throat Diseases, Medipol University Esenler Hospital, Istanbul, Türkiye
[4] Department of Traditional and Complementary Medicine, Graduate School of Health Sciences, Istanbul Medipol University, Istanbul, Türkiye

* Corresponding Author: Ali Timucin Atayoglu, e-mail: atatayoglu@medipol.edu.tr

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Abstract

Objective: Interest in beehive air inhalation therapy, known as Apiair, has grown significantly in recent years. However, clinical studies examining its effects remain limited. Propolis, a key component found in hive air, has been identified as playing an important role in potential therapeutic benefits. This study aims to evaluate the initial safety and physiological impacts of propolis-water extract inhalation in healthy individuals.

Materials and Methods: A total of 20 healthy volunteers were randomly assigned to one of two groups in a double-blind trial. The first group inhaled a saline solution, while the second group received a propolis-water extract. All participants underwent assessments before and after inhalation, including pulmonary function testing, vital signs monitoring, venous blood gas analysis, and electrocardiogram (ECG) recording.

Results: Physiological parameters—including pulmonary function, vital signs, blood gas values, and ECG measurements—remained within clinically normal limits in both groups. No adverse events were observed during the study period.

Conclusion: Propolis-water extract inhalation was found to be safe in healthy individuals. These findings support the safety of propolis inhalation and provide a foundation for further research into its therapeutic potential in respiratory health, contributing to a broader understanding of Apiair applications. Further research is necessary to fully evaluate the long-term efficacy and safety of propolis inhalation.

Keywords: Apiair, Beehive Air, Propolis, Inhalation, Safety

INTRODUCTION

The World Health Organization (WHO) developed a strategy on traditional medicine under the title of the "2014–2023 Traditional Medicine Strategy." A primary goal of this strategy is to facilitate the integration of traditional medicine into national healthcare systems, particularly into primary healthcare services [1].

In Turkiye, the Regulation on Traditional and Complementary Medicine Practices came into force in 2014. Within this framework, apitherapy is defined as the use of bees and bee products to provide protective effects on the human body and as a complementary method for the treatment of certain diseases [2].

One of the application areas of apitherapy is "apiair," also known as "hive air inhalation". Apiair refers to a practice that aims to utilize the air inside the beehive. The harmonious composition of various bee products such as royal jelly, propolis, honey, and pollen found inside the hive contains many compounds. The core concept of the Apiair system relies on inhaling volatile active compounds derived from bee products within the hive[3].

Apiair therapy is considered a potential

complementary method in the treatment of various respiratory tract diseases. This therapeutic approach is widely used, particularly in Germany, Hungary, Slovenia, and Austria [4].

The beehive contains components such as honey, pollen, propolis, beeswax, etc. Therefore, the inhaled hive air is rich in volatile compounds, mainly fatty acids and phenolic acids. Compounds from honey, beeswax, pollen, and propolis have been identified in hive air, with approximately 56 volatile compounds, most of which are short-chain fatty acids [5]. Bee products, especially propolis, exhibit strong antimicrobial, antiviral, antitumor, and anti-inflammatory bioactivities [6].

Propolis is a natural substance produced by honeybees from resins they collect[7]. Honeybees gather these resins from trees such as pine, oak, birch, eucalyptus, poplar, chestnut, and some herbaceous plants [8]. Bees use propolis within the hive for various purposes, including preventing microbial growth, coating the hive walls, sealing cracks and fissures, maintaining the hive's humidity and temperature, and mummifying insects or animals too large to remove from the hive, thus preventing decomposition [7].

Natural composition of propolis generally includes resin and plant balsam, beeswax, essential and aromatic oils, and pollen and various organic substances [9]. Honeybees collect propolis by scraping protective resins from flowers and buds with their mandibles [10,11]. Literature reports indicate that over 300 compounds have been identified in propolis samples from various geographical regions [12–15].

The oral use of propolis has been reported to have beneficial effects in the treatment of certain respiratory diseases, as reported in previous studies[16–19]. However, the efficacy of oral propolis administration in respiratory diseases has been evaluated in only a limited number of studies[19]. Furthermore, none of these investigations have explored the effects of propolis inhalation—a potentially more direct method for targeting respiratory function—through clinical trials.

The purpose of this study is to investigate the initial safety and physiological impacts of propolis inhalation on respiratory functions in healthy individuals, with a particular focus on understanding how this method compares to other forms of propolis administration.

MATERIALS AND METHODS

Participant recruitment

Participants were recruited from the healthy individuals working at the Esenler Health Practice and Research Center of Istanbul Medipol University Hospital. An announcement was made to recruit a group of healthy volunteers from the hospital's staff. The eligibility of participants was evaluated based on specific inclusion and exclusion criteria in coordination with a pulmonologist.

Inclusion and exclusion criteria

The inclusion criteria for this study were as follows: participants must be between the ages of 18 and 45 years, have a Body Mass Index (BMI) ranging from 18.5 to 24.9 kg/m², and exhibit normal pulmonary function test (PFT) values. Additionally, all participants were required to provide written informed consent.

The exclusion criteria included individuals with a history of febrile illness prior to the study, any history of pulmonary disease, recurrent bronchitis, non-allergic drug reactions affecting the bronchial or pulmonary system, or multiple drug allergies. Smoking was also considered an exclusion factor for this study.

The evaluation criteria and reference value ranges used in the study are presented in Table 1.

Table 1. Reference Ranges Used in Evaluation Criteria.

Evaluation Criteria	Reference Value
Venous blood gas measurement [20]	pH 7.32–7.42, HCO_3^- 23 – 27 mmol/L, pCO_2 36–49 mmHg (female), pCO_2 39–52 mmHg (male) and pO_2 43–68 mmHg HCO_3: 24-28 mmol/L
Pulmonary Function Test (Spirometry) [21]	FVC, FEV1, FEV1/FVC, PEF values should be 70-100% of the expected range
Electrocardiogram (ECG) Measurement [22,23]	Heart rhythm should be regular, and all P-wave values should have the same morphological characteristics
Body Mass Index (BMI) [24]	18.5-24.9 kg/m²
Heart Rate [25]	60-100 beats/min
Blood Pressure [25]	Systolic blood pressure: 120-130 mmHg, Diastolic blood pressure: 70-90 mmHg
Body Temperature [26]	36.5°C ± 5°C
Respiratory Rate [26]	12-20 breaths/min

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Randomization and grouping

The study was carried out as a double-blind trial. To ensure proper administration of either propolis or saline inhalation, nurses from the hospital's emergency department randomly assigned volunteers to use inhaler mask sets containing either propolis or saline solution, delivered via a nebulizer. The number on the medication chamber of the inhaler mask set was recorded on the participant's case report form. Neither the nurses nor the participants were aware of the contents of the numbered medication chambers, ensuring double blinding. Volunteers inhaling the propolis solution were assigned to the experimental group, while those inhaling the saline solution were assigned to the control group (Figure 1).

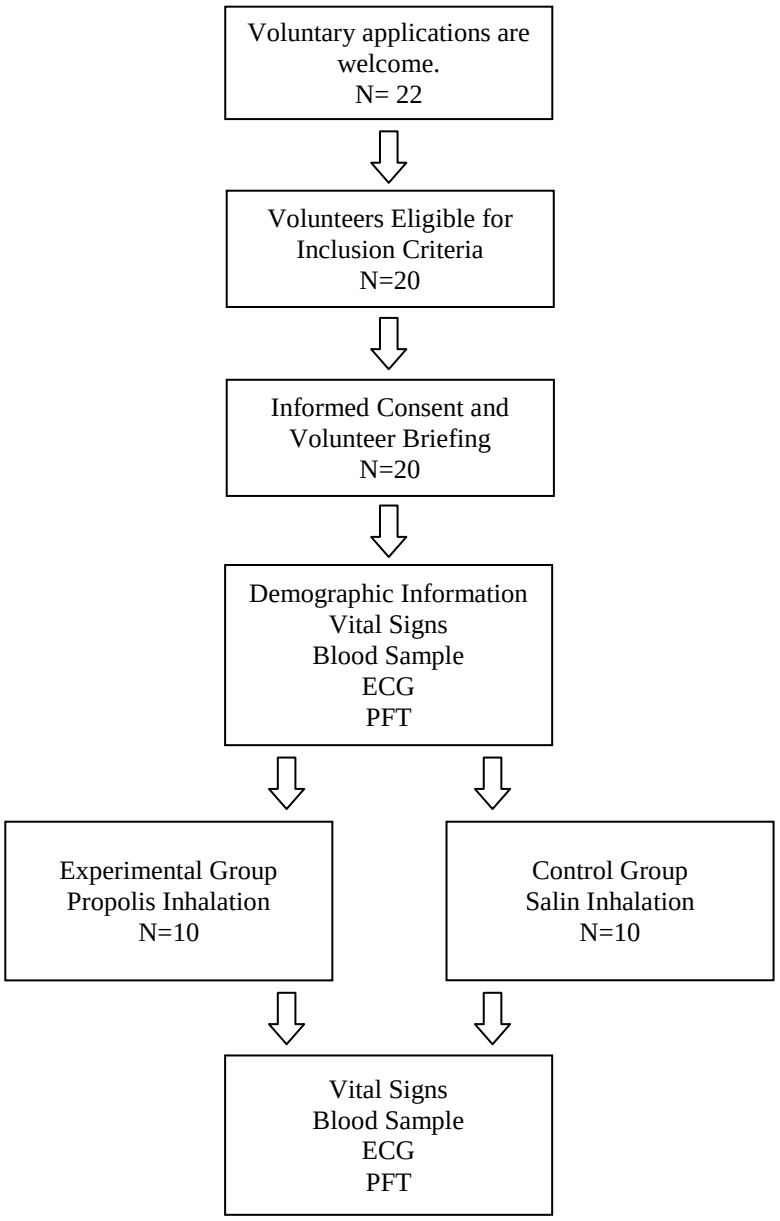

Figure 1. Trial flow-chart.

Chemical composition of propolis by HPLC-DAD

A 10% water-based organic propolis solution was provided by the company, Fanus Food and Organic Products Industry and Trade Ltd. Co. Figure 2 presents the HPLC-DAD chromatograms [27].

Table 2 presents the qualitative and quantitative analysis of the identified compounds in the water-based organic propolis. The amounts of caffeoylquinic acids in propolis are expressed as mg

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of polyphenol or caffeoylquinic acid in the propolis extract. HPLC-DAD analysis revealed that the most abundant compound in propolis was caffeic acid, with a concentration of 204.00 mg/mL Additionally, caffeic acid and trans-cinnamic acid were identified as common compounds in the propolis. Caffeic acid was found in much higher amounts compared to other compounds in the propolis [27].

The HPLC-DAD method was validated by performing replicate analyses and confirming the reproducibility of the results. The linearity and precision of the HPLC method were assessed with multiple samples.

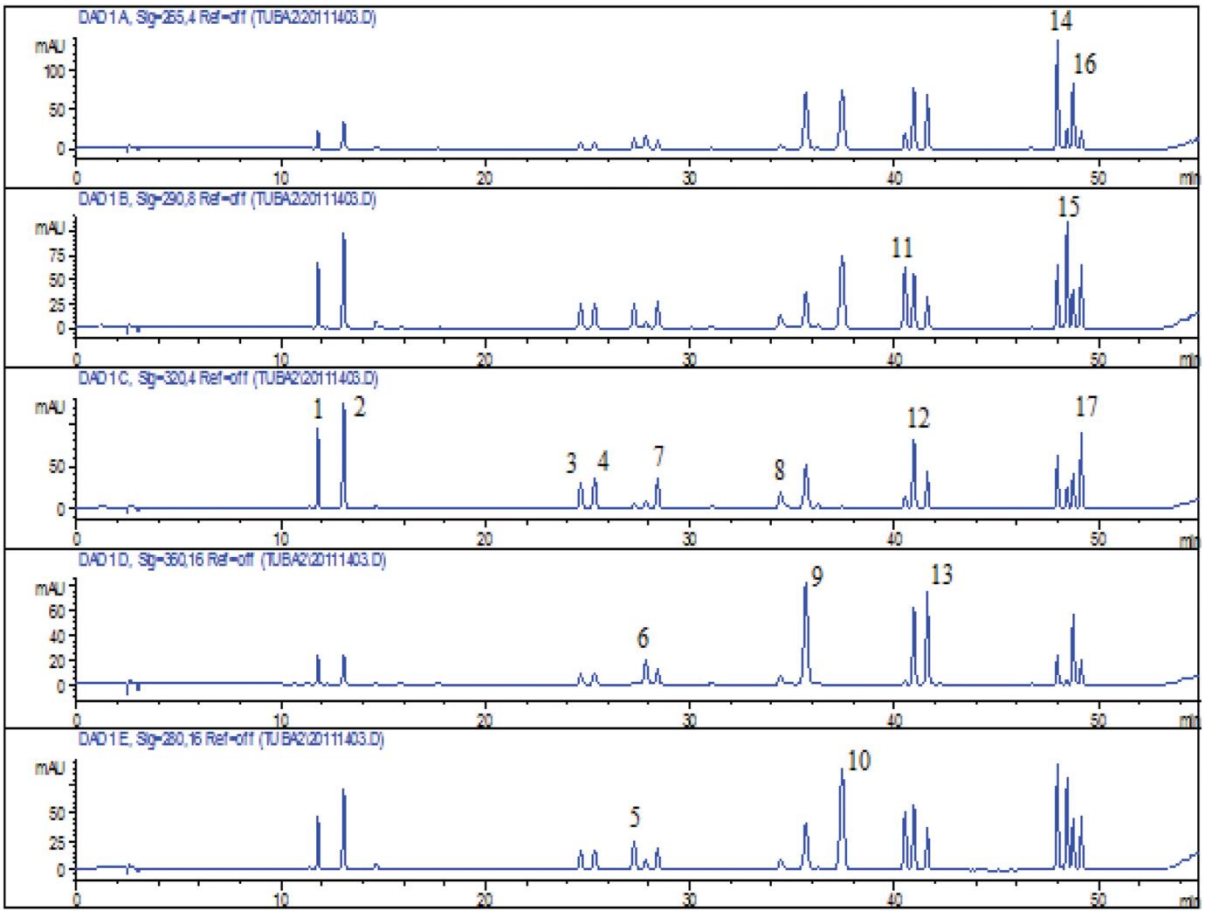

Figure 2. HPLC-DAD chromatograms of a standard solution recorded at wavelengths of 265, 290, 320, 360, and 280 nm [27].

Table 2. HPLC-DAD analysis of water extract of propolis [27].

Number	Compounds	Wavelength (nm)	Retention time (RT)	Concentration (mg/mL)
1	*Chlorogenic acid*	320	*12.484*	*10.20*
2	*Caffeic acid*	320	*13.954*	*204.00*
8	*3,4,5-tri-O-caffeoylquinic acid*	320	*39.694*	*7.75*
11	*Naringenin*	290	*41.309*	*28.90*

Preparation of inhalation products

For the control group, 4 mL of saline solution was added to the medication chamber of the inhalation device using a syringe. The content of the medication chamber was sealed with adhesive tape to prevent visibility, and odd numbers were written on the chamber. For the experimental group, 3 mL of saline solution and 1 mL of 10% water-based organic propolis solution were added to the medication chamber of the inhaler mask set using a syringe. The medication chamber was sealed with adhesive tape to prevent visibility, and even numbers were written on the chamber. The number and content information were documented in an external file.

Pre-inhalation assessment

Volunteers included in the study arrived at the emergency department 30 minutes before inhalation. The informed consent form was signed by the participants, demographic information was recorded by the emergency department nurse, vital signs were measured, an ECG was performed, and a venous blood sample was taken. Then, pulmonary function tests were performed by a pulmonologist using a Spirolab III S/N manual device, and the WinspiroPRO 8.2.0 - Mod.C11 version was used for programming the measurements. Each volunteer was evaluated individually. To obtain expected values, parameters such as age, height, weight, gender, ethnicity, smoking status, and disease groups were entered into the program. Each volunteer was provided with a personal mouthpiece and asked to sit in a chair. Volunteers were instructed to place the mouthpiece between their teeth, take a deep breath, and then exhale forcefully. Measurements were repeated up to three times at the discretion of the specialist based on the expected values.

Application of inhalation protocols

In the experimental group, 3 mL of saline solution and 1 mL of 10% water-based propolis extract, totaling 4 mL of solution, were added to the medication chamber of the inhaler mask set. The chamber was sealed with adhesive tape to obscure the color of the solution. The Omron Compressor Nebulizer (CompAir NE-C28-P) was used for inhalation. Volunteers inhaled the solution for an average of 7 minutes. In the control group, saline solutions were also sealed with adhesive tape to hide the solution's color. The Omron Compressor Nebulizer (CompAir NE-C28-P) was used, and volunteers inhaled for an average of 7 minutes.

Post-inhalation evaluation

After inhalation, volunteers' vital signs were recorded on the case report form by the nurse, an ECG was performed, and a venous blood sample was taken. For pulmonary function testing, the same pulmonologist used the Spirolab III S/N manual device, with measurements programmed using the WinspiroPRO 8.2.0 - Mod.C11 version. Each volunteer was evaluated individually. To obtain expected values, parameters such as age, height, weight, gender, ethnicity, smoking status, and disease groups were entered into the program. Each volunteer was provided with a personal mouthpiece and asked to sit in a chair. Volunteers were instructed to place the mouthpiece between their teeth, take a deep breath, and then exhale forcefully. Measurements were repeated up to three times at the discretion of the specialist, based on expected value ratios.

Statistical analysis

The data obtained in the study were analyzed using the SPSS (Statistical Package for Social Sciences) Windows 22.0 software. Descriptive statistical methods such as number, percentage, mean, and standard deviation were used to evaluate the data. Differences in the proportions of categorical variables between independent groups were analyzed using the Chi-square test. Continuous quantitative variables were compared between two independent groups using the independent samples t-test, while within-group comparisons were conducted using the paired samples t-test.

Ethical approval

This study was conducted following ethical guidelines, with approval from the Istanbul Medipol University Traditional and Complementary Medicine Clinical Research Ethics Committee, dated 19/08/2021, and numbered E-95961207-604.01.01-3898, in accordance with the Declaration of Helsinki.

RESULTS

In the study, 20 healthy individuals were included as participants. No statistically significant difference was observed in gender distribution across the groups ($p=0.763$). The age and BMI scores of the volunteers did not show significant differences between the groups ($p>0.05$) (Table 3).

The pre-inhalation and post-inhalation respiratory rate measurements did not show a significant difference between the groups ($p>0.05$). In the control group, the increase in respiratory rate from the pre-inhalation value ($\bar{x}=19.100$) to the post-inhalation value ($\bar{x}=19.400$) was not found to be significant ($p>0.05$). In the experimental group, however, the increase in respiratory rate from the pre-inhalation value ($\bar{x}=18.800$) to the post-inhalation value ($\bar{x}=19.500$) was found to be significant ($p=0.045$) (Table 4).

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Table 3. Sociodemographic characteristics of the participants (n=20).

		Control Group		Experimental Group		Total		p*
		n	%	n	%	n	%	
Gender	**Male**	1	10.0	1	10.0	2	10.0	X^2=0.000
	Female	9	90.0	9	90.0	18	90.0	p=0.763
		Mean	**SD**	**Mean**	**SD**	**t**	**SD**	**p***
Year		24.800	2.936	27.100	6.757	-0,987	18	0.337
BMI		22.744	2.166	21.670	2.472	1,033	18	0.315

*Chi-square test; SD: Standard Deviation

Table 4. Differences in pre- and post- inhalation respiratory rate measurements between groups (n=20).

Groups	Control Group (n=10)		Experimental Group (n=10)		t[a]	p
	Mean	SD	Mean	SD		
Pre-inhalation respiratory rate	19.100	0.994	18.800	0.919	0.701	0.492
Post-inhalation respiratory rate	19.400	0.843	19.500	0.527	-0.318	0.754
t[b]	-0.709		-2.333			
P	0.496		**0.045**			

[a]Independent samples t-test; [b]Paired samples t-test; SD: Standard Deviation

There were no significant differences between the pre- and post-inhalation values for body temperature, systolic and diastolic blood pressure, pO2, pCO2, SaO2, and HCO3 between the two groups (p>0.05). In the experimental group, a significant increase in heart rate was observed from the pre-inhalation value ($\bar{x}$=77.000) to the post-inhalation value ($\bar{x}$=83.500) (p=0.026), but this increase remained within physiological limits.

The pre- and post-inhalation FVC measurements did not show significant differences between the groups (p>0.05; 95% CI [L/U]=-0.852/16.052). Similarly, the pre- and post-inhalation FEV1 measurements did not show significant differences between the groups (p>0.05).

However, post-inhalation FEV1/FVC measurements showed a significant difference between the groups (p=0.012). The post-inhalation FEV1/FVC measurements in the experimental group ($\bar{x}$=89.970) were higher than those in the control group ($\bar{x}$=86.460). Pre-inhalation FEV1/FVC measurements did not show significant differences between the groups (p>0.05). In the control group, the decrease in FEV1/FVC from the pre-inhalation value ($\bar{x}$=87.730) to the post-inhalation value ($\bar{x}$=86.460) was not significant (p>0.05). In the experimental group, the increase in FEV1/FVC from the pre-inhalation value ($\bar{x}$=88.790) to the post-inhalation value ($\bar{x}$=89.970) was also not significant (p>0.05) (Table 5).

Pre-inhalation blood pH measurements did not show significant differences between the groups (p>0.05) (Table 6).

Table 5. Differences in FEV_1/FVC Measurements Between Groups (n=20).

Groups	Control Group (n=10)		Experimental Group (n=10)		t[a]	p
	Mean	SD	Mean	SD		
Pre-inhalation FEV_1/FVC	87.730	3.164	88.790	4.408	-0.618	0.544
Post-inhalation FEV_1/FVC	86.460	1.490	89.970	3.691	-2.789	**0.012**
t[b]	1.563		-1.807			
P	0.152		0.104			

[a]Independent samples t-test; [b]Paired samples t-test; SD: Standard Deviation

Table 6. Differences in pH Measurements Between Groups (n=20).

Groups	Control Group (n=10)		Experimental Group (n=10)		t[a]	p
	Mean	SD	Mean	SD		
Pre-inhalation blood pH	7.351	0.034	7.336	0.023	1.167	0.258
Post-inhalation blood pH	7.377	0.030	7.348	0.025	2.281	**0.035**
t[b]	-2.082		-1.213			
p	0.067		0.256			

[a]Independent samples t-test; [b]Paired samples t-test; SD: Standard Deviation

DISCUSSION

In our study, no significant differences were found between the control and experimental groups in terms of vital signs, including body temperature, systolic and diastolic blood pressure, and ECG values. However, in the experimental group, the increase in respiratory rate from pre-inhalation to post-inhalation, as well as the similar increase observed in the control group, remained within clinically normal limits. Additionally, the increase in heart rate remained within normal clinical ranges.

Changes in key parameters, such as FEV1/FVC are clinically significant, as they provide insight into the balance between forced vital capacity (FVC) and forced expiratory volume in one second (FEV1). A decrease in the FEV1/FVC ratio may indicate obstructive lung conditions, while a normal or increased ratio suggests preserved lung function. In this study, the observed changes in these parameters could reflect the potential effects of the inhaled treatments on pulmonary function, warranting further investigation. These findings highlight the importance of monitoring these ratios in evaluating the clinical efficacy of inhalation therapies. When analyzing the PFT values, an increase in the FEV1/FVC ratio was observed in the experimental group after inhalation compared to before inhalation. This finding is promising, as it suggests the potential for water-based propolis inhalation to positively impact the FEV1/FVC ratio in future studies targeting diseases such as COPD and asthma. Additionally, blood parameters such as pH, pCO2, pO2, and HCO3 did not show significant changes between pre- and post-inhalation measurements in either group.

Among the bioactive properties of propolis, its antioxidant effect is particularly notable[28,29]. In addition, propolis is known to exhibit anti-inflammatory [6,12], antimicrobial [30], and anticancer [31] properties. Studies have shown that these bioactivities are particularly related to the flavonoid content of propolis [32,33]. Propolis has also been used in the prevention of respiratory infections in children [34].

Studies on hive air are quite limited. The few existing studies have focused on the composition, potential bioactivity, and safety of hive air [4,35]. In one study, the volatile compounds in hive air were identified and categorized as fatty acids, alcohols, aldehydes, esters, ethers, hydrocarbons, phenols, ketones, nitrogen-containing compounds, and terpenes. They were found to be abundant and closely associated with anti-inflammatory, anti-asthmatic, and antimicrobial effects [4,35]. These data support the potential efficacy of hive air inhalation in the treatment of respiratory conditions such as asthma, bronchitis, and pulmonary fibrosis. Apiair therapy has been utilized to promote relaxation, enhance sleep quality and continuity, and facilitate easier breathing. However, given the limited research on this topic in the existing literature, our study aims to be the pioneering clinical trial in this field.

The limitations of this study include its mall sample size, short duration, and focus on immediate effects. Additionally, the absence of a comparative treatment group, lack of control for environmental factors, and limited scope of measurements restrict the generalizability and comprehensiveness of the findings.

CONCLUSION

This study provides evidence that propolis inhalation is safe in the short term, with no significant adverse effects observed on vital signs, venous blood gas parameters, or ECG results. Notably, it also demonstrates a positive influence on pulmonary function test values, suggesting its potential therapeutic benefits. Given the limited existing research on propolis inhalation, particularly in healthy volunteers, these findings contribute valuable insights into the safety and initial effects of propolis inhalation, as a key component of Apiair therapy, pioneering in this field. However, further research with larger sample sizes and longer durations is necessary to fully understand the efficacy and long-term safety of propolis inhalation.

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Conflict of interest: There is no potential conflict of interest among the authors

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