

Combination of *Nephrolepis Exaltata* - *Hibiscus Rosa Sinensis* Herbal Mask Protect The Sinonasal Immune System and Increase Lung Function of Motorcycle Taxi Drivers

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Research

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Abstract

Background:

Combination of *Nephrolepis exaltata* - *Hibiscus rosa sinensis* (NEHRS) herbal mask proved to decrease nasal mucociliary transport time, increase sinonasal IgA and lung function in textile industry workers. Its protection effect on motorcycle taxi drivers exposed to acute motor vehicle exhaust pollutants has not been proven. The study aimed to demonstrate the protective effect of using NEHRS herbal mask both in the sinonasal immune system and lung function.

Methods:

This research was non-blinded randomized control trial with pre-post test design. Thirty two motorcycle taxi drivers were randomly assigned into three groups; positive control (n=11) using regular medical mask, negative control (n=10) using nonwoven cloth mask, and intervention group using NEHRS herbal mask (n = 11). Nasal wash was taken by ENT specialist, and the level of sinonasal IgA and IL-6 was reading by ELISA. Pulmonary function parameters were performed using the MAS-1-A spirometer, Data were analyzed with Wilcoxon, Paired t-test, Mann Whitney and One-way ANOVA

Results:

Sinonasal IgA level were significant decrease in all groups, respectively; intervention 16.31 ± 3.38 ; control (+) 16.24 ± 2.62 ; control (-) 15.28 ± 2.50 . The IL-6 level also decrease in all groups in significantly difference ($p=0.031$), the delta were; intervention -6.92 ± 8.61 ; control (+) -10.23 ± 7.65 ; control (-) -8.56 ± 4.37 . A significant increased of FEV1 ($p=0,005$) and PEF ($p = 0,005$) in the intervention group, but nearly significant of FVC ($p=0,053$), as well as in control (+); FVC ($p=0,017$), FEV1 ($p=0,005$), PEF ($p<0,001$), and control negatif; FVC ($p=0,046$), FEV1 ($p=0,010$), PEF ($p=0,005$). The test for difference of increasing FVC ($p=0,652$), FEV1 ($p=0,696$) and PEF ($p=0,670$) between all groups not significantly different.

Conclusions: NEHRS Herbal mask that worn by motorcycle taxi drivers for 2 weeks decreased IgA and IL-6 sinonasal levels, as well as increased FVC, FEV1, and PEF.

Introduction

In Indonesia, the number of online motorcycle taxi riders is overgrowing and may impact increasing occupational disease. The International Labor Organization (ILO) noted an increase in occupational lung disease due to air pollution exposure, including in Indonesia. However, there is no data for online motorcycle taxi drivers. Land transportation statistics recorded an average increase of 6.61% of motorcycles in 2014–2018, so that by the end of 2018, there were 120,101,047 motorbikes, with more than 2 million motorcycle taxi drivers. The increase in the number of motorized vehicles will affect air quality with the exhaust gases released.

Air chemical pollution due to motor vehicle exhaust, mainly in the form of Volatile Organic Compounds (VOC), i.e., Benzene, Toluene, Ethylbenzene, and Xylene (BTEX), nitrogen oxides (NO₂), as well as other particulates, such as suspended particulate matter (SPM), sulfur oxide (SO₂), carbon monoxide (CO), ozone (O₃), hydrocarbons (HC), Particulate Matter 10 (PM₁₀), Particulate Matter 2,5 (PM_{2,5}), TSP (dust), Pb (lead), dustfall, and others. The concentration of gas from air pollution can be affected by wind, air temperature, and humidity. Increasing vehicle population may cause more traffic jams or occur more frequently, leading to a drastic increase in air pollutant emissions and worsening air quality, especially on main roads that are heavily populated with motorized vehicles.

Airway exposure to VOC such as benzene, formaldehyde, and polycyclic aromatic hydrocarbons (PAHs) produced by motorized machines may interfere with the respiratory system's health, both acute and chronic. These compounds will be inhaled by online motorcycle taxi drivers and cause various respiratory system disorders. Inhalation of VOC causes inflammation of the lung parenchyma airways, which manifests as decreased lung function.

Chronic inhalation of these compounds causes the airways and lung tissue chronic inflammation in decreased lung function. The average operation time of online motorcycle taxi drivers was 12 hours every day, and more exposed to air pollution, which causes Occupational Lung Disease. Decreased lung function parameter values associated with the occurrence of obstruction, restriction, or both. The higher the levels of inhaled pollutants, the greater the symptoms, such as lower respiratory problems, chest tightness, shortness of breath, and coughing.

As foreign particles pass through the nasal and oral mucosa, sinonasal secretory IgA acts as an immune system that reduces colonization of mucosal surface bacteria by decreasing the permeability of bound bacteria. Immunoglobulin A may also capture mucosal bound antigens so that it is recognized and presented by dendritic cells. So, IgA plays a vital role in the sinonasal immune system. Homeostasis of IgA levels in the sinonasal mucosa as protection for foreign objects' entry certainly supports the health of the body.

When exposed to air pollutants such as VOCs and PAHs, the sinonasal mucosa has increased Interleukin (IL) production – 2, IL-4, IL-6, IL-10, IL-13, and INF γ . A study conducted simultaneously in 6 European cities (Helsinki, Stockholm, Augsburg, Rome, Barcelona, Athens) that increased the concentration of CO (340 $\mu\text{g} / \text{m}^3$) in 6–11 hours and NO₂ (15.9 $\mu\text{g} / \text{m}^3$) in 0 hours – 24 has a close relationship in the occurrence of increased plasma IL-6 levels.

Quality of life and productivity has decreased, so it is necessary to reduce the inhaled hazardous material, with tighter protection of the mouth and nose wearing a mask that can filter work dust/vapor entry. Most online motorcycle taxi riders have worn protective medical masks, which filter out particles of more than 3 μm or cloth masks with filter capabilities between 16.9–51.0 μ .

Our study proved the effectiveness of herbal masks made from *Nephrolepis exaltata* extract to filter from 0.7 to 1 micrometer on employees of textile factory dyeing units. Herbal masks can reduce the rate of

nasal mucociliary transport, increase the sinonasal immune system as measured by IgA and IL-6 levels and lung function, such as FVC, FEV1, and PEF.

The combination herbal mask laminated with *N. exaltata* extract has the ability to absorb air pollution, such as volatile organic compounds and formaldehyde, up to 77%. The extract content of *H. rosa sinensis* L. in the form of several antioxidant compounds, such as flavonoids, steroids, alkaloids, phenolics, saponins and tannins, functions as an antimicrobial, anti-inflammatory, which reduces the number of mast cells in patients with respiratory tract infections and is immunoprotective against specific tract antigen infections. inhalation, especially *Mycobacterium tuberculosis*. This extract also increases the function and activity of pulmonary macrophages.”

Our previous study also has developed a prototype herbal mask of *Nephrolepis exaltata* extract, which block sinonasal pathogenesis in a group of mice exposed to BTEX vapors. This herbal mask has high effectiveness in protecting the respiratory system from exposure to volatile organic compounds. *Nephrolepis exaltata* is fast-growing and found in many forest and swamp areas in Indonesia. *Nephrolepis exaltata* can absorb formaldehyde, xylene, trichloroethylene, and carbon monoxide from the air, and the amines in it can react with formaldehyde to produce imine and water.

Those research has never been conducted in an outdoor environment, so further research is needed to assess this combination herbal mask's potential in protecting the respiratory tract of online motorcycle taxi drivers from exposure to chemicals and pollution due to motorized vehicles. This study aims to prove that the sinonasal immune system and lung function of online motorcycle taxi riders who wear combination herbal masks will be better than those who use regular surgical masks or cloth masks.

Methods

Unblinded Randomized Control Trial with pre-post test design was conducted on 32 subjects of online motorcycle taxi riders. The inclusion criteria were: male aged 17–35 years, healthy condition, at least work as an online motorcycle taxi driver for one year, work duration 6–8 hours, no history of chronic lung disease, heart disease, and allergic. Subjects were randomized allocated into 3 (three) groups, i.e; (i) the control (+) wore regular medical masks (n = 11); (ii) the control (-) used cloth masks (n = 10), and (iii) the intervention group used medical masks laminated with a combination of *Nephrolepis exaltata* and *Hibiscus rosa sinensis* extracts (n = 11). They were subjected to a pre-test of spirometry test and nasal wash to measure IgA and IL-6 ELISA levels. Supervision was carried out by the researcher every day for compliance with masks that must be worn during work and replaced every day. After two weeks, the same post-test measurements were taken.

Plant material and Preparation of NEHRS herbal masks

Making extracts of *Nephrolepis exaltata* and *Hibiscus rosa-sinensis* was carried out at the Laboratory of Science and Mathematics, Diponegoro University, Semarang. It processed for patent registration of

Indonesia (Number of Application: S00201909409). Preparation of NEHRS herbal masks by CV. Beauty Kasatama Surabaya.

Spirometry

Lung function parameter values were measured using the "MAS-1-A spirometer type, which has routinely calibrated, so the measurement results are reliable and accurate. Subjects inhaled and exhaled air through a disposable mouthpiece attached to a spirometer. The measurements were as follows: Forced vital capacity (FVC), forced expiratory volume in one second (FEV1), and peak expiratory flow (PEF), before (pre) and after treatment (post-test).

Laboratory analysis

Examination of IgA and IL-6 levels were using nasal wash. Subjects were instructed to sit with the head extended 45°, then took a deep breath and held their breath. The syringe was filled in with 5 ml of isotonic solution at 37°C, inserted into one nose (and the other nose was closed). The subject held the position for a few seconds, then looked down and slowly drained the liquid into place container. The procedure was done in the other nostril and done twice. All nasal wash fluid was stored at 4°C. Each sample was processed for testing by ELISA method. ELISA test for IgA levels uses the Human IgA ELISA Kit ABCLONAL® No: RK00200-96 wells, dan Human IL-6 ELISA Kit 96 ABCLONAL® wells RK00004-96 wells.

Statistical analysis

All data were analyzed with SPSS for Windows version 25. The subjects' characteristics were tested for differentiation using Chi-square and Kruskal Wallis. The primary data on spirometry of FVC, FEV1, and PEF, as well as IgA and IL-6, were analyzed using Kruskal Wallis, Paired t-test, Wilcoxon, and One Way Anova. Data on the IgA level were tested by the Shapiro-Wilk test to see the data distribution, followed by One way ANOVA since the distribution was normal.

Ethical clearance

The health research ethics committee of the Faculty of Medicine, UNDIP and Dr. Kariadi Semarang No.82 / EC / KEPK / FK UNDIP / V / 2020 approved this study.

Results

Two subjects dropped out because they did not attend the post-test, each in control (+) and treatment groups. Finally, the number of participants was 30 people. Table 1 shows the subjects' characteristics, including; age, BMI, presence/absence of respiratory problems, history of smoking, exercise habits, hours of work per hour, and per week. There was no significant difference in all subject's characteristics

The average length of work was 6.32 days (3–7 days per week). Two participants have other jobs that pose a risk of exposure to pollutants. Four subjects stated that they had a history of breathing problems,

and 12 participants had a history of smoking habits.

Spirometry of FVC, FEV1 and PEF

Table 2 shows the increase in the FVC value in all groups, where the control (+) group was the highest compared to the intervention and control (-) groups. The FVC value only increased significantly in the control (+) and control (-) with $p = 0.017$ and $p = 0.046$ respectively.

FEV1 value increased in all groups, where the control group (+) was the highest compared to the intervention and control (-). The PEF value also increased significantly in all groups, where the PEF value in the control (+) group increased the highest compared to intervention and control (-).

Table 3 describes the clinical interpretation of spirometry in all groups, pre-post test measurements. All groups shows the improving spirometry clinical normal status after using masks (post-test). In three intervention group, the post test of spirometry restriction status were zero, as well as decreasing number in control (+) and control (-) groups.

IgA Sinonasal

The pre-test for Sinonasal IgA levels in all groups was not significantly different ($p = 0.376$), as well as after intervention ($p = 0.858$) (Table 4). The results of this study showed IgA levels decreased significantly in all groups ($p = < 0.001$). The largest decrease was in the intervention group, followed by the control (+) and (-) group. The difference in decreasing IgA levels when compared between the three groups was not significantly different.

IL-6 Sinonasal

Table 5 shows that all groups significantly decreased the level of IL-6. However, there were significant difference when comparing the delta between the three groups ($p = 0.031$).

Discussion

This study proves the protective ability of the combination NEHRS herbal mask on the sinonasal immune system and the improvement of pulmonary function parameters for online motorcycle taxi drivers.

NEHRS Herbal Masks Increase FVC, FEV1, and PEF

The results of this study showed an increase in lung function parameters (FVC, FEV1, and PEF) in the treatment, control (+) and control (-) groups, where the use of medical masks showed better results than

NEHRS herbal masks and cloth masks. The increase in FEV1 and PEF values in the use of surgical masks was higher than the NEHRS for herbal masks and cloth masks.

The results also showed a different clinical interpretation after 14 days of mask use. Subjects wearing herbal masks experienced clinical improvement, namely; found a clinical improvement in 4 subjects who had mild restriction disorders, and pulmonary function in 6 other subjects was normal. In the subject who used surgical masks, 3 subjects previously had mild restrictions experienced improvement, but 3 other subjects did not experience clinical improvement. Subjects who wore cloth masks, 2 subjects previously had mild restrictions improving, but 4 other subjects did not experience clinical improvement. The results of clinical interpretation showed that the effectiveness of herbal masks in improving clinical improvement of lung function was better than surgical masks and cloth masks. The results of this study are in synergy with the study of the effects of using masks to filter out pollutants, where masks are able to filter out pollutants and the penetration that occurs is only 0.26-29% based on the type of mask used. Previous studies on the use of herbal masks made from nail blade (*Nephrolepis exaltata*) plant workers found a decrease in nasal mucociliary transport rate, increased levels of IgA, IL-6 and sinonasal TNF- α , and increased lung function.¹⁵

Based on an assessment conducted by the Environmental Protection Agency (EPA) in the United States, it shows that acute exposure to air pollutants can cause obstructive pulmonary disease, asthma to deadly effects. Studies on students who are exposed to air pollution for a month with symptoms of headache, cough, mucus buildup and sore throat prove a significant decrease in PEF scores. Acute exposure to pollutants also results in decreased FVC and FEV1 values in nonsmoking adults. Decreased lung function can be easily detected by decreasing the value of pulmonary function parameters, namely; FVC, FEV1, and PEF by spirometry examination.

The FCV value was found to increase significantly ($p = 0.053$, Paired T-test) in the treatment group, while the control (+) and control (-) groups experienced a significant increase. These different results can be caused due to the influence of environmental factors, such as differences in ambient temperature. Studies examining the effect between acute exposure to pollutants or particulate matter and environmental temperature on lung function found that there was a decrease in FEV1 and PEF values that were higher in the afternoon than in the morning, with insignificant reduction in FVC values, on exposure to pollutant or particulate levels. the same matter.

NEHRS Herbal Mask Decreases IgA and Increases Sinonasal IL-6

This study resulted in a decrease in sinonasal IgA levels in the use of a combination herbal mask of *N. Exaltata* and *H. Rosa sinensis L*, regular medical masks and cloth masks due to acute exposure to air pollution. Herbal masks had the largest decrease compared to medical and cloth masks.

Motor vehicle exhaust contains a large amount of pollutants such as VOC emissions, namely BTEX, NO₂, and other particulates such as suspended particulate matter, SO₂ and CO₂. Inhalation of motor vehicle

exhaust pollutants has the potential to cause respiratory system disorders, such as airway inflammation and lung parenchymal inflammation.

The United States Environmental Protection Agency shows that short-term (acute) exposure to air pollutants can cause obstructive pulmonary disease, asthma to lethal effects.²⁵ Immunoglobulin A is the initial immune system that acts on the mucosa as protection for foreign bodies such as air pollutants and bacterial infections through its role as an anti-inflammatory agent.^{10,11}

Studies on low-dose benzene-exposed fuel tank workers have shown a decrease in IgM, IgA and CD4 T levels due to the suppression of carbon monoxide polychromatic substances contained in benzene solutions. Benzene exposure at a low concentration < 15 ppm resulted in depression of B cells, so that the resulting immunoglobulin secretion also decreased. Chronic inhalation of chemical exposure at older age and longer duration has been shown to further increase IgA.

The decrease in IgA levels in acute exposure can occur due to the bifunction of IgA, which is both anti-inflammatory and pro-inflammatory. IgA function is mediated by the FcR receptor, IgA Fc receptor or FcαRI. The FcαRI receptor with FcRγ activates the inflammatory response with the ITAM tyrosine immune receptor, thereby enhancing various cellular processes. IgA secretion stimulates exposure to pollutants and bacterial infections, causing levels to increase. Immunoglobulin A that binds to antigens (such as pollutants and bacteria) will then bind in the opsonization process, so that it can induce proinflammatory cytokines such as IL-6 and TNF α, while naturally IgA will induce inhibitory signals through FcαRI to reduce excessive immune system, through mediation of ITAMi receptors (inhibitors). Immunoglobulin A can also capture antigens that will be presented to dendritic cells through phagocytosis and inhibit the maturation of pro-inflammatory cytokines, so that the number decreases if it occurs in acute exposure. Acute exposure will cause pro-inflammatory cytokines to increase, as well as increased IgA levels. Simultaneously, IgA will then suppress pro-inflammatory cytokines, so that inflammation will decrease which will be followed by a decrease in IgA levels.^{10,}

IgA levels are also influenced by physical exercise through the activation mechanism of autonomic nerves which reduces the amount and secretion of saliva. Extreme or repeated physical exercise without a resting phase can trigger a decrease in the immune system, including IgA. However, if you do moderate physical exercise, it can actually boost your immune system. This research questionnaire has included exercise habits as a measuring variable, but it has not been classified into light, moderate and heavy levels, so that the physical exercise variable can be a confounder in measuring IgA levels.

Sinonasal IgA levels were decreased in all groups, when compared, there was no significant difference. However, the use of combination herbal masks had the largest difference, followed by regular medical masks and cloth masks. The herbal mask combination of *Nephrolepis exaltata* L. and *Hibiscus rosa-sinensis* L. is able to absorb volatile organic compounds and formaldehyde up to 77%, and its antioxidant content is antimicrobial, anti-inflammatory, reduces the number of mast cells and is immunoprotective of the respiratory tract.^{22,24}

In addition to that ability, the mask density test conducted with Desktop Microscope Electron, showed that the average diameter density of combination herbal masks was 0.7-1.0 μm , better than regular medical masks (3 μm density mean diameter), and cloth masks. (mean diameter density 16.9–51.0 μm). Combined herbal masks and regular medical (surgical) masks consist of 3 layers of protection. The three layers of this mask consist of a waterproof outer layer to protect from droplet splashes, a middle layer as a filter against germs, and an inner layer to absorb the liquid that comes out of the mask user's mouth. This is of course different from cloth masks which only consist of one layer and have low filtration capabilities.

The pretest IL-6 levels of the 3 groups were not different, meaning that the cellular immune conditions and characteristics of the subjects of each group were the same, and confounding variables of the subject characteristics had no effect on the dependent variable, thus eliminating the possibility of bias. The pretest IL-6 level compared to the post-test in all groups had a significant decrease, so it can be said that the three types of masks can improve the sinonasal immune system through decreasing IL-6 levels, within a period of 2 weeks. The difference in pre and post test IL-6 levels, or the degree of reduction of each group when compared to the three at once, was significant ($p = 0.031$, Mann Whitney). This study proves that the use of a combination NEHRS herbal mask has the most potential to improve the sinonasal immune system because the degree of reduction in IL-6 is the largest compared to the regular medical mask and cloth mask groups.

A study to compare the effectiveness of 4 types of masks against air pollution, by conducting 2 experiments first with artificial aerosol particles and second with diesel engine combustion particles proved that ordinary cloth masks had the worst performance in filtering pollutant particles, and were worse again when tested with diesel particles. The performance decreased when tested with diesel particles compared to synthetic aerosol particles due to the nature of the combustion particles from diesel engines which have a very wide size variation of engine combustion. Another study that tested the filtration between an N95 mask, a KF80 mask, a surgical mask, a regular cloth mask, and a handkerchief proved that surgical mask filtration was better than cloth masks and handkerchiefs, but worse than N95 and KF80 masks. This study compared the use of a combination of NEHRS herbal masks, regular medical masks and cloth masks, and showed that the highest decrease in IL-6 levels occurred in the herbal mask group. This study also succeeded in proving the comparison of the potential of masks in reducing IL-6 levels due to differences in diameter, density and ability to absorb the chemical content of inhaled pollutants.

The study time setting in pandemic conditions is a limitation in this study, related to the level of compliance with the use of masks which is only monitored by the WhatsApp media by asking the subject to send a photo while using a mask every day. In addition, the shorter duration of the intervention and the limited number of subjects can also influence the study results. The next study aimed to prove the difference between the potency of the combination of herbal mask NEHR and N95 mask, with a larger subject.

Abbreviations

BMI

Body mass index

BTEX

Benzene, toluene, etilbenzene, xylene

CD4

Cluster of differentiation-4

CO

Carbon monoxide

CONSORT

CONsolidated Standards of Reporting Trials

ELISA

Enzyme-linked immunosorbent assay

EPA

Environmental Protection Agency

FEV1

Forced expiratory volume in one second

FVC

Forced vital capacity

FEV₁

Forced expiratory volume in one second

HC

Hydrocarbon

IgA

Immunoglobulin A

IL-6

Interleukin-6

ILO

International labour organization

INF γ

Interferon gamma

NEHRS

Nephrolepis exaltata - Hibiscus rosa sinensis

NO₂

Nitrogen oxide

O₃

Ozone

PAH

Polycyclic aromatic hydrocarbon

Pb
Plumbum
PM₁₀
Particulate Matter 10
PM₂₅
Particulate Matter 25
PEF
Peak expiratory flow
SO₂
Sulfur oxide
SPM
Suspended particulate matter
VOC
Volatile organic compound
VC
Vital capacity
WHO
World Health Organization

Declarations

Ethics Approval

Ethical clearance was obtained from Commission on Health Research Ethics, Faculty of Medicine, Diponegoro University and Dr. Kariadi Hospital, Semarang, Indonesia. (No. 82/EC/KEPK/FK-UNDIP/V/2020).

Consent to Participate

Consent for publication

Our article contains human data, which through these forms we received informed consent from the subject for we can publish these data while maintaining secrecy of their personal informations.

Availability of Data and Material

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing Interests

The authors declare that they have no competing interest.

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Authors' contributions

AP, US and ALA performed the nasal wash and spirometry examination, and were a major contributor in writing the manuscript. MHA and AD analyzed and interpreted the result of nasal wash examination. AAS analyzed and interpreted the result of spirometry examination. All authors read and approved the final manuscript.

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Tables

Table 1. Subject characteristics

Variables	Groups			<i>p</i>
	Intervention	Control (+)	Control (-)	
Age	26.30 ± 4.83	24.90 ± 5.20	27.60 ± 5.21	0.503 [§]
BMI	27.82 ± 6.47	25.08 ± 3.57	24.69 ± 2.19	0.247 [§]
Respiratory disorders				
Yes	0 (0%)	1 (10%)	3 (30%)	0.133 [¥]
No	10 (100%)	9 (90%)	7 (70%)	
Exercise				
Yes	1 (10%)	5 (50%)	5 (50%)	0.101 [¥]
No	9 (90%)	5 (50%)	5 (50%)	
Smoking				
Yes	3 (30%)	4 (40%)	5 (50%)	0.659 [¥]
No	7 (70%)	6 (60%)	5 (50%)	
Duration of work/daily				
< 8 hours	3 (30%)	1 (10%)	1 (10%)	0.383 [¥]
> 8 hours	7 (70%)	9 (90%)	9 (90%)	
Length of working/week				
5 days	2 (20%)	2 (20%)	1 (10%)	0.380 [¥]
6 days	1 (10%)	4 (40%)	5 (50%)	
7 days	7 (70%)	4 (40%)	4 (40%)	

[¥] *Chi square*; [‡] *Kruskal wallis*

Table 2. FVC, FEV1 and PEF Pre-Test, Post-Test, and the delta in all groups

Variables	Groups	Intervention	Control (+)	Control (-)	<i>p</i>
FCV					
Pre-test	$\bar{X} \pm SD$	2958 ± 332.9	3204 ± 461.6	3069 ± 388.1	0.654 [§]
Post-test	$\bar{X} \pm SD$	3036 ± 457.7	3357 ± 399.4	3256 ± 304.9	0.582 [‡]
	<i>p</i>	0.053 [¶]	0.017 ^{†*}	0.046 ^{¶*}	
	$\Delta \bar{X} \pm SD$	60.00 ± 381.7	153.0 ± 157.3	147.0 ± 255.9	0.696 [‡]
* Significant (<i>p</i> < 0,05); [‡] <i>Kruskal wallis</i> ; [¶] <i>Paired t</i> ; [†] <i>Wilcoxon</i>					
FEV ₁					
Pre-test	$\bar{X} \pm SD$	2777 ± 336.5	2913 ± 341.7	2895 ± 386.7	0.654 [§]
Post-test	$\bar{X} \pm SD$	2968 ± 329.6	3124 ± 353.7	3054 ± 299.5	0.582 [‡]
	<i>p</i>	0.005 ^{¶*}	0.005 ^{†*}	0.010 ^{¶*}	
	$\Delta \bar{X} \pm SD$	191.0 ± 165.7	211.0 ± 144.8	159.0 ± 154.7	0.696 [‡]
* Significant (<i>p</i> < 0,05); [§] <i>One Way Anova</i> ; [‡] <i>Kruskal wallis</i> ; [¶] <i>Paired t</i> ; [†] <i>Wilcoxon</i>					
PEF					
Pre-test	$\bar{X} \pm SD$	7805 ± 582.7	7709 ± 774.1	7857 ± 739.6	<i>p</i>
Post-test	$\bar{X} \pm SD$	8337 ± 361.5	8360 ± 561.8	8362 ± 660.9	0.893 [§]
	<i>p</i>	0.005 ^{¶*}	<0.001 ^{¶*}	0.005 ^{¶*}	0.869 [‡]
	$\Delta \bar{X} \pm SD$	532.0 ± 394.2	651.0 ± 310.6	505.0 ± 439.1	0.670 [§]
* Significant (<i>p</i> < 0,05); [§] <i>One Way Anova</i> ; [‡] <i>Kruskal wallis</i> ; [¶] <i>Paired t</i> ; [†] <i>Wilcoxon</i>					

Table 3. The spirometry clinical interpretation in all groups

		Clinical Interpretation		
Groups		Normal	Obstruction	Restriction
Intervention (n=10)	Pre-test	6	-	4
	Post-test	10	-	-
Control (+) (n=10)	Pre-test	4	1	5
	Post-test	7	1	2
Control (-) (n=10)	Pre-test	4	2	4
	Post-test	6	2	2

Table 4. IgA Sinonasal *Pre-Test*, *Post-Test*, and the delta in all groups

Variable	Groups	Intervention	Control (+)	Control (-)	p
IgA					
Pre-test	$\bar{X} \pm SD$	26.54 ± 1.29	26.65 ± 1.03	26.23 ± 1.03	0.503§
Post-test	$\bar{X} \pm SD$	10.51 ± 3.30	10.18 ± 2.84	10.95 ± 2.57	0.858§
	p	<0.001¶*	<0.001¶*	<0.001¶*	
	$\Delta \bar{X} \pm SD$	16.31 ± 3.38	16.24 ± 2.62	15.28 ± 2.50	0.484‡

* Significant (p < 0.05); § One Way Anova; ‡ Kruskal wallis; ¶ Paired t

Table 5. The IL-6 Level *Pre-Test*, *Post-Test*, and The Delta in All Groups

IL-6	Groups	Intervention	Control (+)	Control (-)	p
Pre-test	$\bar{X} \pm SD$	4.40 ± 8.98	6.07 ± 7.47	4.92 ± 3.39	0.129 ^θ
Post-test	$\bar{X} \pm SD$	-2.51 ± 1.94	-4.16 ± 1.00	-3.64 ± 1.36	0.044 ^{θ*}
	p	0.008 ^{ξ*}	0.008 ^{ξ*}	<0.001 ^{§*}	
	$\Delta \bar{X} \pm SD$	-6.92 ± 8.61	-10.23 ± 7.65	-8.56 ± 4.37	0.031 ^{**}

* Significant (p < 0,05), ^ξ Wilcoxon, [§] Paired T-Test, ^θ Kruskal-Wallis, Mann-Whitney Test[†]