

# Modulation of Lung Function by Increased Nitric Oxide Production

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## ABSTRACT

**Introduction:** Cigarette smoking reduces endogenous Nitric Oxide (NO) production by reducing Nitric Oxide Synthase (NOS) activity, which is one of the probable reason for increased rate of pulmonary diseases in smokers. Nitric oxide/oxygen blends are used in critical care to promote capillary and pulmonary dilation to treat several pulmonary vascular diseases. Among several supplements, the highest NOS activation has been proved for garlic with its unique mechanism of action.

**Aim:** To investigate the effect of dietary supplementation of NO producing garlic on pulmonary function of smokers.

**Materials and Methods:** The study was conducted on 40 healthy non-smoker (Group A) and 40 chronic smoker (Group B) males with matched age, height and weight. The pulmonary function tests- Forced Vital Capacity (FVC), Forced Expiratory Volume in one second (FEV<sub>1</sub>), FEV<sub>1</sub>/FVC ratio and Peak Expiratory Flow

Rate (PEFR) were performed in non-smokers (Group A), smokers (Group B) and smokers after supplementation of approximately 4 gm of raw garlic (2 garlic cloves) per day for three months (Group C). Endogenous NO production was studied in smokers before and after garlic supplementation and in non-smokers without supplementation. The data obtained were compared between the groups using unpaired student's t-test. The p-value considered significant at <0.05.

**Results:** Our results showed that FVC, FEV<sub>1</sub>, FEV<sub>1</sub>/FVC ratio and PEFR were reduced significantly along with a significant decreased NOS activity among smokers (Group B) when compared with non-smokers (Group A). Garlic supplementation significantly improved the pulmonary function tests in Group C in comparison to Group B by increasing NOS activity.

**Conclusion:** Dietary supplementation of garlic, which might be by increasing NOS activity, has significantly improved pulmonary functions in smokers.

**Keywords:** Garlic, Pulmonary function, Nitric oxide, Smoking

## INTRODUCTION

Tobacco smoking, one of the major risk factors for respiratory diseases including airway obstruction, is one of the leading causes of morbidity and mortality all over the world [1]. It affects pulmonary functions by destructing alveolar wall and narrowing of small airways leading to reduced elastic recoil, increased compliance, increased physiological dead space including residual volume with a greater decline in forced expiratory volume and vital capacity [2]. This obstruction in airways invariably affects the parameters of pulmonary function that is FVC, FEV<sub>1</sub>, PEFR and FEV<sub>1</sub>/FVC ratio [3].

Endogenous NO is involved in the mechanical defense of the airways since it affects the beating frequency of cilia and also plays a modulatory role in inflammatory response [4]. It has been shown that levels of exhaled nitric oxide are decreased in smokers [5]. Moreover, production of endogenous nitric oxide of airways is reduced in smokers [6]. Since, nitric oxide is involved in the immunological defense system, one would expect that smokers with lower production of nitric oxide in their airways would have more episodes of upper airway infection and associated airways obstruction than non-smokers [7,8]. In view of the many effects that nitric oxide acts as a modulator of the airway defense system, it would seem favourable to have intact endogenous nitric oxide production. Cigarette smoking reduces endogenous NO production by reducing NOS activity, which is one of the probable reason for increased rate of pulmonary diseases in smokers. Nitric oxide/oxygen blends are used in critical care to promote capillary and pulmonary dilation to treat several pulmonary vascular diseases [9].

For the years, scientists have been trying for NOS activation using some supplementation and medication to improve pulmonary health

in smokers [10]. Among several supplements, the highest NOS activation has been proved from garlic with its unique mechanism of action [11,12].

Studies have reported that garlic supplementation may be beneficial in patients of pulmonary and cardiac diseases along with Hepatopulmonary Syndrome (HPS) for the reversal of intrapulmonary shunts as well as reducing hypoxemia and mortality [13,14]. However, reports lack any comment on beneficial effect of raw garlic supplementation in improvement of lung functions in chronic smokers and other inflammatory airways obstruction. Keeping in view the above facts, this study was aimed to evaluate and compare the pulmonary function test parameters in chronic smokers, non-smokers and chronic smokers with garlic supplementation to see the effect of garlic supplementation in chronic smokers.

## MATERIALS AND METHODS

This cross-sectional comparative study was conducted on 40 male subjects in each group over years (March 2015- April 2016) that were randomly selected by convenient sampling technique. Forty healthy non-smoker males formed the control group (Group A). Age, height and weight matched [Table/Fig-1] male chronic smokers indulging in heavy tobacco smoking mostly in the form of cigarettes 80 to 100 per week for the last 8-10 years formed the experimental group (Group B). The subjects of both groups recruited were from the hospital workers and attendants of the patients having lower socioeconomic status. Both the hospital workers and attendants of the patients were lower grade office staffs by occupation with almost sedentary lifestyle having no physical exertion.

The subjects with acute illness or any respiratory illness at the time of the study were excluded. Subjects were also excluded if satisfactory

measurements could not be taken because of lack of cooperation or an inability to perform the tests. Prior to the study, all subjects were informed of the study procedure, purpose and known risks and thereby obtained their informed written consent. This study was approved by the Institutional Ethical Committee, Chitwan Medical College, Nepal.

All the subjects of Group A and Group B were subjected to pulmonary function tests- FVC, FEV<sub>1</sub>, FEV<sub>1</sub>/FVC ratio and PEFR in sitting upright posture. Computerized equipment "MEDSPIROR" (MED SYSTEMS Pvt. Ltd., Chandigarh, India) was used in this study. Standard methods and precautions outlined by Miller M et al., have been followed. Pulmonary function tests were carried out at a fix time of the day (10.00 – 12.00 noon) to minimize any diurnal variation [15]. All values were corrected for body temperature and pressure saturated with water vapour.

For this study, some food items (vegetables, fruits and spices) were screened for the production of nitric oxide in vitro which has been described below in detail in the headings with "Preparation of extract of various vegetables, fruits and spices" and "Assay of nitric oxide synthase activity of the extract". Among them, garlic has shown the highest NOS stimulating activity [Table/Fig-2]. In this experimental design, the subjects of Group B were then supplemented with approximately 4 gm (2 cloves) of raw garlic per day for three months, garlic dose recommended in the study by Tattelman E [16] and the University of Maryland Medical Center [17], referred as experimental group II (Group C). At the end of three months of garlic supplementation the subjects of Group C were again subjected to pulmonary function tests. Endogenous nitric oxide production was studied in smokers before (Group B) and after (Group C) garlic supplementation. Endogenous nitric oxide production was also studied in non-smokers (Group A) at the start of the study.

### Collection of Blood and Preparation of Red Blood Cell Membrane

Blood was collected in plastic tubes containing 0.13 M sodium citrate. Intact red blood cells were isolated and suspended in 0.9% NaCl; RBC membranes were prepared by ultra-centrifugation of red blood cell lysate for the measurement of nitric oxide level by activation of nitric oxide synthase enzyme present in RBC cell membrane [18]. For estimation of endogenous production of nitric oxide, the blood was collected before (Group A and Group B) and after (Group C) garlic supplementation and the RBC was processed as described above for in vitro production of nitric oxide.

### Preparation of Extract of Various Vegetables, Fruits and Spices

Various vegetables, fruits and spices were homogenized in glass Potter Elvehjem homogenizer by suspending in Kreb's buffer, pH 7.4 (5 mg/ml) at 0°C. Some vegetables, fruits and spices were homogenized after boiling in test tube with a tight cork. The homogenized mass was then centrifuged at 60,000 g at 0°C for 60 minutes in separate tubes. Supernatant hereafter called as "extract" was collected and protein concentration was determined according to Lowry OH et al., [19].

### Assay of Nitric Oxide Synthase Activity of the Extract

Different extracts were separately incubated at 37°C with constant stirring for 30-45 minutes with Kreb's buffer. The incubation mixture was centrifuged at 0°C at 8,000 gm for 20 minutes and supernatant was collected for determination of nitric oxide synthase, which was expressed as nmol NO/ mg protein/hr [20].

### Determination of Nitric Oxide

Nitric oxide was determined according to Jia L et al., by using Scanning Beckman Spectrophotometer (Model DU6, USA) [21]. Nitric oxide content of the supernatant was quantitated by determining the conversion of oxyhaemoglobin to methaemoglobin.

## STATISTICAL ANALYSIS

All values were expressed as mean±SE, Unpaired student's t-test was used for groupwise comparisons and p-value of <0.05 was considered statistically significant.

## RESULTS

### Nitric Oxide Production of Vegetables, Fruits and Spices

Some vegetable, fruits and spices were screened for the production of nitric oxide in vitro. The nitric oxide production ranged from 0.00 to 20.08 (nmol NO/mg protein/hr). Those items produced more nitric oxide is presented in [Table/Fig-2]. Among them, garlic has shown the highest (20.08 nmol NO/mg protein/hr) nitric oxide production capability.

### Nitric Oxide Synthase Activity (NOS Activity)

Nitric oxide synthase activity showed a significant (p<0.01) decrease in smokers (Group B) compared to non-smokers (Group A) [Table/Fig-3]. Garlic supplementation had significantly (p<0.05) increased the nitric oxide synthase activity of the smokers (Group C).

### Forced Vital Capacity (FVC)

[Table/Fig-4,5] show that forced vital capacity was markedly reduced (22%) in smokers (Group B) as compared to non-smokers (Group A) which was statistically significant (p<0.01). Supplementation of garlic significantly (p<0.05) improved the forced vital capacity of the smokers (Group C).

### Forced Expiratory Volume in One Second (FEV<sub>1</sub>)

As for FVC, the FEV<sub>1</sub> was also significantly (p<0.01) reduced by 16% in smokers as compared to non-smokers [Table/Fig-4,5]. Dietary supplementation of garlic has significantly (p<0.01) improved (13%) the FEV<sub>1</sub> in smokers (Group C) as compared to Group B.

### Peak Expiratory Flow Rate (PEFR)

PEFR of Group B was significantly (p<0.01) reduced by 17% as compared to Group A [Table/Fig-4,5]. After supplementation

Parameter	Group A (n=40)	Group B (n=40)	Group C (n=40)
<b>Gender</b>	<b>Male</b>	<b>Male</b>	<b>Male</b>
Age in years (ranges)	30-40	30-40	30-40
Height in cm (ranges)	165-175	165-175	165-175
Body weight in kg (ranges)	60-70	60-70	60-70

[Table/Fig-1]: Subject characteristics.

Name of the plant	NOS activity (nmol NO /mg protein/hr)
1. Pea (boiled) ( <i>Pisum sativum</i> )	3.6 ± 0.014
2. Gourd (boiled) ( <i>Cucurbita pepo</i> )	6.8 ± 0.034
3. Spinach (boiled) ( <i>Spinacia oleracea</i> )	1.42 ± 0.034
4. Tomato ( <i>Lycopersicon esculentum</i> )	8.40 ± 0.055
5. Lemon ( <i>Citrus limon</i> )	12.08 ± 0.060
6. Orange ( <i>Citrus sinensis</i> )	5.16 ± 0.053
7. Banana ( <i>Musa paradisiacasapientum</i> )	16.5 ± 0.046
8. Apple ( <i>Malus sp.</i> )	3.05 ± 0.044
9. Onion ( <i>Allium cepa</i> )	6.23 ± 0.030
10. Garlic ( <i>Allium sativum</i> )	20.08 ± 0.055

[Table/Fig-2]: NOS activity of various food items (vegetables, fruits, spices). Values are expressed as mean±SE. Number of observations = 6

Non-Smokers	Smokers	Significance level	% Decrease		% Increase	
Group A	Group B	Group C	A vs B	B vs C	A vs B	B vs C
1.765±0.30	0.725±0.32	1.575±0.20	p<0.05	p<0.05	58%	117%

[Table/Fig-3]: Effects of dietary supplementation of garlic on NOS activity of smokers in vivo. Values are expressed as mean±SE. Number of observations = 40. NOS activity is expressed as nmol NO/mg protein/hr.

Parameter	Group A	Group B	Group C
FVC (ml)	3600 ± 169.0	2900 ± 187.6	3400 ± 158.8
FEV <sub>1</sub> (ml)	2880 ± 101.07	2470 ± 110.1	2800 ± 45.2
PEFR (L/min)	350 ± 13.87	290 ± 12.75	320 ± 7.61
FEV <sub>1</sub> /FVC (%)	80 ± 3.3	67 ± 3.6	78 ± 2.1

**[Table/Fig-4]:** Effects of garlic supplementation on pulmonary function test of smokers.

Values are expressed as mean±SE. Number of observations = 40.

Parameter	% Decrease A vs B	% Increase B vs C	Significance level	
			A vs B	B vs C
FVC	22.0	17.0	p<0.01	p<0.05
FEV <sub>1</sub>	16.0	13.0	p<0.01	p<0.01
PEFR	17.0	10.3	p<0.01	p<0.05
FEV <sub>1</sub> /FVC ratio	16.3	14.0	p<0.05	p<0.05

**[Table/Fig-5]:** Percent change in pulmonary function test in various groups and level of significance.

of garlic, PEFR was improved significantly ( $p<0.05$ ) in Group C compared to Group B.

### FEV<sub>1</sub>/FVC ratio

[Table/Fig-4,5] shows that FEV<sub>1</sub>/VC ratio was marked reduced (16.3%) in smokers (Group B) as compared to non-smokers (Group A) which was statistically significant ( $p<0.05$ ). Supplementation of garlic significantly ( $p<0.05$ ) improved the FEV<sub>1</sub>/FVC ratio of smokers (Group C).

## DISCUSSION

Our findings showed that FVC, FEV<sub>1</sub>, FEV<sub>1</sub>/FVC ratio and PEFR were decreased significantly in smokers compared to non-smokers [Table/Fig-4,5]. Though, these reductions are quite significant statistically in all these parameters in smokers, they were clinically asymptomatic during the study, which might be due to their sedentary lifestyle and concurrent effect of chronic smoking. These findings are well supported by findings by Bohadana A et al., and Baraldo S et al., with explanation to the possible mechanism of these reductions of pulmonary function test in tobacco smoking due to narrowing of small airways [1,2]. This causes reduced elastic recoil, increased compliance and increased physiological dead space [1]. These smokers with reduced PFT parameters did not visit to any clinicians as they did not have clinically apparent difficulties in respiration with daily normal life activities. This interpretation finds support from earlier reports that this might be due to undiagnosed respiratory problems, as in UK, it is estimated that more than 3 million people currently have COPD, and an estimated 2 million people have COPD which remains undiagnosed because they do not visit physician due to absence of apparent respiratory problems in them and/or most patients mainly with mild-to-moderate disease, are thought to remain undiagnosed or not diagnosed until they are in their fifties [22,23].

Apart from the destruction of alveoli and smaller airways, cigarette smoking induces changes in the larger airways, with impairment of ciliary function and hypertrophy of mucosal glands in the bronchi [2]. These findings are consistent with the earlier report, which shows that smoking reduces endogenous production of nitric oxide [6]. This is due to the reduction in the activity of the enzyme nitric oxide synthase, which is caused by an inadequate supply of the coenzyme tetrahydropterine [24]. Hence, the enzyme is present, but is unable to produce nitric oxide. In view of the above-mentioned facts, it is reasonable to assume that increasing the production of endogenous nitric oxide should improve the pulmonary functions in smokers. Therefore, it was aimed to increase the production of endogenous nitric oxide by stimulating the activity of NOS enzyme.

In the present study, some common vegetables, fruits and spices listed in [Table/Fig-2] are shown that produce nitric oxide in vitro. Now, it raises a question, whether these diet also increase the

production of nitric oxide endogenously. Indeed, in the present study, garlic supplementation in smokers has significantly increased the endogenous production of nitric oxide [Table/Fig-3] and also significantly improved the impaired pulmonary function tests of smokers [Table/Fig-4,5]. These findings are supported by the earlier report that nitric oxide synthase activation is a unique mechanism of garlic action [25,26].

The most likely explanation is that supplementation of garlic, which increases endogenous production of nitric oxide, might be doing so by stimulating nitric oxide synthase, a membrane bound enzyme. Though, reports lack studies regarding the effects of garlic on smokers' pulmonary function test parameters, it is well reported with the therapeutic health benefits of garlic explaining the possible reason for enhanced pulmonary functions mentioning that increased endogenous production of nitric oxide causing pulmonary vasodilatation and also has a modulatory role of the airway defense system affecting the beating frequency of cilia [4,27]. According to a study carried out at the Jiangsu Provincial Center for Disease Control and Prevention in China that people who ate raw garlic at least twice a week had a 44% lower risk of developing lung cancer [28]. Throughout history in the Middle East, East Asia and Nepal, the beneficial effects of the garlic supplementation have been reported in several studies to treat several lung diseases including common cold, bronchitis, tuberculosis, inflammatory disorders of the lungs and several other diseases with explaining the possible mechanism by decreasing the leukocyte inflammatory mediators [29-31].

Thus, garlic may offer relief from airway restriction and impaired lung function by improving the clearance of mucus from the airways, help airways relax, and improve airflow into the lungs. These could be the possible explanation for uplifting the reduced pulmonary function test parameters in chronic smokers after garlic supplementation in our study.

Interestingly, exclusively garlic supplementation in our study has improved pulmonary functions significantly in chronic smokers, which seems promising in its medical effects on respiratory system, yet to be confirmed by further detailed studies. This study would have been strongly conclusive if larger numbers of samples had been included. Moreover, it would be more clarifying if we could study the animal model with histological changes in respiratory system brought about by garlic supplementation.

## CONCLUSION

In conclusion, we can say that dietary supplementation of garlic significantly improved pulmonary functions in smokers. Therefore, it can be suggested that garlic should be incorporated in common food items daily and may reduce the risk factors for causing impaired pulmonary function in smoker. However, keeping in mind the deleterious effects of smoking of tobacco in the form of cigarettes, an anti smoking awareness should be launched in the society.

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