Negilla Sativa Lipids a Potential COPD Treatment Through Elastase Activity Inhibition

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Submitted on 12 May 2023, Accepted on 24 July 2023, published on 30 October 2023.

Abstract

This study aims to research an inhibitor of elastase produced during the activation of neutrophils and macrophages or under the effect of reduced levels of α1 antitrypsin from *Nigella sativa L.* (NS) seeds, in the case of Chronic Obstructive Pulmonary Disease (COPD). In this context, the effects of polar and non-polar oils from NS seeds on elastase activity *in vitro* were evaluated. The oil extracted from NS seeds yielded 29.21 ± 2.012%. The oil was fractionated using column chromatography (CC), resulting in five nonpolar fractions (92.12%) and three polar fractions (7.83%). Gas chromatography analysis showed that these oils are rich in unsaturated fatty acids. The inhibition of pure elastase activity was tested using the total oil and different fractions. The total oil was found to have the most potent inhibitory effect with an IC_{50} of 129.29 ± 1.4 µg/ml, while the different fractions exerted a synergistic effect. The average concentrations of elastase in bronchoalveolar lavage fluid from patients with COPD were found to be 39.19 ± 19.54 nM, with a detected enzymatic activity of 0.0149 ± 0.0037 IU/ml. The total NS oil powerfully inhibits this activity with an IC_{50} of 104.69 ± 3.58 µg/ml. The inhibitory effect of the total oil on elastase in bronchoalveolar lavage fluid (leukocyte elastase) is relatively superior to that exerted on pure elastase (porcine pancreatic elastase). The study results indicate that the polar and nonpolar oils extracted from *Nigella sativa* seeds possess significant inhibitory activity against elastase. This activity is primarily located in the unsaturated fatty acids of the oils.

Keywords: Bronchial lavage, COPD, Elastase, Emphysema, Inflammation, *Nigella sativa.*

Tob Regul Sci.™ 2023;9(2): 2959 - 2976

DOI: doi.org/10.18001/TRS.9.2.192

Introduction

Chronic obstructive pulmonary disease (COPD) is the leading cause of morbidity and mortality and is expected to be the third leading cause of mortality worldwide in the near future. It is the fourth leading cause of death in the United States and Europe causing 3.23 million deaths in 2019 (WHO, 2023; Barnes *et al.*, 2003). Smoking is the main cause of this disease. Studies have shown that increased airway neutrophil levels in patients with chronic bronchitis (Riise*et al.,* 1997) and a strong correlation between neutrophil recruitment and the severity of airway obstruction have been reported (Di Stefano *et al.*, 1998). These neutrophils produce serine proteases such as elastase, involved in the production of several COPD devices including pulmonary emphysema (Surkova*et al.*, 2005), intensive destructive processes in the lung parenchyma (Komlev*et al.*, 2005), reperfusion damage (Mori *et al.*, 2005), the function of causing mucus hypersecretion and inactivation and alteration of lung defense systems (Stockley, 1999). Emphysema is a slowly progressive disease characterized by permanent distension of the distal airspaces downstream of the terminal bronchioles and destruction of the alveolar walls without fibrosis (Komlev*et al.*, 2005). COPD has been relatively neglected and there is no common therapy that prevents the inevitable progression of the disease. There is now a growing interest in research at the cellular and molecular level aimed at new therapies (Barnes, 2000; Barnes, 2001).

For several years researchers in the pharmaceutical industry have been interested in the development of protease inhibitors as a treatment to prevent the onset or slow down COPD and emphysema. The benefit of antiprotease therapy is to slow the rate of lung function decline by stopping the apparent damage. Reports from the World Health Organization estimate that more than 80% of the world's population uses traditional extracts of medicinal plants as therapeutic substances against many diseases. Then it is necessary to develop the natural resources of medicinal plants. The advantage of these products is the absence of the chemicals causing the side effects. *Nigella sativa* plant is among the most used medicinal plants.

Extracts of this plant have been used in traditional medicine against certain symptoms and respiratory diseases such as cough, asthma and lung inflammation. Given the lack of in the ethnopharmacology of natural products against COPD, we tried to evaluate the effects of polar and non-polar oils of *Nigella sativa* L seeds on elastase activity: in application to chronic obstructive pulmonary disease (COPD) and pulmonary emphysema.

Materials and Methods

Materials

Nigella sativa seeds

Nigella sativa seeds are local, grown and harvested in the Maguera region (State of M'SILA). The seeds are cleaned from plant debris and kept away from light until use.

Bronchoalveolar lavage

Samples of Bronchoalveolar lavage of emphysematous patients are obtained from different university hospitals in Algeria, carefully divided into aliquots and then stored at -70°C until use.

Chemicals

The solvents (methanol, chloroform, hexane, acetone and acetic acid) used for the preparation of polar and non-polar oils are analytical grade, from Sigma and Fluka. The salts used for the preparation of the buffers as well as pancreatic porcine elastase, Suc-Ala-Ala-Ala-pNA substrate and α-1 antitrypsin are subsequent to Sigma.

Methods

Extraction of *Nigella sativa* total oil

The extraction of the total oil was carried out according to the protocol of Ramadan and Mörsel (2003) with slight modifications; the use of pure methanol instead of hexane or chloroform/methanol during hot extraction.

Obtaining the methanolic extract by soxhlet

The previously cleaned *Nigella sativa* seeds are ground into a powder. The powder obtained is subjected to extraction by soxhlet (hot extraction), using methanol as solvent. To carry out the extraction, 40 g cartridges of NS seed powder were used, 200 ml of methanol is added (1/5: w/v), then the powder is subjected to hot extraction for 8 hours.

The methanol is removed by evaporation under reduced pressure at 40°C using a rotary steamer (BÜCHI). This operation a thus makes it possible to obtain an extract characterized by a dark brown color, which is considered to be the methanolic extract.

Total Oil Extraction

The methanolic extract was mixed in a counting funnel with hexane (50 ml). After stirring, two phases were obtained; (i) a denser aqueous phase that appears below, (ii) an organic phase,

containing lipids.

The upper organic phase was recovered. This step was repeated several times with renewal of the solvent until it became transparent. The hexane was subsequently evaporated at 30°C, the resulting extract is considered to be the total oil of *Nigella sativa* seeds characterized by a greenish color.

Fractionation of total oils

The fractionation of the total oil of *Nigella sativa* seeds was carried out by column chromatography (CC). The column used is 30 cm high and 30 mm in diameter. The stationary phase is silica gel (60G, 70-230 Mesh, Merck). The elution of the different fractions was done in an increasing order of polarity thanks to the mobile phase consisting of: 100% chloroform followed by 50% chloroform – 50% Acetone. While the polar fractions are eluted with methanol, the last fraction is eluted by adding 2ml of acetic acid.

The total oil and all other fractions are vaporized in a temperature chamber at 40°C and then stored at -20°C until use.

Yield Calculation

The yield of the extraction of the total oil is expressed as a percentage relative to the weight of the seeds and calculated according to the following formula:

a) Yield for total oil:

Yield = (oil weight/seed weight) × 100

b) Fraction Yield:

Yield = (weight of fraction / weight of total oil) × 100

Fatty acid composition of *Nigella sativa* oils

Gas chromatography analyses coupled with a mass spectrometer (GC/MS) were carried out at the Bab EzzouarPhysico-chemical Research and Analysis Center (CRAPC) (Algiers). The results were expressed as a percentage of different compounds and fatty acids from the total area of the chromatogram peaks by the device software.

Preparation of the injection solution (methyl esters)

The method applied is that of Morrison and Smith., (1964). 1 ml of BF3 (boron trifluoride in 14% methanol) is added to the dry lipid extracts. The mixture is transmethylated at 100°C to remove non-polar lipids. The methyl esters are then extracted twice with hexane in the presence of

water and then evaporated to dryness. The fatty acid methyl esters are analyzed by gas chromatography coupled with mass spectrometry. The compounds in the extracts are analyzed using a gas chromatograph coupled to an electron impact mass spectrometer (HP/MSD Agilent 5890) operating in the scan mode (m/z =25-2000). Samples are injected (injection temperature 250°C) into an HP-5MS capillary column (length: 30 m, internal diameter: 0.25 mm, film thickness: 0.25µm) eluted with helium at 0.3 ml/min with a split ratio of 1/10. The temperature of the column is programmed at 50°C for 2 min, then the temperature is raised to 240°C with a gradient of 4°C/min. The sequence ends after 40 min at 240°C. The analysis is carried out under an ionization energy of 70 eV (filament temperature: 150°C, current: 600 mA, PM potential: 600 V). The identification is made by comparing the mass spectra with the database provided by the Chemstation software (NIST 2002 and Wiley version 7.0).

Inhibition of elastase activity by *Nigella sativa* oils

The inhibitory effect of *Nigella sativa* oils on elastase activity was studied spectrophotometrically by monitoring the amount of p-nitroanilide (pNA) produced by the hydrolysis of Suc-Ala $_3$-pNA dissolved in buffer (Tris-HCl, 0.1M, pH 8, containing 0.1 M NaCl), in the presence of increasing concentrations of each fraction of *NS* oils (prepared in Tris-HCl buffer, 0.1M, pH 8, containing 0.1 M NaCl, 0.05% Triton-X100, 1% DMSO). Increasing concentrations of polar and non-polar oils and total oil were incubated with 50 µl elastase (200 nM stock solution) in a reaction volume of 200 µl, for 20 min at 25°C. The reaction is initiated by the addition of 0.9 mM substrate. The absorbance was recorded at 405 nm after a second incubation under the above conditions (Moroy*et al.*, 2011), α-1 antitrypsin is used as a positive control.

The inhibitory activity of NS oils was expressed as a percentage inhibition (I %) calculated asfollows: I % = [1- (B / A)] × 100

A: absorbance in the absence of the inhibitor (negative control).

B: absorbance in the presence of the inhibitor.

The 50% inhibitory concentration of the enzyme activity (IC_{50}) of each fraction was calculated from the equation that determines the percentage inhibition as a function of the concentration of the inhibitor (Y = a X + b). It was expressed in µg / ml and compared with that of α-1antitrypsin; IC_{50} = (50 – b) / a.

Test of elastase activity in bronchoalveolar lavage

After the collection of the AML samples of the 24 COPD patients from different university hospitals in the country. The test for protease activity of elastase in bronchoalveolar lavage (Bal) (n = 24) was carried out according to the protocol of Duvoix et al. (2011). Using Succ-Ala $_3$-pNA as

substrate. The LBAs, diluted ten-fold in 0.1M Tris-HCl buffer, pH 7.5, containing 0.5 M NaCl, 0.05% Triton- X100, incubated in the presence of 1 mM of the substrate in a final volume of 200 µl of 96 wells of the microplate. The absorbance was recorded at 405 nm during times t_1= 1h and t_2= 1.30 h. A range of increasing concentrations of pure elastase (10-50 nM) was incubated with the substrate to obtain the elastase calibration line.

Determination of elastase activity in Bal

The number of elastase units per milliliter of Bal was calculated by the following using the following relationship:

IU/ ml enzyme = (d Abs x V_F x FD) /$\varepsilon_{405(pNA)}$x V_{Bal} x dt

d Abs: difference in absorbance between t_2 and t_1.

V_F: the final volume of the reaction.

DF: Bal Dilution Factor.

ε_{405}(pNA) : Molar extinction coefficient of pNA at 405 nm.

V_{Bal}: volume of Bal in each trial.

dt: t2 - t1

Determination of enzyme concentration in Bal

Elastase concentration in Bal was determined from the elastase calibration curve equation.

(Y = a X + b). It was expressed in nM, calculated as follows: X = (Y - b) / a.

The Bals of patients with COPD were distributed as a percentage according to elastase concentration in nM and elastase activity in IU per milliliter of the Bal.

Inhibition of elastase activity in Bal by *Nigella sativa* total oil

The inhibitory effect of NS oils on elastase activity was studied spectrophotometrically by monitoring the amount of p-nitroanilide (pNA) produced by the hydrolysis ofSuc-Ala $_3$-PNA dissolved in 0.1M Tris-HCl buffer, pH 7.5, containing 0.5 M NaCl, 0.05% Triton X-100), in the presence of increasing concentrations, of each fraction of the NS oil (prepared in 0.1M Tris-HClbuffer, pH 7.5 , containing 0.5 M NaCl, 0.05% Triton-X100, 1% DMSO. increasing concentrations of polar and non-polar oils and total oil were incubated in the presence of 25 µl of Bal in TrisHCl buffer (15 to 2000 µg/ml) for 2 h at 37°C. After incubation, 1 mM of substrate was added and the absorbance is recorded at 405 nm after a second incubation under the same

conditions as the first incubation. The inhibitory activity of NS oils was expressed as a percentage of inhibition (I %) (I % = [1- (B / A)] × 100).

Statistic alanalysis

Results were expressed as mean ± SD (standard deviation), and mean ± SEM (standard error of mean) (n=3). The results were analyzed by the univariate ANOVA test (one-way ANOVA) followed by the Tukey test for the comparison of the results in the presence of the extracts with the negative controls (in the absence of the extracts), and the comparison of the different extracts with each other. The difference was considered statistically significant when the p-value is < 0.05.

Results and discussion

Extraction of *Nigella sativa* total oil

The extraction of the total oil from the seeds of *Nigella sativa* was carried out in three main steps, the first is a hot extraction with pure methanol to obtain the methanolic extract. The second step is cold extraction by using hexane to obtain the total oil. The third step is fractionation by column chromatography (CC) was carried out by a series of solvents whose polarity is increasing (chloroform, a chloroform/ acetone mixture (1/1: V/V), methanol then methanol with a few drops of acetic acid) thus making it possible to separate the compounds from the total oil according to the degree of solubility in the extraction solvents and therefore according to their degree of polarity. Nine fractions were obtained, total oil, five non-polar fractions (three eluted with chloroform and two eluted with chloroform/acetone (50%: V/V) and three polar fractions (two eluted with methanol and the third fraction eluted by the addition of a few drops of acetic acid to methanol). The colour, appearance and yield of each fraction relative to the total oil are shown in Table I.

The obtained results show that among the different fractions obtained from the total oil of the *NS* seeds, which represents 29.21 ± 2.012% of the total weight of the seeds, the non-polar fraction F1 represents the highest yield (49.17%), followed by the non-polar fraction F2 (39.76%), the polar fractions F7 (4.69%), F8 (1.82%), the non-polar fraction F4 (1.43%), the polar fraction F6 (1.32%), the non-polar fractions F3 (1.14%) and F5 (0.62%). As a result, the combined non-polar fractions represent the highest yield (92.12 %), while the polar fractions represent 7.83% of the total weight of the total oil of *NS*.

The total oil yield of *NS* seeds in this study is less than 39.2 % and 37.9% reported by Ramadan and Mörsel (2003). These authors used the same extraction technique but the hot extraction solvents were chloroform/ methanol (2/1: V/V) and hexane, respectively. Knowing that, in the present work, the solvent used is pure methanol, which is more polar than the solvents used by Ramadan and Mörsel, which can explain the performance of this study.

Table I: **Appearance, colour and yield of the various polar and non-polar fractions extracted from the NS seeds as a percentage of the total weight of the total oil**

fractions		*Colour*	*Appearance*	*Performance*
Non-polar fractions	F 1	Dark Green	Oily	49.17%
	$ → F2	Light Green	Oily	39.76%
	F3	Yellow	Oily	1.14%
	F4	Green	Paste	1.43%
	F5	Dark Brown	Paste	62
Polar Fractions	F6	brown	Paste	1.32%
	F7	Yellowish Brown	Paste	4.69%
	F8	Dark Brown	Paste	1.82%

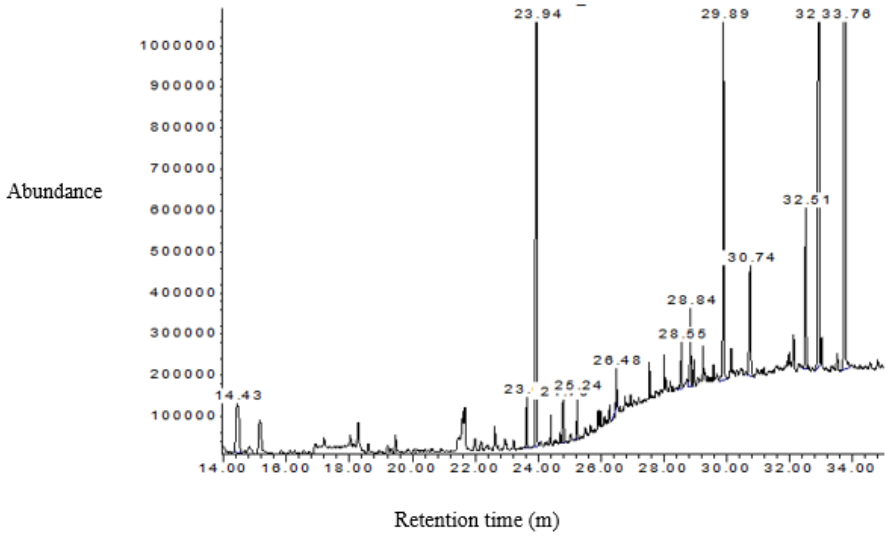
Comparing our results with those reported by Ramadan and Mörsel. It is pointed out that our yield of non-polar fractions (92.12%) is slightly lower than the yields of 97.2% and 96.1% reported. This variation is probably due to the solvent used. On the other hand, the polar fraction yield (7.83%) in this study is higher than the reported yields (3.64% and 2.5%). This is due to the use of methanol, during hot extraction which is more polar than the chloroform/methanol and

hexane used by Ramadan and Mörsel (2003). However, it is difficult to compare these results with those of the bibliography, because the yield is only relative and seems to be related to the genetic properties of the see the geographical origin, storage conditions and duration of the harvest and also the extraction methods applied.

Fatty acid composition of *Nigella sativa* oils by GC/MS

The fatty acid composition of *Nigella sativa* oils is obtained after methylation, analyzed by gas chromatography coupled with a mass spectrometer (GC/MS). The total oil fatty acid chromatographic profiles are shown in Figure 01 and the Compounds identified in the total oil of NS seeds by GC/MS are shown in Table II.

Figure 01: Chromatogram of compounds and fatty acids of *Nigella sativa* oil determined by gas



chromatography coupled with a mass spectrometer.

Table II:Compounds identified in the total oil of NS seeds by GC/MS.

Time of Retention	*Components*	*Grade (%)*
terpenoideshydrocarbons		
14.44	o-cymene	3.50
15, 15	Gamma-terpinene	1.04
15.82	p-cymene	0.19

16.54	m-cymene	0.19
19.32	Alpha-Longipinene	0.16
21.67	(+)-Longifolene	1.59
22.95	Squalene	0.53
Non-terpenoidehydrocarbons		
16.25	Cumene	0.12
16.92	1-ethyl-3,5-dimethyl-Benzene	51
18.59	Prehnitol	0.38
22.62	(E) 5-Octadecene	0.75
23.64	Heptadecane	1.04
23.94	Not identified	26.21
30.74	Sandaracopimaradiene	3.28
FattyAcids		
29.89	Palmiticacid	8.81
32.51	Stearicacid	4.09
32.90	Oleicacid	22.58
33.72	Linoleicacid	25.03

These results were mentioned in our previous study (Mosbah*et al.*, 2023). They show that the total oil of *Nigella sativa* seeds is very high in unsaturated fatty acids (47.61% of the total oil). Comparing our results with those reported by Ramadan and Mörsel (2003). It is pointed out that our oleic acid content (22.58%) is slightly less than 24.1 and 23.9%, and linoleic acid (25.03%) is less than 57.3 l % and 57.0 l % reported. While the content of stearic acid (4.09%) is greater than 3.16% and 3.22%, and palmitic acid (8.81%) is less than 13.0 l % and 13.1% reported, therefore saturated fatty acids represent 12.9% of the total oil. This variation is likely due to the solvent used and appears to be related to the genetic properties of the seeds and geographical origin. The other compounds identified are part of the essential oil (Longifolene, O-cymene, Gamma-terpinene and p-cymene).

Inhibition of elastase activity by *Nigella sativa* oils

The inhibitory effect of polar, non-polar, total seed oil of NS and α1- antitrypsin on elastase protease activity was evaluated spectrophotometrically by monitoring the amount of para nitroanilide (pNA) produced by the hydrolysis of Suc-Ala 3-pNA at 405 nm. The results show that all fractions exert a significant inhibitory effect on elastase protease activity in a dose-dependent manner. The IC50 values of polar and non-polar fractions, total oil and elastase specific inhibitor (AAT) show that total oil has the strongest inhibitory effect ($p < 0.001$), with an IC50 of 129.29 ± 1.40 µg /ml, followed by non-polar fraction F5 (IC50 = 347.15 ± 6.74), polar fraction F7 (IC50 = 355.32 ± 4.54), non-polar fraction F2 (IC50 = 363.02 ± 0.35), non-polar fraction F1 (IC50 = 375.85 ± 2.96), non-polar fraction F3 (IC50 = 512.46 ± 5.1), non-polar fraction F4 (IC 50 = 542.9 ± 3.64), polar fraction F6 (IC50 = 784.28 ± 2.2) and polar fraction F8 (IC50 = 1438 ± 4.40) µg / ml. All fractions have IC50s 1 to 20 times higher than the IC50 of α1- antitrypsin (IC50 = 70.88 ± 0.56) µg / ml (Table III). The various polar and non-polar fractions inhibit the activity of the elastase enzyme in a significant ($p < 0.001$) and dose-dependent manner (Figure 04 and 05)

***Table III*:** IC_{50} of polar and non-polar oils of *NS* seeds on elastase activity.

Samples	**AAT**	**HT**	**F1**
IC50 (µg / ml)	70.88± 0.56	129.29± 1.40	375.85 ± 2.96
Samples	F2	F3	F4
IC50 (µg / ml)	363.02± 0.35	512.46± 5.19	542.9± 3.64
Samples	F5	F6	F7
IC50 (µg / ml)	347.15 ± 6.7	784.28 ± 2.2	355.32± 4.5
Samples	F8		
IC50 (µg / ml)	1435 ± 4.4		

Each value represents the mean ± SD (n = 3).

Table III: IC_{50} of polar and non-polar oils of *NS* seeds on elastase activity.

The results show that total oil inhibits elastase almost 2 times less than AAT. The non-polar fractions F1, F2, F5 and the fraction F7 are 5 times lower. On the other hand, fractions F3 and F4 are almost 8 times lower, while fractions F6 and F8 are almost 11 and 21 times lower, respectively. These results explain the synergistic effect of the deferent fractions of the oil with respect to the inhibition of the protease activity of elastase.

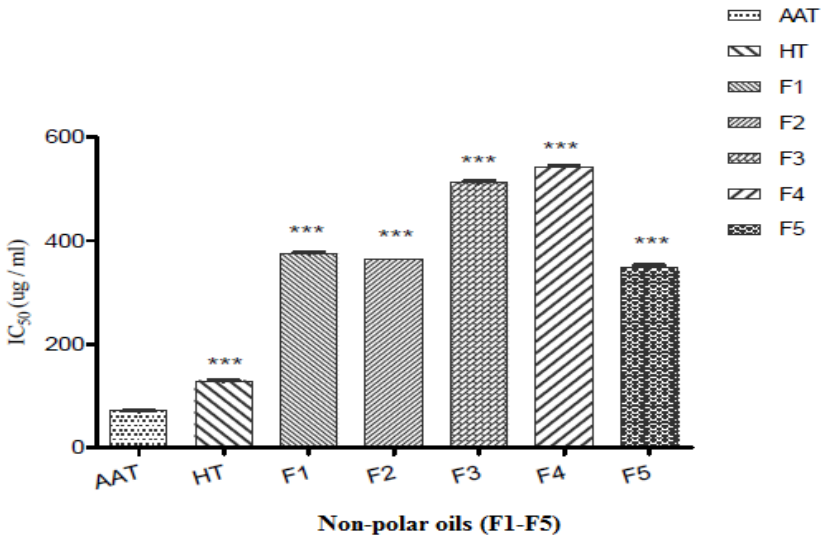
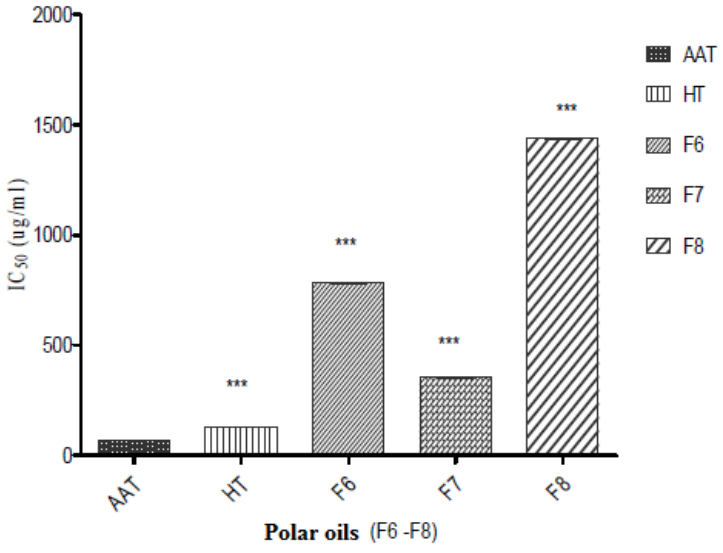


Figure 04: The IC50 of the non-polar fractions of NS oil on elastase activity.

Each value represents the mean ± SD (n = 3). *** $p < 0.001$.

Figure 05: The IC50 of the polar fractions of NS oil on elastase activity.Each value represents the mean ± SD of three representations. *** $p < 0.001$.



Inhibition of elastase activity by *Nigella sativa* oils is reflected by the presence of one or more

compounds acting on the active sites of the enzyme. Recently, Moroy*et al.* 2011 evaluated the inhibitory effect of oleic acid (the predominant compound in total black seed oil) on elastase activity which has an IC_{50} of 3 - 16 µM (Hornebeck*et al.*, 1985; Tyagi and Simon., 1990). They proposed the following inhibition mechanism:

1. The carboxylic group of oleic acid forms a salt bridge with Arg^{217} from the active site of elastase and this binding will prevent the interaction of the enzyme with its substrate.

2. The double bond of oleic acid interacts with amino acids Phe^{192} and Val^{216} of the S_3 site.

3. The end of the aliphatic chain occupies the S_1 site of the enzyme.

The total oil of *Nigella sativa* as well as the different polar and non-polar fractions exert an inhibitory effect on elastase activity thanks to their richness in unsaturated fatty acid, particularly oleic acid, which represents 20-25% of the total oil. The richness of *Nigella sativa* oil in unsaturated fatty acid (73 – 77%) (Atta., 2003; Nickavar*et al.*, 2003; Cheikh-Rouhou*et al.*, 2007) could be at the origin of the most powerful inhibitory effect on elastase activity (Moroy*et al.*, 2011).

Test of elastase activity in bronchoalveolar lavage

The obtained results of the activity of elastase in bronchoalveolarlavage (Bal) show that all samples from patients with COPD tested have elastase activity. The activity is quantified in the form of the number of enzyme units per ml of Bal and quantified by determining the concentration of elastase in nM.

Determination of elastase activity in Bal

The results of the calculation of the number of elastase units per milliliter of Bal a allow the distribution of Bal of patients with COPD into two categories, the first category with a number of elastase units is less than 0.01 unit/ ml of enzyme and whose percentage is 41.67% and the second category which represents 58.33% has more than 0.01 unit / ml (Figure 06). This difference in the number of enzyme units between the different patients can be at the stage of the disease (mild, moderate or severe). The average number of units of enzyme per millilitre of Bal is 0.01486 ± 0.0037 units/ml.

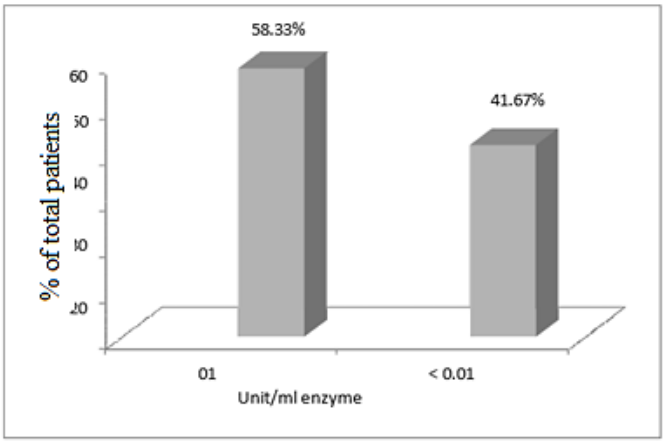


Figure 06: Distribution of bronchoalveolarlavage ofpatients with COPD according to elastase activity per ml. Each value represents the average of three determinations.

Determination of enzyme concentration in Bal

The concentration of elastase in the Bal of the different patients was determined from the elastase calibration curve using the equation Y = a X + b and expressed in nano molar (nM). The obtained results allow the total of patients to be divided into three categories; 37.5% of patients have concentrations below 20 nM, 16.67% have 20-50 nM and 45.83% have more than 50 nM (Figure 07). Mean elastase concentrationsin Bal are 39.19 ± 19.54 nM.

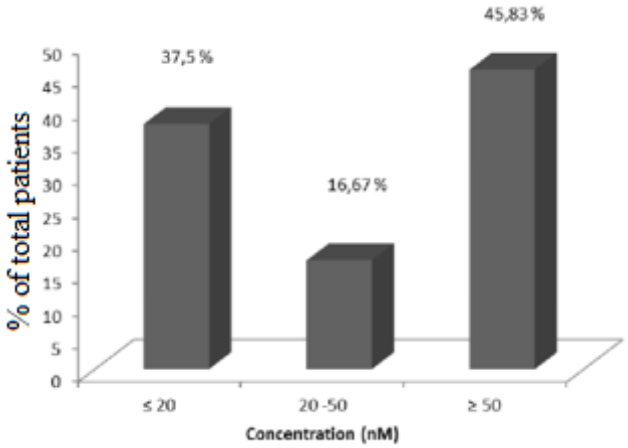


Figure 07: Distribution of bronchoalveolar lavage of patients with COPD according to elastase concentration. Each value represents the average of three determinations.

The obtained results highlight the detection of elastase activity and elastase in bronchoalveolarlavage of COPD patients. In the literature, several studies confirm the presence of elastase and elastase activity in AML. In 1986, Damiano et al. demonstrated that there is certainly an increase in neutrophils associated with an increase in neutrophil elastase activity in Bal in smokers and neutrophil elastase was detected in the human emphysematous lung. The

destruction of air spaces in smokers is negatively correlated with the number of neutrophils while it is positively correlated with the number of alveolar macrophages (Finkelstein *et al.*, 1995) which is very high in smokers. In addition, alveolar macrophages have the ability to degrade elastic fibers (Senior *et al.*, 1989) under the action of metalloproteases with elastolytic activity that they secrete (macrophage elastase (MMP-12), cathepsin L and S).

3. Inhibition of elastase activity in bronchoalveolarlavage by total oil of*Nigella sativa*

The inhibitory effect of *Nigella sativa* total oil on elastase activity in Bal was evaluated spectrophotometrically at 405 nm. The obtained results show that it inhibits elastase activity, in a dose-dependent manner. The inhibitory effect of polar and non-polar oils has not been studied sincethey have a lesser inhibitory effect compared to that of total oil on the one hand and by the synergistic effect on the other hand.

The IC_{50} of the enzyme activity of elastase in Bal of five patients with COPD of the total oil of *NS* range from 100 to 108 µg/ml which means that there is no difference in the inhibition of elastase in Bal. The mean IC_{50} for the five washes is 104.69 ± 3.583 µg/ml (Table V), twice the IC_{50} for α1 antitrypsin (51.41 ± 2.12 ug/ml) (Table VI).

***Table V*: IC_{50} of total *Nigella sativa* oil on elastase activity in Bal of five patients with COPD.**

Samples	S1	S2	S3
IC_{50} (µg / ml)	100.15 ± 4.24	102.08 ± 0.88	108.35± 0.27
Samples	S4	S5	
IC_{50} (µg / ml)	104.94 ± 2.29	107.93 ± 3.18	

Each value represents the mean ± SD (n = 3).

***Table VI:* IC_{50} of α1 antitrypsin on elastase activity in Bal of five patients with COPD.**

Samples	S1	S2	S3
IC_{50} (µg / ml)	51.21± 0.69	52.26 ± 2.68	48.27 ± 0.89
Samples	S4	S5	
IC_{50} (µg / ml)	51.14±2.32	54.12 ± 1.89	

Each value represents the mean ± SD (n = 3).

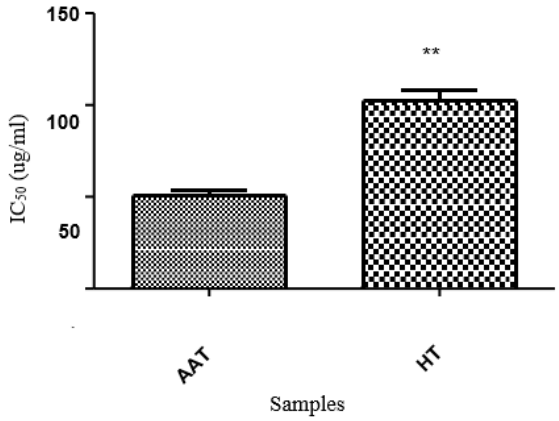


Figure 08: The IC50 of total *NS* oil on elastase activity in Bal. Each value represents the mean ± SD (n = 3). ** p < 0.05.

The obtained results show that the total oil of the nigella as well as the α1 antitrypsin inhibits the elastase of bronchoalveolar lavage, which represents the leukocyte elastase, in a dose-dependent manner, the inhibitory effect of which is better than that exerted on the pancreatic porcine elastase, due to the fact that the IC_{50} on the elastase activity by the total oil is 104.69 µg/ml and 129.29 µg/ml for the leukocyte elastase and the porcine pancreatic elastase, respectively. Thus, α1 antitrypsin has an IC_{50} of 70.88 µg/ml and 51.41 µg/ml for marketed pure elastase and leukocyte elastase (Bal), respectively.

This inhibition is due to the richness of black seed oil by unsaturated fatty acids, particularly oleic acid, which is considered a powerful inhibitor of leukocyte elastase (Moroy*et al.*, 2011).

The inhibition of leukocyte elastase in Bal by the total oil of *NS* explains their effectiveness as an inhibitor of this enzyme because Bal are very rich in different proteins of inflammation, despite that the total oil exerts their significant inhibitory effect.

According to the results obtained, the inhibitory activity of the elastase of Bal by the total oil shows a relatively greater inhibitory effect than that presented by pure elastase, therefore the oils of the seeds of *Nigella sativa* seem to present a real and potential interest in the inhibition of elastase produced by polynuclear neutrophils and macrophages in COPD patients, involved in the destruction of elastin of the elastic fibers that are present in large quantities in the lung and that give it the characteristic elasticity properties. Therefore, *Nigella sativa* oils can be used as a treatment for COPD.

IV. Conclusion

This study suggests that the oils extracted from Nigella sativa seeds have significant potential treatment for COPD, this due to their inhibitory activity on pure elastase (porcine pancreatic

elastase) and leukocyte elastase in bronchoalveolar lavage. The total oil from Nigella sativa seeds is highly effective in inhibiting the proteolytic activity of elastase. The non-polar fractions (F5, F2, and F1) and the polar fraction F7 exhibit a significant inhibitory effect on the enzyme. This inhibitory effect is likely due to the presence of unsaturated fatty acids.

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