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Nigella sativa oil effects on the non-enzymatic defense statute and the histopathological changes induced by smokeless tobacco in a model of allergic asthma in wistar rats

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Abstract

In our present study, we aimed to investigate the non-enzymatic defense system, as well as the histopathological changes, induced by ST at the dose of 40 mg/kg. Furthermore, the preventive and ameliorating effects of oral administration of *Nigella sativa* oil (NSO) at a dose of 4 ml/kg/day are evaluated. The rats were immunized by an intraperitoneal injection of 10 µg ovalbumin (Ova) adsorbed to 1 mg aluminum hydroxide. The administration of ST to Ova-sensitized rats caused significant increase of MDA levels in the lung and the protein carbonyl groups in erythrocytes and lung. In addition, we noticed a significant decrease of GSH in the lung and liver and NPSH in the lung. Moreover, Ova/ST co-exposed rats' lung and liver revealed an inflammatory cells infiltration, cellular hyperplasia with mucus hypersecretion compared with Ova-sensitized rats. In contrast, NSO administration showed significant improvement of levels of MDA, carbonyl groups, GSH and NPSH. Furthermore, we observed reduction of inflammatory cell infiltration, a lesser degree of cells hyperplasia, and many cells having normal morphology. The present study indicates that smokeless tobacco enhances airway inflammation and oxidative stress. Also, these results suggest that the treatment with oral NSO could be a promising treatment for allergic asthma.

Introduction

Allergic inflammation associated with airway hyperreactivity is the main feature of allergic asthma, that affects about 300 million people of all ages worldwide and is increasing by 50% per decade (Abdel-Aziz *et al.*, 2014). The inflammatory response is characterized by an increase in the numbers of eosinophils and mast cells, mucus hypersecretion and activation of T cells, development of various structural alterations in airway wall and smooth muscles of blood vessels in lung tissues (Raza *et al.*, 2010). As inflammation is frequently associated with an increased generation of reactive oxygen species (ROS), and the biochemical environment in the asthmatic airways is favorable for free radical mediated reactions, it has been shown that inflammation caused by increased oxidative stress occurs in the airways of patients with asthma (Dworski, 2000). During those two last decades, the toxicological research has focused on the induction of oxidative stress (OS) after consumption of smokeless tobacco (ST) as a possible mechanism of toxicity in lung, liver and kidney. However, exactly how ST mediates lung damage is not well defined, but

presumably direct contact of the respiratory epithelium with ST toxic compounds, even when not burned or volatilized, can cause inflammatory changes (Schivo *et al.,* 2014).

Nigella sativa commonly known as black seed is an annual herbaceous plant belonging to the *Ranunculaceae* family. It has been widely studied for its pharmacological and therapeutic effects and shown to have extensive range of activities as antibacterial, antifungal, antidiabetic, antioxidant, anti-inflammatory and analgesic, anticancer and immunomodulatory properties (Aftab *et al.,* 2013). Many active compounds have been isolated and identified through phytochemical investigations such as phenolic acid, epicatechin, quercetin and flavones. Moreover, most of the therapeutic properties of this plant are related to the presence of thymoquinone (TQ) which is the main active chemical component of essential oil (Aftab *et al.,* 2013). Therefore, the present study aimed at investigating, on the one hand, the effects of ST in the aggravation of inflammation, and on the other hand, preventive and ameliorating effects of NSO on allergen-induced airway inflammation in a rat model of allergic asthma.

Materials and methods

Animals

Twenty-Four Wistar albino male rats (6–8 weeks old), obtained from Pasteur institute (Algiers, Algeria) were used. The animals were housed in polypropylene cages that were sanitized every 48 hours. The rats were fed a standard laboratory diet and clean tap water *ad libitum.* They were exposed to a natural photoperiod, at temperature of 25±1 °C and a relative humidity of 40±5% and allowed to acclimatize in this condition for 2 weeks prior to experimental use. All protocols in this study were used in accordance with the guidelines of the Committee on Use of Laboratory Animals and approved under the CNEPRU project by the Ethical Committee of DGRSDT at Algerian Ministry of Higher Education and Scientific Research.

Sensitization and Aerosol exposure

The rats were immunized by an intraperitoneal injection of 10 mg Ova adsorbed to 1 mg aluminum hydroxide in a volume of 1mL PBS on Day 0 and boosted on Day 7. At Days 14, 16, 18, 21, and 24, rats were placed in a plexiglass exposure chamber connected to the outlet of an ultrason aerosol generator (OMRON, NE-C29-E) for 30 min. Ova challenges were performed with a mean particle size of 3.2 µm and with an output of 3 mL/min. The last aerosol exposure was done 72h before the end of the experiment (Moerloose *et al.,* 2005). The animals in the other groups were challenged with PBS.

ST and NSO administration

The lethal dose (LD50) concentration of the ST was calculated using LD50 for nicotine in rats (50 mg/kg b.w.) as standard. The used ST concentrations were calculated as 80% of the LD50, which is 40 mg/kg (b.w.). An amount of 1 mL from the stock solution was administered by oral gavage (force-feeding) once per day for 15 days (Adias *et al.,* 2014).

The NSO was administered orally by gavage for 31 days, at a dose of 4 mL/kg/day (Balaha *et al.,* 2012).

Oxidative stress parameters were measured using spectrophotometric methods.

Results and discussion

Ova- sensitization, ST exposure and Ova/ST co-exposure considerably increased the levels of MDA and protein carbonyl groups and decreased levels of GSH and NPSH in the lung, liver and erythrocytes of the sensitized group compared with the control group (Table 1). Moreover, the administration of ST to Ova-sensitized rats caused significant increase of MDA levels in the lung and the protein carbonyl groups in erythrocytes and lung. In addition, we noticed a significant decrease of GSH in the lung and liver and NPSH in the lung. However, NSO administration resulted in a significant improvement of all these parameters in the studied organs (Table 1).

Table 1. Antioxidant defense status in lung, liver and erythrocytes of treated and control rats

Parameters and Treatments	C	Ova	Ova/ST	Ova/ST/NSO
MDA (lung) (nmol/mg prot.)	25.09 ± 0.90	40.82 ± 1.22 ***	43.98 ± 1.22 ***#	34.54 ± 2.06 *** §§§
Protein carbonyl groups (lung) (µmol/mg prot.)	47.58 ± 1.88	53.21 ± 1.79 *	62.56 ± 1.98 ***##	54.58 ± 1.96 ** §§
Protein carbonyl groups (Erythrocytes) (µmol/mg prot.)	20.78 ± 1.24	26.12 ± 1.14 **	30.64 ± 0.82 ***##	26.98 ± 1.12 **§§
GSH (lung) (µmol/mg prot.)	0.72 ± 0.08	0.55 ± 0.03 *	0.32 ± 0.03 ***###	0.47 ± 0.07 *§
GSH (liver) (µmol/mg prot.)	1.53 ± 0.08	1.31 ± 0.03 *	1.04 ± 0.09 ***#	1.25 ± 0.07 **§
NPSH (lung) (µmol/mg prot.)	132.24 ± 6.45	98.73 ± 4.32 ***	87.20 ± 4.15 ***#	115.74 ± 4.04 * §§§

Values are given as mean ± S.E.M for groups of six animals each. Significant difference: all treated groups compared to the control

one (*p≤0.05, **p≤0.01, ***p≤0.001), compared to the Ova sensitized one (#p≤0.05, ##p≤0.01, ###p≤0.001), compared to the Ova/ST treated one (§p≤0.05, §§p≤0.01, §§§p≤0.001).

The histopathological examination of Ova-sensitized and Ova/ST-co-exposed rats' lung revealed an inflammatory cell infiltration (yellow arrow), goblet cells hyperplasia (red arrow) with mucus hypersecretion (circle) (Figure 1). However, NSO administration revealed a reduction of inflammatory cell infiltration (white arrow), a smaller degree of goblet cells hyperplasia (star) and normal cells morphology compared to Ova-sensitized and Ova/ST-co-exposed groups (Figure 1).

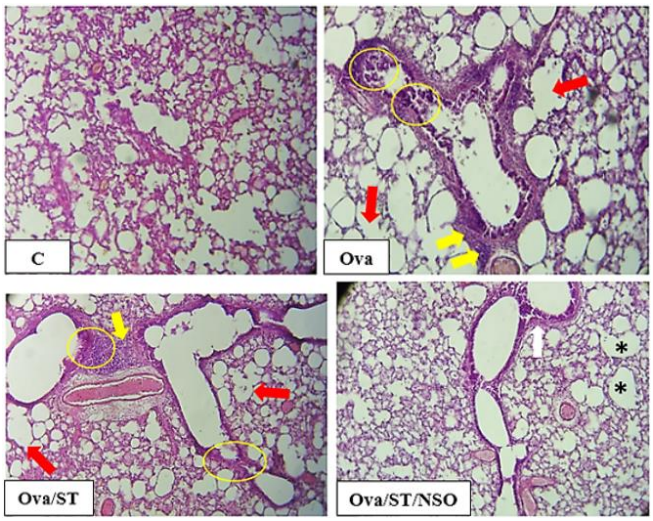

Figure 1. Photomicrographs of H&E stained sections of lung (×400).

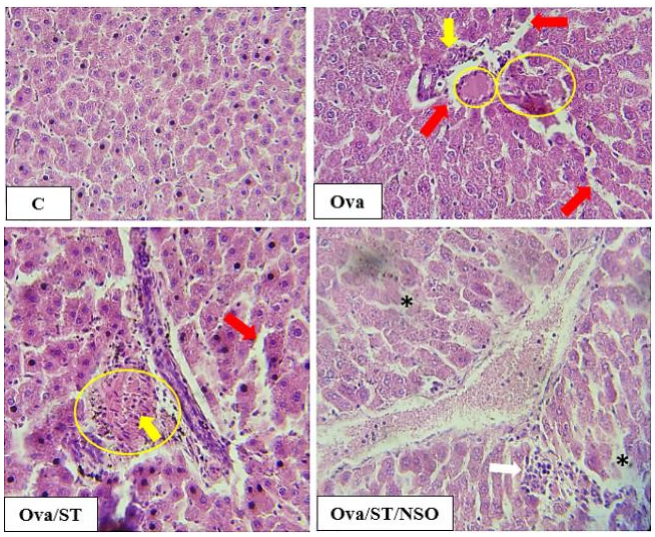

Figure 2. Photomicrographs of H&E stained sections of liver (×400).

Histopathological examination of Ova-sensitized and Ova/ST co-exposed rats' liver revealed an inflammatory cell infiltration (yellow arrow) with degenerative changes in hepatocytes (circle), loss of typical hepatic cord organization and sinusoidal dilatation (red arrow) (Figure 2). In contrast, NSO administration revealed reduction inflammatory cell infiltration (white arrow), a lesser degree of sinusoidal dilatation (star) and normal cells morphology compared to Ova-sensitized and Ova/ST co-exposed groups (Figure 2).

Several studies have shown that subsequent administration of the allergen by inhalation induced inflammatory airway response by overproduction of free radicals, which in turn can initiate lipid peroxidation (Dworski, 2000). Furthermore, it has been shown that ST administration in rats induce oxidative stress resulting in the enhanced levels of MDA, as well as in human consumers of ST (Schivo *et al.,* 2014). The elevated level of MDA in treated rats could be linked to the increasing number of activated inflammatory cells in the pulmonary alveoli, which release large quantities of superoxide anion and hydrogen peroxide by various mechanisms, superoxide anion can interact with NO• to generate peroxinitrite (ONOOH) with high toxicity (Raza *et al.,* 2010). Although, NSO supplementation causes a significant decrease of MDA levels in Ova/ST co-exposed rats. These findings are consistent with several studies which have demonstrated that NSO prevents the formation of reactive oxygen species, causes reduction of lipid peroxidation and stimulates antioxidant defense system (Aftab *et al.*, 2013).

Protein carbonyl content is actually the most general indicator and by far the most commonly used marker of protein oxidation, and accumulation of protein carbonyls has been observed in several human diseases including inflammatory and oxidative lung injury. Protein carbonyls have a major advantage over lipid peroxidation products as oxidized proteins are generally more stable, form early and circulate in the blood for longer periods. The intracellular level of oxidized proteins reflects the balance between the rate of protein oxidation and the rate of oxidized protein degradation. This balance is a complex function of various circumstances that lead to the generation of ROS, on the one hand, and of multiple factors determining the concentrations and/or activities of proteases involved in degradation of oxidatively modified proteins (Sitte N., 2003). Although, NSO pretreatment in Ova/ST co-exposed rats showed a significant decrease in protein carbonyl content. This improvement could be explained by the fact that NSO possesses components such as polyphenols and

flavonoids which prevents the formation of ROS. Also, it has been demonstrated that NSO enhance the tissue capacity for detoxifying ROS (Ebru U. *et al.*, 2008).

Reduced glutathione is only one among many potential antioxidant defenses involved in the protection of various organs against oxidant-induced injury in inflammation (Dworski, 2000). during the metabolism of smoked tobacco many electrophiles are generated which are detoxified by the use of GSH. The decreased GSH levels increase the free radical burden due to ineffective removal of ROS from the tissues, which results in increased lipid peroxidation and protein oxidation. In addition, enhanced lipid peroxidation and protein oxidation with concomitant decrease in reduced GSH is indicative of oxidative stress that provides evidence of the relationship between lipid peroxidation, protein oxidation, tissue damage, and inflammation (Ebru U. *et al.,* 2008).

NPSH is an intracellular antioxidant and in some body compartments, such as the epithelial lining fluid of the lung, is present in high concentrations. NPSH in the lung lining plays a critical role in protecting the lung from oxidative stress by detoxifying exogenous and endogenous toxicants, and quenching ROS. In agreement with these data, various experimental research studies have demonstrated that oxidative stress, provoked by tobacco, causes redox instability and activation of body protective mechanisms, which result in a decrease in the concentration of some antioxidants (Schivo *et al.*, 2014).

All the previous results are in agreement with the histopathological study of lung and liver. In fact, contraction, thickening and abnormality of smooth muscle of airways are mainly responsible for the AHR in asthma. Intranasal challenge with Ova are known to cause goblet cell hyperplasia, mucus hypersecretion and inflammatory cells infiltration in lung tissues. Moreover, NSO administration revealed reduction inflammatory cell infiltration, a lesser degree of goblet cells hyperplasia and normal cells morphology in the present study. This may be linked to the anti-inflammatory and the antioxidant potential of NSO through restoration of local Th1/Th2 cytokine balance, reducing NO levels in lung and restoration of the balance between ROS and the antioxidant defense system (Aftab *et al.*, 2013).

Conclusion

Taken together, the findings of the present study indicate that short-term administration of ST in a rat model of allergic asthma, clearly enhances airway inflammation and oxidative stress. Also, these results suggest that the treatment with oral NSO could be a promising treatment for allergic asthma.

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