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ORIGINAL ARTICLE

# Ameliorating effects of *Nigella sativa* oil on aggravation of inflammation, oxidative stress and cytotoxicity induced by smokeless tobacco extract in an allergic asthma model in Wistar rats

**T. Khaldi, N. Chekchaki, M. Boumendjel, F. Taibi, M. Abdellaoui, M. Messarah, A. Boumendjel***

*Laboratory of Biochemistry and Environmental Toxicology, Department of Biochemistry, Faculty of Sciences, Badji Mokhtar University, Annaba, Algeria*

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**KEYWORDS**
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**Abstract**
*Background:* The comparison of smokeless tobacco (ST) exposure versus Ovalbumin (Ova) sensitized rats or asthmatic patients has hardly been studied in the literature. Thus, the present study aims to investigate the aggravation of inflammation, exacerbation of asthma, oxidative stress and cytotoxicity induced by ST.
*Methods:* ST was given at the dose of 40 mg/kg in an allergic asthma model in Wistar rats. Furthermore, the effects of oral administration of *Nigella sativa* oil (NSO), at a dose of 4 mL/kg/day, were investigated.
*Results:* The obtained results showed that ST clearly enhanced lung inflammation through interleukin-4 (IL-4) and Nitric oxide (NO) increased production. Actually, ST was found to intensify the oxidative stress state induced by Ova-challenge in rats, which was proven not only by augmenting lipid peroxidation and protein oxidation, but also by altering the non-enzymatic and enzymatic antioxidant status. Furthermore, the aggravation of inflammation and oxidative stress was obviously demonstrated by the histopathological changes observed in lung. In contrast, NSO administration has shown anti-inflammatory effects by reducing IL-4 and NO production, restoring the antioxidant status, reducing lipid peroxidation and improving the histopathological alterations by both protein oxidation and NSO treatment.

*Abbreviations:* AHR, airway hyperresponsiveness; b.w., body weight; BALF, BronchoAlveolar Lavage Fluid; CAT, catalase; DGRSDT, (in Frensh Direction Générale de la Recherche Scientifique et du Développement Technologique) General Directorate for Scientific Research and Technological Development; DNPH, 2,4-dinitrophenylhydrazine; DTNB, 5,5′-dithiobis-(2nitrobenzoic acid); ELISA, enzyme-linked immunosorbent assay; GPx, glutathione peroxidase; GSH, reduced glutathione; IFN-γ, interferon gamma; IgE, immunoglobulin E; IL-4, interleukin-4; LD50, median lethal dose; LPO, lipid peroxidation; MDA, MalonDiAldehyde; NBT, nitro blue tetrazolium; NO, nitric oxide; NPSH, non-protein thiols; NSO, *Nigella sativa* oil; ONAB, (in Frensh Office National des Aliments de Bétails) National Livestock Feed Office; Ova, Ovalbumin; PBS, phosphate buffer solution; ROS, reactive oxygen species; SEM, standard error mean; SOD, superoxide dismutase; ST, smokeless tobacco; STE, smokeless tobacco extract; TBA, thiobarbituric acid; TBARS, thiobarbituric reactive species; Th1, T helper cell 1; Th2, T helper cell 2; TQ, thymoquinone.

* Corresponding author.
*E-mail addresses:* amelibis@yahoo.fr, amel.boumendjel@univ-annaba.org (A. Boumendjel).

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*Conclusions:* Our data have proven that severe concurrent exposure to allergen and ST increases airway inflammation and oxidative stress in previously sensitized rats. They also suggest that the oral NSO treatment could be a promising treatment for asthma.
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## Introduction

Over the past two decades, toxicological research has been interested in the induction of oxidative stress after the consumption of smokeless tobacco (ST) as a possible mechanism of toxicity in liver, kidney and lung.[1–3] Presumably, the direct contact of the respiratory epithelium with ST toxic compounds, even when it is not burned or volatilized, can cause inflammatory changes.[1]

Allergic asthma affects about 300 million people of all ages worldwide. It is increasing by 50% per decade[4] and is considered a very serious public health problem. Allergic inflammation is also frequently associated with an increased generation of reactive oxygen species (ROS),[5] 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.[5]

Moreover, it would be interesting to study the ST additive effects on an experimental asthma model, while seeking a natural way to remedy the two sources generating inflammation and oxidative stress. Therefore, we investigated the traditional remedies of pulmonary pathologies and chose *Nigella sativa*, since it is known in the prophetic writings that it heals all the evils.[6] This plant, commonly known as black seed, is an annual herbaceous plant belonging to the Ranunculaceae family.

As previously mentioned, *N. sativa* is known for its pharmacological and therapeutic effects, namely due to its antibacterial, antifungal, antidiabetic, antioxidant, anti-inflammatory, anticancer and immunomodulatory properties.[7,8] Many active compounds have been isolated and identified through phytochemical investigations such as phenolic acid, epicatechin, quercetin and flavones.[9] Moreover, most of the therapeutic properties of this plant are related to the presence of thymoquinone (TQ) which is the main active chemical component cold-pressed black cumin seed oils[10] when linoleic, oleic and palmitic acids were the main fatty acids in the oils tested. Otherwise, in Ramadan study,[11] the author showed that cold-pressed oils of *N. sativa* were healthy oils and had good anti-free radical properties and good oxidation stability, probably due to changes in the fatty acid profile and tocols.[11]

The present study explores not only the effects of ST in the aggravation of inflammation, but also the preventive and ameliorating effects of *N. sativa* oil (NSO) on allergen-induced airway inflammation in a rat model of allergic asthma associated with taking ST.

## Materials and methods

### Animals

Forty-eight Wistar albino male rats weighing 160 ± 10 g (6–8 weeks old), obtained from the Pasteur Institute (Algiers, Algeria) were used. The animals were housed in polypropylene cages that were sanitized every 48 h. The rats were fed a standard laboratory diet (standard food, supplied by the ''ONAB of Bejaia'', Algeria) and clean tap water ad libitum. They were exposed to a natural photoperiod, at a temperature of 25 ± 1 °C and a relative humidity of 40 ± 5% and allowed to acclimatize in this condition for two 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 (D01N01UN230120150006) by the Ethical Committee of DGRSDT at the Algerian Ministry of Higher Education and Scientific Research.

### Sensitization and aerosol exposure

The rats were immunized by an intraperitoneal injection of 10 mg Ovalbumin (Ova) adsorbed to 1 mg aluminum hydroxide in a volume of 1 mL phosphate buffered saline (PBS) on day 0 and boosted on day 7[8,12] (Fig. 1).

At days 14, 16, 18, 21, and 24, the rats were placed in a plexiglass exposure chamber connected to the outlet of an ultrason aerosol generator (OMRON, NE-C29-E) for 30 min. Ovalbumin (1 g OVA in 100 mL PBS) (Grade III; Sigma Chemical Co., Poole, UK) 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 72 h before the end of the experiment[13] (Fig. 1). The animals in the other groups were challenged with PBS.

### Administration of smokeless tobacco extract (STE)

Commercially-prepared ST was bought from the local market (Ets El-Nardjass Company zone 06, Sidi Chehmi-Oran, Algeria). The lethal dose (LD50) concentration of the ST was calculated as 50 mg/kg rat body weight (b.w.) using LD50 for nicotine in rats as standard.[14] The used ST concentrations were calculated as 80% of the LD50, which is 40 mg/kg (b.w.).[14]

An amount of 1 mL from the stock solution was administered by oral gavage (force-feeding) once per day for fifteen (15) days.[14] On the days of the challenge, STE was given 30 min after the aerosol exposure.[8]

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