

Antioxidant Effects of *Nigella Sativa* in the Treatment of Experimentally Induced Rhinosinusitis

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Abstract

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Aim. The objective of this study was to investigate the effect of *Nigella sativa* (NS) in experimental bacterial rhinosinusitis.

Material and Methods. Bacterial rhinosinusitis was induced with *Staphylococcus aureus*. Rabbits were divided into control, NS 50, NS 100 and NS 200 mg/kg/d groups. NS was given orally for 7 days. The same volume of normal saline was given as a vehicle to the control group for the same period. At 7 days post-treatment, mucosal samples were excised from the treated and control groups for measurements of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), myeloperoxidase (MPO) and malondialdehyde (MDA).

Results. SOD and GSH-Px activities significantly increased in the NS 50, NS 100 and NS 200 mg/kg/d groups when compared with the saline treated group. MPO activity and MDA levels significantly decreased in the NS 50, NS 100 and NS 200 mg/kg/d when compared with the saline treated group.

Conclusion. These findings show that administration of NS increased the SOD, GSH-Px activities and decreased the lipid peroxidation and MPO activity in experimental rhinosinusitis in rabbits. NS prevented oxidative stress by scavenging reactive oxygen species generated in rhinosinusitis model in rabbits.

Introduction

Rhinosinusitis is one of the most frequently reported chronic diseases. In contrast to acute rhinosinusitis, where the bacterial or viral etiology is well established, chronic rhinosinusitis has been defined as an inflammation of the mucous membrane of the paranasal sinuses resulting from impaired transport mechanisms [1]. Local and systemic host immune responses interact under such conditions, leading to bacterial and respiratory virus effects in the pathophysiological events which is

characterized by hyperemia, hemorrhage and submucosal edema with polymorphonuclear infiltration of rhinosinusitis [2]. Therefore, treatment of rhinosinusitis has to break the vicious cycle of inflammation, edema formation and mucous hypersecretion, before antibiotic treatment. Although treatment of rhinosinusitis is usually based upon use of antibiotics and/or surgeries, it may both results in high medical costs and development of multiple drug resistance in sinusitis-causing pathogenic microorganisms in human [3-5]. On this account new antimicrobial substances from

various sources like medicinal plants excite scientist's interests to the highest pitch.

The seeds or oil of *Nigella sativa* (NS), belonging to the Ranunculaceae family, commonly known as black seed or black cumin, have been used as a natural remedy for a number of diseases and conditions such as asthma, cough, bronchitis, headache, eczema, fever, dizziness and influenza [6]. NS contains >30 w/w of fixed oil and 0.45 w/w of a volatile oil. The volatile oil has been shown to contain thymoquinone and monoterpenes such as *p*-cymene and *alpha*-pinene. Recently, clinical and animal studies have shown that an extract of black seed has many therapeutic effects, including anti-inflammatory, antiviral, antibacterial, antitumor, and antihistaminic effects [7-13].

Oxygen metabolism in aerobic organisms has obvious beneficial effects, but adverse effects of oxygen also occur because of the generation of reactive oxygen species (ROS). Most macromolecules can undergo oxidative reactions that are mediated by ROS. The adverse effects of ROS on biological systems have become a major focus of current biomedical research [14].

Cells have developed different antioxidant systems and various antioxidant enzymes to defend themselves against free radical attacks. Superoxide dismutase (SOD) catalyses the dismutation of the $O_2^{\cdot-}$ into hydrogen peroxide (H_2O_2). The glutathione-dependent antioxidant system consisting of reduced glutathione and an array of functionally related enzymes plays a fundamental role in cellular defense against reactive free radicals and other oxidant species. Of these enzymes, glutathione peroxidase (GSH-Px) is a selenoprotein that reduces hydroperoxides as well as H_2O_2 while oxidizing glutathione [15-17].

Although the plant has many different effects, to our best knowledge there are no reports on the protective effects of NS in experimental rabbit model of rhinosinusitis. The aim of this study was to document the effects of three different doses of oral administration of NS in a well characterized rabbit model of rhinosinusitis, and investigate the effects of these antioxidant substances on antioxidant (SOD, GSH-Px) and oxidant parameters (MDA, MPO) in the nasal mucosa.

Materials and methods

Animals

Thirty male white rabbits weighing an average of 3.5-3.6 kg were obtained from Ataturk University

Experimental Animal Laboratory of Medicinal and Experimental Application and Research Centre. Rabbits were divided into four equal groups and were maintained in our laboratory under controlled environmental conditions. Animals were fed *ad libitum* consumption of pelleted feed mixture that was formulated to meet or exceed National Research Council recommendations. Animal experiments were performed in accordance with the national guidelines for the use and care of laboratory animals and were approved by the local animal care committee of Ataturk University.

Drugs

The seeds of NS were purchased from a local market. The taxonomic identification of the seeds was confirmed by a senior plant taxonomist, Dr. Y. Kaya, in Department of Biology, faculty of Science, Ataturk University, Erzurum, Turkey. NS seed samples (200 g) were separately extracted with ethanol at room temperature four times. After the organic solvents were evaporated to dryness under vacuum at low temperature using a rotary evaporator, 51.8 g liquid ethanol extract (25.9% yields) were obtained.

Preparation of Bacterial Suspension and Induction of Rhinosinusitis

S. aureus strain ATCC 25923 was suspended at a concentration of 900×10^6 cells per milliliter using a McFarland Nephelometer Standard III at the Department of Microbiology, School of Medicine, Ataturk University, Erzurum, Turkey. After administration of sedative, nasal cavities were filled with Merocels^R for indirect obstruction of the maxillary sinus ostium [18]. The nasal dorsum was aseptically prepared with povidone-iodine before administering local anesthetic to the skin and adjacent soft tissue. After that, 0.5 ml of bacterial suspension was injected to the right maxillary cavity using a hypodermic syringe.

Experimentally Induced Rhinosinusitis and Treatment

Animals were inoculated with *Staphylococcus aureus* (*S. aureus*) after the right nasal cavity was blocked by the Merocels^R (Medtronic Xomed, Jacksonville, FL, USA). Twenty-four hours after bacterial inoculation, the Merocels^R in the right nasal cavities were removed. Thirty animals were assigned randomly to receive control (per-oral, Group 1), NS (per-oral, 50 mg/kg/d) (Group 2), NS (per-oral, 100 mg/kg/d) (Group 3); NS (per-oral, 200 mg/kg/d) (Group 4) daily for 7 days. Twenty-four hours after

ending the treatment period, the rabbits were sedated with a respiratory failure dose of pentobarbital sodium (120 mg/kg, iv). The external nasal dorsum was sterilized by swabbing with povidone. After skin elevation, the upper wall of the maxillary sinus was excised and mucosa samples were taken for biochemical studies.

Biochemical analyses

Each sample of sinus mucosa was homogenized (Omni Tissue Homogenizer, Omni Corp., USA) and the homogenate was centrifuged at 10,000 g for 60 min. The clear supernatant was removed and assayed for SOD, GSH-Px, and MPO activities and for MDA and protein concentrations.

Malondialdehyde (MDA) determination

MDA levels in sinus mucosa homogenate were measured with the thiobarbituric acid reaction according to the method of Ohkawa and coworkers [19]. The values of MDA were expressed as nmol mg⁻¹ protein.

SOD activity determination

SOD activity was determined according to the method of Sun and coworkers [20]. The principle of the method is based on the inhibition of NBT reduction by the xanthine-xanthine oxidase system as a superoxide generator. One unit of SOD was defined as the amount of enzyme causing 50% inhibition in the NBT reduction rate. SOD activity was also expressed as unit per mg⁻¹ protein.

Determination of glutathione peroxidase activity

GSH-Px activity was measured by the method of Paglia and Valentine, 1967 [21]. The enzyme reaction in a tube containing NADPH, reduced glutathione (GSH), sodium ascorbate, and glutathione reductase was initiated by addition of H₂O₂, and the change in absorbance at 340 nm

was monitored with a spectrophotometer. GSH-Px activity was given as mUmg⁻¹ protein.

Determination of myeloperoxidase enzyme activity

MPO activity was determined using a 4-aminopyrrole/phenol solution as the substrate for MPO-mediated oxidation by H₂O₂, and changes in absorbance at 510 nm were recorded [22]. One unit of MPO activity was defined as the amount of protein that degrades 1 mmol min⁻¹ H₂O₂ at 25°C. MPO activity was presented as mUg⁻¹ protein. The protein was determined using the Bradford method [23]. Biochemical measurements were carried out using a spectrophotometer (CECIL CE 3041, Cambridge, UK).

Statistical analyses

Results are presented as mean ± S.D. All parameters were analyzed using a one-way ANOVA. Least significant difference multiple range test was used to compare the mean values. Acceptable significance was recorded when *P* values were <0.05. Statistical analysis was performed with Statistical Package for the Social Sciences for Windows (SPSS, version 10.0, Chicago, IL, USA).

Results

All parameters are shown in Table 1. SOD and GSH-Px activities significantly increased in the NS50, NS 100 and NS 200 groups when compared with the saline treated group. MPO activity and MDA levels significantly decreased in the NS50, NS 100 and NS 200 groups when compared with the saline treated group. However, there were no statistically significant differences among the NS 50, NS 100 and NS 200 groups regarding to oxidative and antioxidative parameters used in this study. Inflammation

Table 1: GSH-Px, CAT, SOD, MPO activities and MDA levels in control and treated maxillary sinus mucosae of rabbits.

	MPO (mU/g protein)	SOD (U mg ⁻¹ protein)	GSH-Px (mUmg ⁻¹ protein)	MDA (nmol mg ⁻¹ protein)
Saline treated group	378.1 ± 9.3	7.7 ± 2.1	148.5 ± 19.1	260.4 ± 21.3
Nigella sativa (50 mg/kg/d)	217.9 ± 11.3 ^a	15.4 ± 4.2 ^a	241.3 ± 29.0 ^a	203.2 ± 9.9 ^a
Nigella sativa (100 mg/kg/d)	211.7 ± 7.2 ^a	16.5 ± 4.6 ^a	245.1 ± 29.5 ^a	200.4 ± 12.2 ^a
Nigella sativa (200 mg/kg/d)	220.6 ± 10.3 ^a	17.5 ± 4.7 ^a	242.3 ± 31.8 ^a	202.5 ± 12.3 ^a

a: p<0.001 in comparison with control group. All data were expressed as mean±SD. There were 7 animals in each group.

and various degrees of exudation were grossly evident in the treated maxillary sinuses, but not in the control sinuses.

Discussion

Nasal and paranasal sinus mucosa have a highly efficient system for the physiologic functions of olfaction, respiration, and protection [24]. The respiratory epithelial cell layer presents a physical barrier that prevents invasion by microorganisms, and the mucociliary action prevents bacterial infection and protects the mucosa from injury and drying [25]. Sinusitis is one of the most frequently reported acute or chronic and heterogeneous diseases, which shows variations of etiology. Where the bacterial or viral etiology is well established, this has been defined as an inflammation of the mucous membrane of the paranasal sinuses resulting from impaired transport mechanisms [1]. Various systemic and local factors are known to be associated with nasal and sinus infections [2, 26]. To maintain the physiologic condition of the nasal cavity and sinuses, it is known that nasal airflow, anatomical conditions, patency of the natural ostium, oxygen saturation in sinuses and mucociliary clearance all play important roles. When one of these physiologic conditions is changed, these abnormal conditions cause inflammatory reactions, due to an abnormal mucous membrane immunity, phagocytosis and bacteriologic action of the nasal secretion enzymes. The local and systemic host immune responses interact under such conditions, leading to bacterial and respiratory virus effects in the pathophysiological events, which are characterized by hyperemia, hemorrhage and submucosal edema with polymorphonuclear infiltration of rhinosinusitis [2, 26].

The aim of this study was to compare the effectiveness of three different doses of *NS*, which was of natural herbal origin. Our results demonstrate that post-treatment with *NS* has protective effects in experimental rabbit model of rhinosinusitis. One of the potential properties of *NS* is the ability of one or more of its constituents to reduce toxicity due to its antioxidant activities. The majority of studies that have been performed to evaluate the different effects of *NS* have been confined to address its antitoxic properties [9, 10, 26, 27]. In addition to this, many investigators have determined the antioxidant activities of *NS in vivo* [6-8].

Although many factors are involved to inflammation in rhinosinusitis, ROS play a major role in the pathogenesis of rhinosinusitis. ROS reversibly or irreversibly damage compounds of all biochemical classes, including nucleic

acids, proteins and free amino acids, lipids and lipoproteins, carbohydrates, and connective tissue macromolecules. ROS may impair such cell activities as membrane function and gene expression. When ROS are not removed by natural scavengers, damage occurs through peroxidation of structurally important polyunsaturated fatty acids within membrane phospholipids. The peroxidative damage is propagated by a repeated chain reaction [14].

Excessive lipid peroxidation in the experimental sinus mucosa can arise due to factors favoring the formation of ROS. Doner et al. [28] reported that MDA levels of serum and infected maxillary sinus mucosa were significantly higher than that of controls. However, in the present study, we found that MDA level was significantly lower in the *NS* 50, *NS* 100 and *NS* 200 groups when compared with the saline treated group. Our findings suggest that oxidative stress in tissue of rabbits with rhinosinusitis may cause an increase in MDA level, which arises as a result of tissue damage. *NS* may be given to decrease the oxidative stress in patients with rhinosinusitis. *NS* decreases the tissue level of MDA via anti-inflammatory, microbiocidal or antioxidant functions even at the low dosages like *NS* 50. Although we used *NS* in different dosages, we could not find statistically significant difference between its effects on the used oxidative and antioxidative parameters. Therefore, this is the first study showing that extract of *NS* can be used for treatment of bacterial rhinosinusitis in addition to other ailments as reported in literature.

We found that GSH-Px and SOD activities were significantly higher all *NS* groups when compared with the saline treated group. SOD dismutates $O_2^{\cdot -}$ to H_2O_2 . GSH-Px detoxifies the H_2O_2 by converting it into H_2O and molecular oxygen. Although H_2O_2 is weakly reactive, its major toxicity derives from its conversion to the highly toxic $OH^{\cdot -}$ via the Fenton or Haber-Weiss reactions. In the study, it seems that increased $O_2^{\cdot -}$ radical is converted to H_2O_2 due to increased activity of SOD. These antioxidant enzymes protect the cell constituents from damage by OFR [14].

Two sources of oxidants which may contribute to injuries in experimental rabbit model of rhinosinusitis include the mitochondrial electron transport chain, which leaks superoxide radical and neutrophils which may secrete the superoxide radical and H_2O_2 through the action of the NADPH oxidase system. MPO activity provides good evidence for arguing that there may be extensive migration of neutrophils to that area. The present study has demonstrated one important result to explain the sources of the ROS: high MPO activity in the

experimental induced rhinosinusitis. It seems that the source of ROS is mostly due to neutrophil activity rather than from the experimental induced rhinosinusitis mucosa itself [29].

In conclusion, NS has clear antioxidant properties and can be used as an antioxidant against oxidative stress. By increasing antioxidant enzymes activities and decreasing oxidant enzymes activities and decreasing lipid peroxidation, NS prevented oxidative stress by scavenging reactive oxygen species generated in experimental rabbit model of rhinosinusitis. These results also show the need for further studies on this subject.

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