Assessment of In vitro Antibacterial Activity and Cytotoxicity Effect of Nigella sativa Oil

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ABSTRACT

Background: Methicillin resistance is a serious health concern since it has spread among Staphylococcus aureus and coagulase-negative Staphylococci (CoNS) that are frequent community and nosocomial pathogens worldwide. Methicillin-resistant strains are often resistant to other classes of antibiotics, making their treatment difficult. Nigella sativa oil is known to be active against Gram-positive cocci, yet its in vitro cytotoxicity is rarely investigated, is a proper and powerful candidate for treatment of methicillin-resistant isolates. Objectives: The aim of this study is to evaluate the in vitro antibacterial activity and cytotoxicity effect of N. sativa oil. Materials and Methods: The minimal inhibitory concentrations (MICs) of N. sativa oil were determined by broth microdilution method against four different American Type Culture Collection strains, 45 clinical isolates of methicillin-resistant S. aureus (MRSA), and 77 methicillin-resistant CoNS (MRCoNS). The effects of different dilutions (0.25 μg/mL, 0.5 μg/mL, and 1 μg/mL) of N. sativa oil on the proliferation of gingival fibroblasts were evaluated. Results: The MIC values of N. sativa oil against clinical isolates of Staphylococci were between <0.25 μg/mL and 1.0 μg/mL. Compared to the control group, there was no cytotoxic effect on the proliferation of the gingival fibroblasts. Conclusion: In the present study, the oil of N. sativa was very active against MRSA and MRCoNS and had no in vitro cytotoxicity at relevant concentrations. These findings emphasize that there is a requirement for further clinical trials on N. sativa oil for “safe” medical management of infections caused by methicillin-resistant Staphylococci. Key words: Cytotoxic effect, methicillin-resistant, Staphylococci, microdilution, Nigella sativa

INTRODUCTION

Methicillin resistance is a serious health concern since it has spread among Staphylococcus aureus and coagulase-negative Staphylococci (CoNS) that are frequent community and nosocomial pathogens worldwide. Methicillin-resistant strains are often resistant to other classes of antibiotics making their treatment difficult. In recent years, the need for new antimicrobial agents because of the rise in antibiotic resistance has led to a search for alternative sources of antimicrobials. Medicinal plants offer a wide range of biodiversity of great value for pharmacology. It has been known since antiquity that herbs and their essential oils have varying degrees of antimicrobial and therapeutic activity. The World Health Organization has been recently supporting countries to integrate traditional medicine with their national health care systems. Nigella sativa that belongs to family Ranunculaceae is commonly known as black seed or black cumin. It has been shown to possess antimicrobial, immunomodulatory, anti-inflammatory, and antioxidant properties. The antimicrobial activity of the oil and its constituents has been frequently studied so far. Although there have been varying ranges of susceptibility results in the literature, Gram-positive bacteria such as Bacillus cereus, S. aureus, and Staphylococcus epidermidis...
have been commonly designated as the most susceptible species to \textit{N. sativa} oil.\cite{8} Moreover, significant antimicrobial activity against multidrug-resistant clinical bacterial isolates has been also reported.\cite{11-13}

In this study, we aimed to investigate \textit{in vitro} antibacterial activity and \textit{in vitro} cytotoxicity effect of \textit{N. sativa} oil, which has been so far merely studied.

**MATERIALS AND METHODS**

**Antibacterial activity**

The \textit{in vitro} antibacterial activity of \textit{N. sativa} oil was evaluated against following strains of \textit{S. aureus} American Type Culture Collection (ATCC) 29213, \textit{Enterococcus faecalis} ATCC 29212, \textit{Escherichia coli} ATCC 25922, \textit{Pseudomonas aeruginosa} ATCC 27853, 45 clinical isolates of methicillin-resistant \textit{S. aureus} (MRSA), and 77 clinical isolates of methicillin-resistant CoNS (MRCoNS).

The minimal inhibitory concentrations (MICs) of \textit{N. sativa} oil were determined by broth microdilution method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.\cite{14} 10.24 mg of cold pressed \textit{N. sativa} oil (purchased from ZADE Vital, Konya, Turkey) was dissolved in 10 mL of dimethyl sulfoxide (DMSO) to prepare a stock solution. The serial dilutions from the stock solution were made ranging from 256 \(\mu\)g/mL to 0.25 \(\mu\)g/mL using Mueller-Hinton broth (Becton Dickinson, Sparks, MD, USA) in 96-well microplates. The bacterial suspension containing approximately \(5 \times 10^8\) colony-forming units/mL was prepared from a 24 h culture plate. From this suspension, 100 \(\mu\)L was inoculated into each well. A sterility control well and a growth control well were also studied for each strain. The plates were incubated at 35°C for 24 h. The MICs were read as the lowest concentrations of \textit{N. sativa} that inhibit the appearance of visible growth. These experiments were carried out in duplicate.

**Cell culture**

The optimal seeding concentration (10,000 cells/well) for proliferation experiments of the human gingival fibroblasts (HGFs) was determined, and then, cells were allowed to adhere for 19 h in Dulbecco’s modified Eagle’s medium (DMEM) with 10% fetal bovine serum (FBS). The media were changed to DMEM with 5% FBS containing \textit{N. sativa}. xCELLigence cell index (CI) impedance measurements were performed according to the instructions of the supplier.

**Proliferation experiments**

Cytotoxic effect of the selected dilutions of \textit{N. sativa} oil (0.25 \(\mu\)g/mL, 0.5 \(\mu\)g/mL, and 1 \(\mu\)g/mL), equal to the MICs at which the \textit{Staphylococci} were susceptible to, were evaluated on the proliferation of gingival fibroblast cells by a real-time cell analyzer (xCELLigence, Roche Diagnostics GmbH, Penzberg, Germany). The cells were suspended in DMEM with 10% FBS. Then, 200 \(\mu\)L of the suspensions was seeded into wells (10,000 cells/well) of the E-plate 16. The gingival fibroblast cells were observed every 15 min during 95 h. After seeding, cells were held to attach to the E-plate for 19 h; then, the cells were exposed to 100 \(\mu\)L of medium containing dilutions of the \textit{N. sativa} oil.

**RESULTS**

**Antibacterial activity**

The MIC values of \textit{N. sativa} oil against \textit{S. aureus} ATCC 29213, \textit{E. faecalis} ATCC 29212, \textit{E. coli} ATCC 25922, and \textit{P. aeruginosa} ATCC 27853 standard strains were 0.5 \(\mu\)g/mL, 2 \(\mu\)g/mL, 64 \(\mu\)g/mL, and 64 \(\mu\)g/mL, respectively.

The MIC values of \textit{N. sativa} oil against clinical isolates of \textit{Staphylococci} were between <0.25 \(\mu\)g/mL and 1.0 \(\mu\)g/mL. Of 45 MRSA strains, MICs of 41 isolates were <0.25 \(\mu\)g/mL, two were 0.5 \(\mu\)g/mL, and two were 1 \(\mu\)g/mL. Of 77 MRCoNS strains, MICs of 53 isolates were <0.25 \(\mu\)g/mL, 19 were 0.25 \(\mu\)g/mL, two were 0.5 \(\mu\)g/mL, and three were 1 \(\mu\)g/mL.

**Proliferation experiments**

For the \textit{N. sativa} oil applications, cytotoxic effect at concentrations up to 1 \(\mu\)g/mL was not observed on the gingival fibroblasts when compared to the control group. CI of all NS oil concentrations was not significantly different after the treatment [Figure 1].

**DISCUSSION**

\textit{N. sativa} seed oil consists of oleosins and essential oil components, including thymoquinone, dithymoquinone, thymohydroquinone, p-cymene, carvacrol, 4-terpineol, \(\alpha\)-thujene, \(\tau\)-anethol, longifolene, thymol, and pinene.\cite{10} Thymoquinone (30–52.6%) and p-cymene (7–25.8%) were reported as its major components.\cite{15,24} Antimicrobial activity of \textit{N. sativa} oil is attributed mainly to its phenolic constituents of the essential oil compartment. Thus, thymoquinone followed by its related compounds such as thymohydroquinone, dithymoquinone, and thymol along with carvacrol plays major role in antimicrobial activity.\cite{16,27,30} Other constituents, oleosins, linoleic acid, and oleic acid, may also have minor antimicrobial activity.\cite{15} Indeed, whole essential oil was reported to have higher antibacterial activity than the combinations of its prominent constituents, suggesting that the minor components potentiate the antimicrobial activity.\cite{19-22}

Although our findings regarding the antibacterial activity of \textit{N. sativa} oil against Gram-positive and Gram-negative bacteria were in agreement with other studies in terms of Gram-positive bacteria being more susceptible, our results were inconsistent with most of previously published works in terms of much lower MICs against Gram-positive ATCC strains and clinical isolates of \textit{Staphylococci}. This discrepancy may be explained by several factors such as differences in the extraction methods, antibacterial assay methods used, percentage of active components in the oils, quality and composition of the active constituents, and type of microorganisms selected.\cite{8,19,23,24}

In fact, the oil composition and antimicrobial activities of a specific plant may differ depending on geographical locations where it is cultivated and on harvesting periods.\cite{24,25} It was reported that genetic differences between \textit{N. sativa} seeds grown in the different countries exist and the genetic polymorphisms took place over time seem to cause distinct

![Figure 1: Dynamic monitoring of cell adhesion and proliferation using the xCELLigence system. The effects of different dilutions of \textit{Nigella sativa} oil on the proliferation of human gingival fibroblasts at a density of 10,000 cells per well in E-Plates 96 were observed during 95 h](image-url)
The seed extracts of *N. sativa* oil deserves further investigations on methcillin-resistant *Staphylococcus*.

The seed extracts of *N. sativa* are characterized by a low level of toxicity. Although potential toxicity of the *N. sativa* seed oil was investigated in animal experiments to determine LD₅₀ values, its *in vitro* cytotoxicity effect has been rarely studied. It was reported that the extract of *N. sativa* seed was not toxic when administered to rats intraperitonally at a daily dose of 50 mg/kg. In addition, experimental animals were not affected when *N. sativa* oil at doses of 10 mL/kg was administered orally. The present study, *in vitro* cytotoxicity assay of *N. sativa* oil on the fibroblast cells did not represent cytotoxic effect at the relevant dilutions when compared to the control group. Kadan et al. reported that cytotoxic effect of 50% ethanol/water extract of *N. sativa* on the human hepatocellular carcinoma and the rat L6 muscle cell line exhibited at concentrations higher than 500 µg/mL. Although this concentration was much higher than concentrations we investigated, the results should not be compared with each other because the subject materials are different in composition and nature.

**CONCLUSION**

The oil of *N. sativa* was very active against MRSA and MRCoNS and had no *in vitro* cytotoxicity at concentrations up to 1 µg/mL in the present study. These findings emphasize that there is a requirement for further clinical trials on *N. sativa* oil for "safe" medical management of infections caused by methcillin-resistant *Staphylococci*.

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**Conflicts of interest**

There are no conflicts of interest.

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