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ORIGINAL ARTICLE

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

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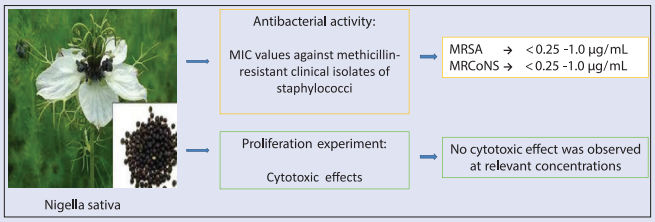
ABSTRACT

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*

SUMMARY

- The minimal inhibitory concentration (MIC) values of *Nigella sativa* oil against *Staphylococcus aureus* American Type Culture Collection (ATCC) 29213, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853 standard strains were 0.5 µg/mL, 2 µg/mL, 64 µg/mL, and 64 µg/mL, respectively
- The *N. sativa* oil showed an excellent antibacterial activity against clinical isolates of methicillin-resistant *S. aureus* and methicillin-resistant coagulase-negative *Staphylococci* with very low MIC range of <0.25–1.0 µg/mL
- The *N. sativa* oil exhibited no cytotoxic effect on the proliferation of the gingival fibroblasts.



Abbreviation used: ATCC: American Type Culture Collection; CLSI: Clinical and Laboratory Standards Institute; CoNS: Coagulase-negative Staphylococci; DMEM: Dulbecco's modified Eagle's medium; DMSO: Dimethyl sulfoxide; FBS: Fetal bovine serum; HGF: Human gingival fibroblast; MIC: Minimal inhibitory concentration; MRCoNS: Methicillin-resistant CoNS; MRSA: Methicillin-resistant S. aureus

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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.[1,2] Methicillin-resistant strains are often resistant to other classes of antibiotics making their treatment difficult.[2] In the 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.[3] 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.[4] The World Health Organization has been recently supporting countries to integrate traditional medicine with their national health care systems.[5]

Nigella sativa that belongs to family *Ranunculaceae* is commonly known as black seed or black cumin.[6] It has been shown to possess antimicrobial, immunomodulatory, anti-inflammatory, and antioxidant properties.[7] The antimicrobial activity of the oil and its constituents has been frequently studied so far.[8-10] Although there have been varying ranges of susceptibility results in the literature, Gram-positive bacteria such as *Bacillus cereus, S. aureus*, and *Staphylococcus epidermidis*

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have been commonly designated as the most susceptible species to *N. sativa* oil.[8] Moreover, significant antimicrobial activity against multidrug-resistant clinical bacterial isolates has been also reported.[11-13]

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

MATERIALS AND METHODS

Antibacterial activity

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

The minimal inhibitory concentrations (MICs) of *N. sativa* oil were determined by broth microdilution method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.[14] 10.24 mg of cold pressed *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 μg/mL to 0.25 μg/mL using Mueller–Hinton broth (Becton Dickinson, Sparks, MD, USA) in 96-well microplates. The bacterial suspension containing approximately 5×10^5 colony-forming units/mL was prepared from a 24 h culture plate. From this suspension, 100 μL 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 *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 *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 *N. sativa* oil (0.25 μg/mL, 0.5 μg/mL, and 1 μg/mL), equal to the MICs at which the *Staphylococci* were susceptible to, were evaluated on the proliferation of gingival fibroblast cells by a real-time cell analyzer (xCELLigence, Roche Diagnostics GmbH, Penzbeerg, Germany). The cells were suspended in DMEM with 10% FBS. Then, 200 μL 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 μL of medium containing dilutions of the *N. sativa* oil.

RESULTS

Antibacterial activity

The MIC values of *N. sativa* oil against *S. aureus* ATCC 29213, *E. faecalis* ATCC 29212, *E. coli* ATCC 25922, and *P. aeruginosa* ATCC 27853 standard strains were 0.5 μg/mL, 2 μg/mL, 64 μg/mL, and 64 μg/mL, respectively.

The MIC values of *N. sativa* oil against clinical isolates of *Staphylococci* were between <0.25 μg/mL and 1.0 μg/mL. Of 45 MRSA strains, MICs of 41 isolates were <0.25 μg/mL, two were 0.5 μg/mL, and two were 1 μg/mL. Of 77 MRCoNS strains, MICs of 53 isolates were <0.25 μg/mL, 19 were 0.25 μg/mL, two were 0.5 μg/mL, and three were 1 μg/mL.

Proliferation experiments

For the *N. sativa* oil applications, cytotoxic effect at concentrations up to 1 μg/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

N. sativa seed oil consists of oleoresins and essential oil components, including thymoquinone, dithymoquinone, thymohydroquinone, p-cymene, carvacrol, 4-terpineol, α-thujene, t-anethol, longifolene, thymol, and pinene.[15] Thymoquinone (30–52.6%) and p-cymene (7–25.8%) were reported as its major components.[15,16] Antimicrobial activity of *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.[8,17,18] Other constituents, oleoresins, linoleic acid, and oleic acid, may also have minor antimicrobial activity.[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.[19-22]

Although our findings regarding the antibacterial activity of *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 *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.[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.[24-27] It was reported that genetic differences between *N. sativa* seeds grown in the different countries exist and the genetic polymorphisms took place over time seem to cause distinct

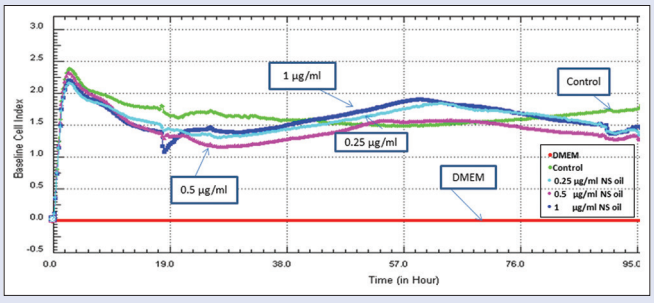

Figure 1: Dynamic monitoring of cell adhesion and proliferation using the xCELLigence system. The effects of different dilutions of *Nigella sativa* oil on the proliferation of human gingival fibroblasts at a density of 10,000 cells per well in E-Plates 96 were observed during 95 h

varieties.[28] Moreover, differences in the antimicrobial activities of the essential oils may be obtained depending on the species, subspecies, or varieties.[25] Indeed, comparison of data belonging to previously published works becomes more complicated because of the lack of a standardized method for investigating the antimicrobial activity of natural compounds such as oils obtained from various herbs. Yet, disk diffusion and broth microdilution methods are most applied technics to investigate the antimicrobial activity of plant and seed oils.[8,15,17,29] The solvents utilized to dissolve these oils or their constituents are also quite diverse.[8,16,29,30] CLSI recommends DMSO, ethyl alcohol, polyethylene glycol, and carboxymethyl cellulose as solvents for water-insoluble drugs without mentioning naturally existing antimicrobial compounds.[14] The units used for the MIC values that are reported as mg/mL, µg/mL, µL/mL, ppm, µL/well, and %(v/v) also differ between articles making comparison of results very difficult.[16,19] Considering vast studies in the literature, it is obvious that there is a need to standardize every step of antimicrobial susceptibility tests for essential oils and their components. However, this subject is beyond our scope.

In the present study, we investigated the antibacterial activity of the whole *N. sativa* oil by broth microdilution method. Our findings presented at least three-fold lower MIC values against *Staphylococci* when compared to previously published works, most of which have studied active components instead of the entire oil of the *N. sativa* seed.[12,17,30-32] It was previously reported that the MIC value of 12.5 µg/mL of the *N. sativa* seed extract was the lowest concentration at which all the tested microorganisms (*E. coli, B. subtilis, S. aureus, P. aeruginosa, Candida albicans, Aspergillus niger*) were inhibited suggesting that further dilutions may possess antimicrobial activity against *Staphylococci*.[29] In point of fact, it has been severally shown that *Staphylococci* are more susceptible to *N. sativa* oil and its components than other bacteria.[8,32,33]

In the present study, an excellent activity of the *N. sativa* oil was observed with very low MICs against clinical isolates of MRSA and MRCoNS. Our findings were in agreement with literature reporting thymoquinone, the most active constituent, to have substantial antimicrobial activity against MRSA.[34,35] Although Hannan *et al*.[12] reported higher MIC ranges (0.2–0.5 mg/mL) against MRSA, their results indicated that *N. sativa* has inhibitory effect on MRSA. In another study, the multidrug-resistant *S. aureus* isolates from diabetic wounds were susceptible to various concentrations of *N. sativa* oil.[11] Some experiments on animals show that *N. sativa* oil has significant *in vivo* antibacterial activity on *S. aureus* infections.[9,36] It is also noteworthy to mention that MICs against methicillin susceptible and methicillin-resistant *Staphylococci* did not differ significantly in our study ($P > 0.05$) as in several other works.[17,30] Moreover, a few studies demonstrated that *N. sativa* essential oil and thymoquinone can effectively inhibit *S. aures* biofilm formation, suggesting that *N. sativa* oil deserves further investigations on methicillin-resistant *Staphylococci*.[31,37]

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 LD_{50} 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 intraperitoneally at a daily dose of 50 mg/kg.[38] In addition, experimental animals were not affected when *N. sativa* oil at doses of 10 mL/kg was administered orally.[39,40] In 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*.[41] 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 methicillin-resistant *Staphylococci*.

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The authors declare that they have no competing interests.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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