Anti-proliferative Effect of Niaouli and Elemi Essential Oils on MDA-MB-231 Breast Cancer Cell Line

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Abstract

Objective: Recently, there has been an increasing number of studies investigating the role of essential oils in cancer treatment. In our study, we aimed to investigate the cytotoxicity of Niaouli and Elemi essential oils against human breast cancer MDA-MB-231 cells.

Material and Methods: The breast cancer cell line MDA-MB-231 was used and cells were cultured in Dulbecco's modified eagle medium (DMEM) in an incubator with 37°C and 5% CO_2. Cells were treated with essential oil at different concentrations (100, 210, 430 and 650 µg/mL for Niaouli and 105, 210, 420 and 630 µg/mL for Elemi) for 24 hours. After that, cytotoxicity was determined by the MTT test.

Results: In our study results showed that Niaouli and Elemi essential oils had cytotoxic activity for the breast cancer cells line. For Niaouli and Elemi essential oils, 50% inhibition concentration (IC_{50}) values were determined as 280±1.29 µg/mL and 214±1.01 µg/mL, respectively.

Conclusion: As a conclusion, it is thought that there is a need for research using molecular analyzes to discover which molecular signaling pathways are involved and *in vivo* studies to support these activities.

Keywords: Niaouli, Elemi, Essential oils, Breast cancer, Cytotoxicity, MTT

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Introduction

Breast cancer is among the most common causes of death in the world, and the need to support conventional treatment and develop new agents for treatment continues (1,2). To date, we know 3000 essential oils, but only 300 of them are consumed as pharmaceuticals, cosmetics, and food (3). The fact that different essential oils obtained from aromatic medicinal plants have many bioactive makes them among the candidates that can be used in the treatment of breast cancer (2). Studies about plants have demonstrated that essential oils have better activities than intact plants (4). Studies have reported that essential oils have antioxidant, antimicrobial, antifungal, antiviral, and anticancer properties (2,5–7). Phytochemical compounds in essential oils lead to protective and cytotoxic effects against diseases (2,7,8). These compounds have been shown to increase the effectiveness of commonly used chemotherapy drugs (7). Niaouli essential oil is extracted from *Melaleuca viridiflora* which is a plant in the myrtle family Myrtaceae and elemi essential oils extracted from *Canarium luzonicum* plant have industrial importance and are used for medicinal purposes (9–11). 3-(4,5-dimethylthiazolyl)-2,5-diphenyltetrazolium bromide (MTT) assay is the most common method used to determine whether an active substance has anti-cancer properties. This method is a colorimetric method and gives results of cell viability according to the color change (12). In this study, it was investigated of Niaouli and Elemi essential oils' cytotoxic activities in MDA-MB-231 breast cancer cells.t

Materials and methods

Cell culture

The breast cancer cell line MDA-MB-231 (The American Type Culture Collection (ATCC) was used and the cells delivered as a cold chain were thawed and grown in a cell culture medium. Cells were grown using a medium containing Dulbecco's modified eagle medium (DMEM) (Capricorn Scientific), 10% heat-inactivated fetal bovine serum (FBS) (Sigma Aldrich), and 1% penicillin-streptomycin antibiotic solution (Sigma Aldrich). $7x10^3$ cells per well were seeded in 96-well plates to apply essential oils and form experimental groups. 24 hours after cell cultivation, control and essential oil groups were exchanged with normal medium and medium

containing essential oil at different concentrations (100, 210, 430, and 650 µg/mL of Niaouli and 105, 210, 420, and 630 µg/mL of Elemi). Essential oils were dissolved in %0,5 DMSO. To evaluate the acute effect of essential oils, cells were incubated for 24 hours in an incubator (37°C, 5% CO_2). At the end of the experiment, cell viability/cytotoxicity was determined based on the mitochondrial activity by the MTT test. DMEM, FBS, and penicillin-streptomycin antibiotic solution (Wisent-Canada), MTT, (Sigma-Aldrich, Germany), and essential oils used in the experiments were commercially available.

Cell viability/cytotoxicity analysis

The tetrazolium ring of MTT (3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide), a tetrazolium salt, is cleaved by the succinate-dehydrogenase enzyme in the mitochondria of living cells, resulting in water-insoluble formazan salts. As cell proliferation increases, the absorbance value increases due to the formation of formazan salt. Cell viability/cytotoxicity depending on mitochondrial activity is determined by this method. In our study, mitochondrial activities of breast cancer cells treated with essential oils were evaluated with this method. At the end of the experiment, the medium was removed from the cells in the 96-well plates incubated with essential oils. Then, 100 µL of fresh medium and 10 µL (5 mg/mL) of MTT solution were added to the wells and incubated for 3 hours. At the end of the incubation period, 100 µL of DMSO was added to each well and absorbance values were determined at 570 nm in an ELISA microplate reader (Multiskan GO-Thermo).

Statistical analysis

The data obtained from the study were evaluated with the IBM SPSS 21 package program. The normal distribution of the data was determined by the Shapiro-Wilk test. Comparisons between groups were made with One-way ANOVA, one of the independent sample tests. Results were given as mean ± standard deviation (mean ±SD). The statistically significant level of $p<0.05$ was accepted.

Results

In our study, the cytotoxic effect of Niaouli essential oil on MDA-MB-231 breast cancer cells was evaluated, and it was determined that all concentrations of Niaouli decreased viability significantly compared to the control group ($p<0,001$) (Figure 1). It was found that there was no statistical difference between the group treated with DMSO used as a solvent and the control group ($p=0,719$). When the groups administered Niaouli essential oil were compared with each other, there was no statistical difference between N1 and N2 ($p=0.069$), but a statistically significant difference was found between N1 and N3 ($p=0.000$) and N4 ($p=0.000$). It was determined that N3 ($p=0.008$) concentration decreased cell viability less than N2 concentration, and a statistically significant difference was observed between these two concentrations. There was no statistical difference between the N2 and N4 ($p=0.019$) groups and between the N3 and N4 ($p=0.999$) groups.

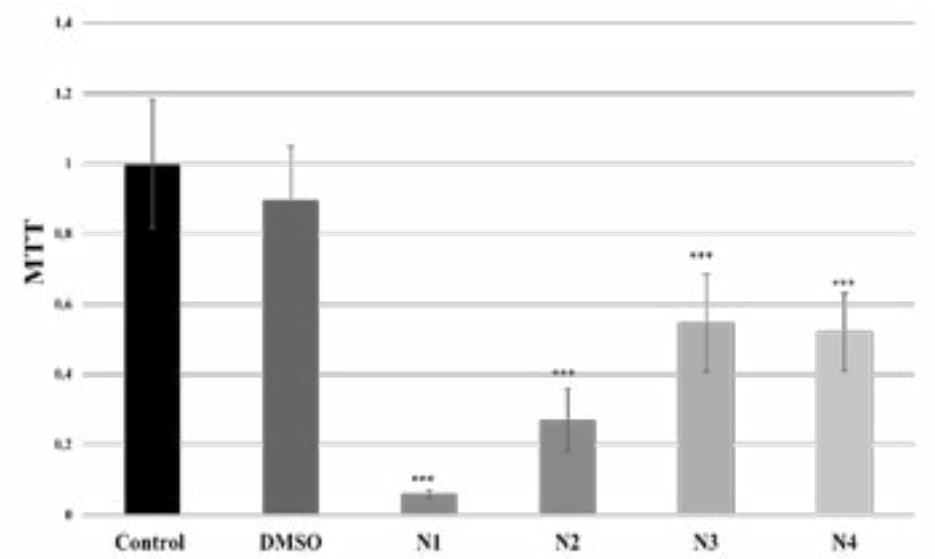

Figure 1. *The effect of Niaouli essential oil applied to MDA-MB-231 breast cancer cells. N1=650 µg/mL N2=430 µg/mL N3=210 µg/mL N4=100 µg/mL. p<0.001****

Similar to Niaouli essential oil, Elemi essential oil has been found to have cytotoxic effect on cell viability of MDA-MB-231 breast cancer (p<0,001) (Figure 1). There was no statistical difference between the group treated with DMSO as a solvent and the control group (p=0,601). When Elemi essential oil groups are compared with each other, it was found that there was no statistical difference between E1 and E2 (p=1,000) groups, and there was a statistically significant difference between E1 and E3 (p=0.000) and E4 (p=0.000) groups. Compared to the E2 group, viability decreased less in the E3 (p=0.000) and E4 (p=0.000) groups. However, it was determined that there was a statistically significant difference between the concentrations. It was found that cell viability decreased less in the E4 (p=0.000) group compared to the E3 group, and there was a statistically significant difference between the groups.

According to the cytotoxic effects of Niaouli and Elemi essential oils, 50% inhibition concentration (IC_{50}) values were calculated and determined as 280±1.29 µg/mL and 214±1.01 µg/mL, respectively.

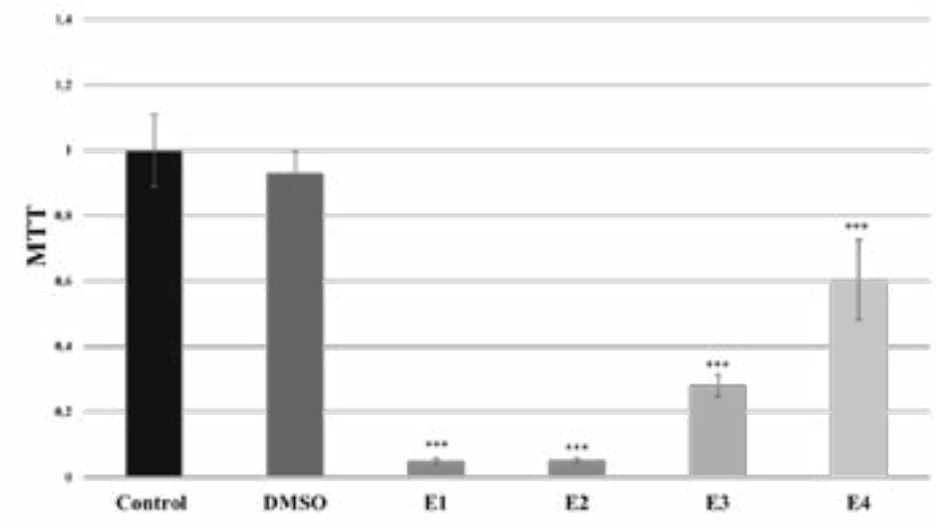

Figure 2. *The effect of Elemi essential oil applied to MDA-MB-231 breast cancer cells on proliferation. E1=630 µg/mL E2=420 µg/mL E3=210 µg/mL E4=105 µg/mL. p<0.001****

Discussion

Essential oils have been shown to have antioxidant, antibacterial, antifungal, antiviral, and anticancer effects in studies. Essential oils contain phytochemical components that have both preventive and cytotoxic effects against diseases. These compounds have been demonstrated to improve the efficacy of commonly used chemotherapy medicines (2,5–8). In comparison to synthetic medications, plant-derived products are believed to cause less adverse effects. The number of new cancer therapy options based on endemic plants is growing every day. Essential oils are lipophilic, meaning they pass the plasma membrane to affect the cell. Many studies have shown that essential oils can contribute to cancer treatment due to their anti-cancer effects (13–15). Biological activity varies depending on the way the oil is obtained, its content and usage doses. It also varies according to the cell line used (16). For example, essential oil is not cytotoxic in a normal fibroblast cell but may have cytotoxic activity in a cancerous cell line (3,16). Tea tree oil (TTO) obtained from *Melaleuca alternofiolia*, which belongs to the same genus as the plant from which Niaouli is obtained, has been studied intensively and according to the results obtained, it has been determined that it causes both digestive problems and skin irritation when used in high concentrations (17). However, it does not cause genotoxic effects in cancer cells and human lymphocytes (17,18). In our study, the concentration of Niaouli that caused the death of 50% of cells was 280 mg/mL and had a relatively higher concentration than Elemi's concentration (214 mg/mL). The dose range in which TTO caused 50% cell death of various cells (HeLa, K562, CTVR-1, Molt-4, Hep G2, HL-60, fibroblast, and epithelial cells) was determined as 20-2700 mg/mL (16). Aromatic essential oils are oils that can be applied especially on the skin by massage. In an *in vivo* study, topically applied TTO essential oil has also been reported to have antitumoral properties (3,19). The other essential oil obtained from *Canarium luzonicum* is a valuable oil that can be used for human health with its ingredients (20). It has been reported that Elemi has low cytotoxic activities in B16 melanoma cells, but can suppress melanogenesis, therefore, elemi can be used as a depigmentation agent for the cosmetic industry (21). In our study, we have found that Elemi essential oil also had cytotoxic activity for breast cancer cells.

Conclusion

Taken together, the results obtained in the present work suggest that the Niaouli and Elemi essential oils have cytotoxic effect on cell viability of breast cancer for 24h.

It may be possible to develop drugs with high efficacy and less side effects by using essential oils. However, it was concluded that there is a need for studies to determine which molecular signaling pathways affect by using molecular analyzes and to support these activities with *in vivo* studies.

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