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Beta-Sitronellol ve (-)-Menton Monoterpenlerinin İnsan Meme Kanseri (MCF-7) Hücre Hattında Sitotoksik Etkilerinin İncelenmesi

Investigation of Cytotoxic Effect of Monoterpenes Beta-Citronellol and (-)-Menthone in Human Breast Cancer (MCF-7) Cell Line

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ÖZ

Amaç: Monoterpenler, ilaç, gıda veya kozmetik endüstrisinde kullanılan, bitki esans yağlarının ana bileşenleridir. Birçok terpenoid, antialerjik, antiviral, anti-inflamatuar ve antikanser gibi çeşitli farmakolojik aktiviteler sergiler. Bununla birlikte, bu bileşiklerin insan meme kanseri (MCF-7) hücreleri üzerine sitotoksik etkileri ile ilgili yeterli çalışma bulunmamaktadır. Bu çalışmanın amacı, Beta-Sitronellol (BS) and (-)-Menton (MNT) monoterpenlerinin potansiyel sitotoksik etkilerini MCF-7 hücre hattında 3-(4,5-dimethylthiazolyl-2)-2,5 diphenyl tetrazolyum bromid (MTT) testi ile incelemektir.

Yöntem: Bu çalışmada BS ve MNT monoterpenlerinin sitotoksik etkileri, MCF-7 hücre hattında 24 ve 48 saat muamele sürelerinde MTT testi ile analiz edilmiştir. Bunun için hücrelere BS (2, 10, 50, 250, 1250 µg/mL) ve MNT (16, 80, 400, 2000, 10.000 µg/mL)'nin farklı konsantrasyonları uygulanmıştır.

Bulgular: BS ve MNT, 24 saatlik uygulama süresinde tüm konsantrasyonlarda, (%) hücre canlılığını önemli düzeyde azaltmıştır. Ayrıca 48 saatlik uygulamada, BS (250, 1250 µg/mL) ve MNT (16, 400, 2000, 10.000 µg/mL), (%) hücre canlılığını anlamlı oranda düşürmüştür.

Sonuç: Sonuç olarak; BS ve MNT, her iki muamele süresinde de MCF-7 hücreleri üzerinde sitotoksik etki göstermiştir. Ancak sitotoksisitenin belirlenmesine yönelik farklı hücre hatlarında daha fazla çalışma yapılmalıdır.

Anahtar Kelimeler: Monoterpen, Beta-Sitronellol, (-)-Menton, MCF-7, MTT testi

ABSTRACT

Objective: Monoterpenes are the primary components of plant essential oils which can use in medicines, food or cosmetic industries. Many terpenoids exhibit a various pharmacological activities such as antiallergic, antiviral, anti-inflammatory and anticancer. However no enough data are found about cytotoxicity of these compounds on human breast cancer (MCF-7) cells. The aim of this study was to evaluate the potential cytotoxic effects of monoterpenes Beta-Citronellol (BC) and (-)-Menthone (MNT) using 3-(4,5-dimethylthiazolyl-2)-2,5 diphenyl tetrazolium bromide (MTT) assay in MCF-7 cell line.

Methods: In this study, the cytotoxic effects of monoterpenes BC and MNT were analyzed by MTT assay at 24 and 48 hour treatment times in the MCF-7 cell line. MCF-7 cells were treated with different concentrations of BC (2, 10, 50, 250, 1250 µg/mL) and MNT (16, 80, 400, 2000, 10.000 µg/mL).

Results: BC and MNT were significantly decreased cell viability (%) at all concentrations at 24-h treatment period. Furthermore, BC (250, 1250 µg/mL) and MNT (16, 400, 2000, 10.000 µg/mL) were significantly reduced the cell viability at 48-h treatment.

Conclusion: As a result, BC and MNT showed cytotoxic effect on MCF-7 cells in both treatment times. Further studies should be conducted to determination cytotoxicity using different cell lines.

Key words: Monoterpene, Beta-Citronellol, (-)-Menthone, MCF-7, MTT assay

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1. INTRODUCTION

Essential oils are synthesized by aromatic plants as secondary metabolites (1, 2). The most compounds in the essential oils are monoterpenes (3). Monoterpenes are widely used in medicines, preservation, food flavoring and fragrances or cosmetic industries (4, 5). They exert a wide range of pharmacological activities such as antiallergic, antiviral, anti-inflammatory and anticancer (6, 7). Some researchers revealed that terpenes are important cancer chemopreventive and chemotherapeutic agents (8-10).

Beta-Citronellol is a monoterpene naturally found in the essential oil of various plant worldwide (11). This monoterpene is a widely used as a fragrant constituents in perfumes (12) and a flavoring agent in food and beverages (12, 13). It has an antihypertensive effects (11) and anti-inflammatory effects in rodent (14).

(-)-Menthone along with Menthol and other compounds are a cyclic monoterpene which is generally members of the Mentha genus (15). (-)-Menthone is synthesized in the 8-step pathway of menthol biosynthesis described in great detail by Croteau et al. (16). It is a major essential oil constituent of a very limited number of aromatic plants, known to shown various biological activity such as antimicrobial, antifungal, anticancer and anti-inflammatory effects (15). (-)-Menthone, isomenthone and other compounds impart the cooling minty taste and smell to plants (15, 17). Therefore they are major flavouring additives besides vanilla and citrus. Also it is used in a variety of consumer products ranging from oral-care products such as toothpaste to confections such as chocolate and chewing gum as well as in over-the counter medicinal products for its cooling and biological effects (15).

Many terpenes have cytotoxic effect and they can inhibit the survival of cancer cells (18-21). Breast cancer is one of the most common female malignant tumors which primary leads cancer deaths in worldwide (22, 23). However no data are found about the cytotoxicity of these compounds in human breast cancer (MCF-7) cell line. The aim of this study was to evaluate the cytotoxic effects of monoterpenes Beta-citronellol (BC), and (-)-Menthone (MNT) using 3-(4,5-dimethylthiazolyl-2)-2,5 diphenyl tetrazolium bromide (MTT) assay in MCF-7 cell line.

2. METHODS

Chemicals

The test substances Beta-citronellol (3,7-dimethyl-6-octen-1-ol, Cas No: C83201-5G, %95) and (-)-Menthone [(1R,4S)-p-Menthan-3-one, (2S,5R)-2-Isopropyl-5-methylcyclohexanone, Cas No: 218235-25G, %90] were obtained from Sigma. Dulbecco's Modified Eagle Medium with phenol red (DMEM, Cas. No: F0445), Dulbecco's Modified Eagle Medium without phenol red (DMEM, Cas. No: F0475), PBS (L1825), foetal bovine serum (FBS, Cas. No: S0613), penicillin/streptomycin (Cas. No: A2213), L-glutamine (Cas. No: K0283) and trypsin (Cas. No: L2163) were obtained from Biochrome. In vitro toxicology assay kit (MTT based, Tox-1), DMSO Hybrimax (Cas. No: D2650) were obtained from Sigma.

The molecular structure of Beta-citronellol and (-)-Menthone were respectively shown in Figure 1.

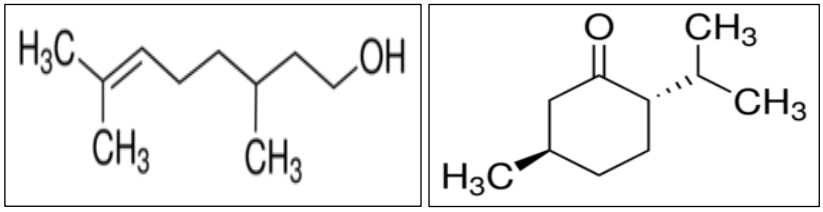

Figure 1. Molecular Structure of Monoterpenes a) Beta-citronellol (24) b) (-)-Menthone (25)

Cell Culture

Human breast cancer (MCF-7) cells were cultured at 37 °C and 5% CO2 in Dulbecco's Modified Eagle Serum (DMEM) medium supplemented with 10% fetal bovine serum (FBS), 1 (%) penicillin/streptomycin and 2 mM L-glutamine. The cells were seeded in T75 flaks to grown. After the cells were reached a sufficient number, the culture medium was transferred to 96 multiwell plates and incubated 24 h for the adhesion of cells.

MTT Cell Viability Assay

The 3-(4,5-dimethylthiazolyl-2)-2,5 diphenyltetrazolium bromide (MTT) assay was applied according to the methods of Mossman et al. (26) with some modifications Mamur et al. (27). Firstly, the cells were plated on 96-multiwell plates, including to per well 5x103 cells. And then MCF-7 cells were treated with different concentrations of BC (2, 10, 50, 250, 1250 µg/mL) and MNT (16, 80, 400, 2000, 10.000 µg/mL) for 24 and 48 hours. Afterwards the processes was finished, the cell viability (%) and the half of inhibitory (IC50) values were calculated.

Statistical Analysis

MTT cell viability assay was analyzed by One Way ANOVA, followed by Dunnet's multiple comparison test and P value less than 0.05 was considered as the statistically significance level.

3. RESULTS

Beta-citronellol (BC)

The cytotoxicity result of Beta-citronellol (BC) shows in Table 1. BC was significantly decreased the cell viability (%) at all concentrations at 24-h treatment period. Furthermore, BC was significantly reduced the cell viability at 250, 1250 µg/mL for 48-h treatment on human breast cancer (MCF-7) cell line. The half of inhibitory value (IC50) evidenced by BC was 1250 µg/mL for 24-h and at 250 µg/mL concentration for 48-h (Figure 2).

Table 1. Cytotoxic effect of Beta-citronellol on MCF-7 cell line

Test substance			24 hour	48 hour
	Concentrations (µg/mL)	N	Mean±SD	Mean±SD
Control	0.00	3	1.2690±0.1443	1.1570±0.2136
Beta-citronellol	2	3	0.8767±0.1460*	0.6950±0.0580
	10	3	0.8560±0.0812*	0.7877±0.2112

Table 1. Cytotoxic effect of Beta-citronellol on MCF-7 cell line (continued)

	50	3	0.6840±0.1298 *	0.7070±0.4171
	250	3	0.6720±0.0752 *	0.5443±0.0155 *
	1250	3	0.6420±0.1857 *	0.3297±0.1715 *

MCF-7: human breast cancer cell line, SD: standart deviation
* Significantly different from the control P< 0.05 (One way ANOVA-Dunnet Test)

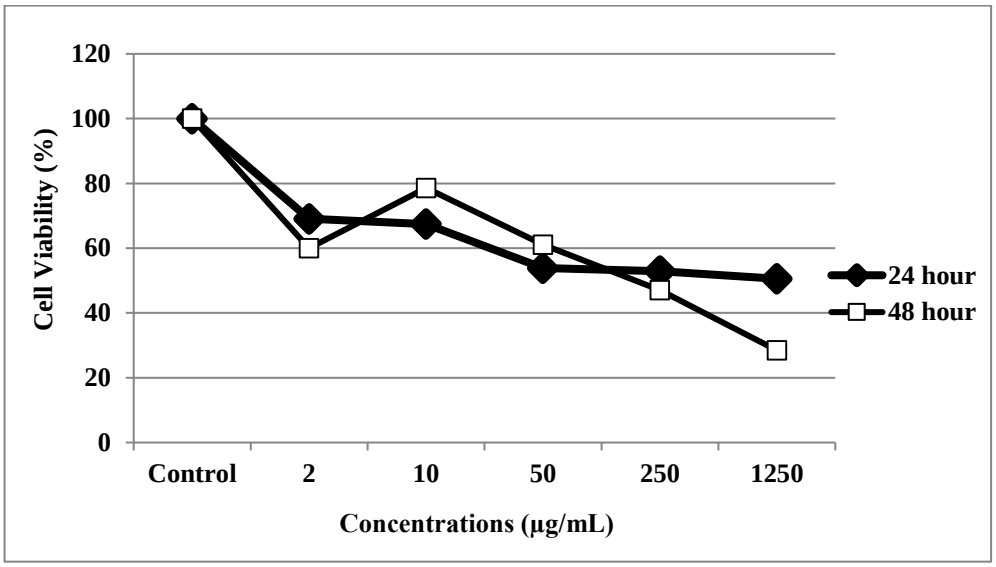

Figure 2. Cell viability (%) results of Beta-citronellol on MCF-7 cell line

(-)-Menthone (MNT)

Table 2 summarizes the results of cytotoxic effect of a treatment with (-)-Menthone (MNT) on MCF-7 cell line. MNT was significantly reduced the cell viability (%) at all concentrations at 24-h treatment period. In addition, MNT was significantly decreased the cell viability at 48-h treatment (16, 400, 2000, 10.000 µg/mL). Moreover, IC50 value for MNT was determined as 80 µg/mL concentration for 24-h and at 2000 µg/mL concentration for 48-h treatment (Figure 3).

Table 2. Cytotoxic effect of (-)-Menthone on MCF-7 cell line

Test substance			**24 hour**	**48 hour**
	Concentrations (µg/mL)	**N**	**Mean±SD**	**Mean±SD**
Control	0.00	3	1.4210±0.5214	1.037±0.030
(-)-Menthone	16	3	0.7957±0.0965 *	0.7723±0.1057 *
	80	3	0.7243±0.1668 *	0.8520±0.2119
	400	3	0.6010±0.0898 *	0.6640±0.0034 *
	2000	3	0.5483±0.0319 *	0.6270±0.1490 *
	10.000	3	0.2097±0.0560 *	0.7280±0.0511 *

MCF-7: human breast cancer cell line, SD: standart deviation
* Significantly different from the control P< 0.05 (One way ANOVA-Dunnet Test)

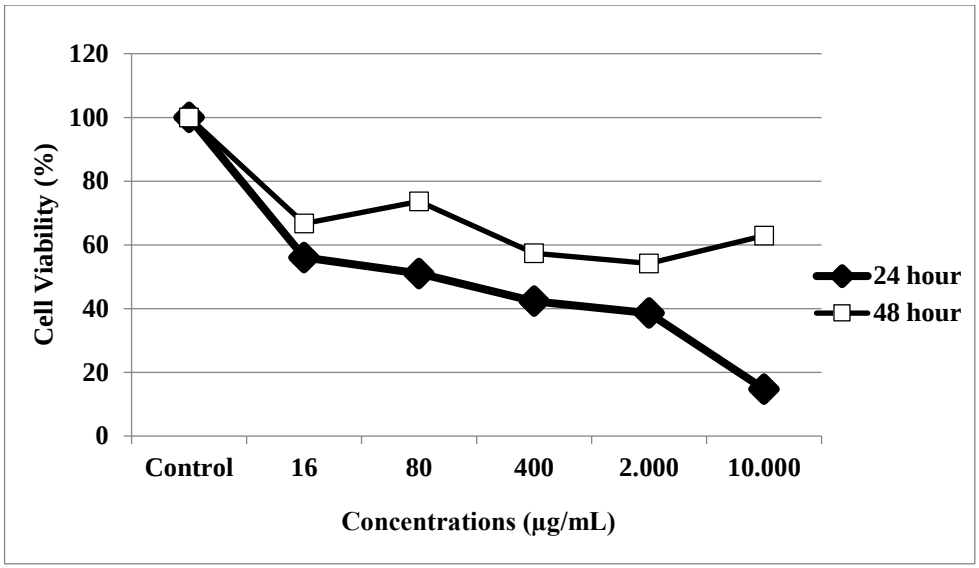

Figure 3. Cell viability (%) results of (-)-Menthone on MCF-7 cell line

4. DISCUSSION

Monoterpenes are an essential oils of many aromatic plants. They are provided active components exhibiting antibacterial, antiviral, antifungal, antioxidant and anticancer effects (3, 28). Recently, monoterpenes have become of clinical relevance due to their ability to suppress cancer development (21). Various studies have indicated that many terpenes possible use in primary prevention and therapy of many types of cancer (18, 20, 21, 29) and their ability to suppress cancer development, e.g., in the breast (30), lung (31, 32), pancreas (33), prostate (34), colon (19). Monoterpenes are widely used in cosmetic, pharmaceutical and medical applications, as well as in the food industry (4). For this reason, it is necessary to determine their safety of terpenes for human health.

Cytotoxicity is an necessary parameter for evaluating chemical substances for toxicity and health risks (35). MTT assay is mostly used colorimetric analysis and it used to detect cytotoxicity following an exposure to toxic substances measured mitochondrial activity (36). According to MTT protocol, the living cells to reduce MTT into a blue formazon product (37).

In this study results indicated that Beta-citronellol (BC) (250, 1250 µg/mL) and (-)-Menthone (MNT) (all treatment except at 80 µg/mL) were significantly decreased the cell viability (%) for 48-h treatment on human breast cancer (MCF-7) cells line. Moreover, both monoterpenes had cytotoxic effects in all concentrations for 24-h treatment period on MCF-7 cells. The half of inhibitory value (IC50) evidenced by BC was 1250 µg/mL for 24-h and at 250 µg/mL for 48-h. Furthermore, the IC50 value of MNT was determined as 80 µg/mL for 24-h and at 2000 µg/mL for 48-h. According to our results observed that BC is more cytotoxic effect than MNT on MCF-7 cells.

To the best of our knowledge, no studies have been conducted on cytotoxicity of Beta-citronellol and (-)-Menthone on MCF-7 cell line. However, the cytotoxic effects of these terpenes and their derivatives in several cell line have been previosly reported. Citronellol exhibited a weak cytotoxic effects against HL60 tumor cells (38). Some studies have demonstrated citronellal affect hepatocellular processes in rodents and humans (21). Maßberg et al. (21) investigated that physiological effect upon long-term stimulation with (-)-citronellal,

a proliferation assay was performed. (-)-Citronellal (500 μM) treated with Huh7 cells for 24 h, 48 h and 5 day. According to their results, stimulation over a period of 5 day resulted in a significant inhibition of cell proliferation (52%) compared to the control. (-)-Citronellal does not affect the cell viability of Huh7 cells with propidium iodide staining. Another monoterpenes is a (-)-Menthone, which is a derivatives from Menthol. Menthol was a cytotoxic effect on murine leukemia WEHI-3 cells in a concentration-dependent manner (39). Lin et al. (40) showed menthol induced cytotoxicity by inhibiting the expression of topoisomerases I, II alpha and II beta and promoting the expression of NF-κB in SNU-5 cells. In addition this terpene induced the antiproliferative activity of 1α,25-dihydroxyvitamin D3 in LNCaP cells (41). Wang et al. (42) found that menthol was inhibited the proliferation and motility of prostate cancer DU145 cells.

Researches with other terpenes have shown that many monoterpenes were determined to have cytotoxic effect in different cell line. Slamenova et al. (4) observed that eugenol had a cytotoxic effect in VH10 human fibroblasts, in Caco-2 human colonic cells, but not in HepG2 human hepatoma cells. Thymol exhibited cytotoxic effect against P815 mastocytoma cells (43), human colonic cells (Caco-2), human hepatoma cells (HepG2), and hamster lung cells (V79) (44). Similarly, Jaafari et al. (45) determined that carvacrol, thymol, carveol, carvone, eugenol and isopulegol were cytotoxic activity on murine mastocytoma (P-815), human chronic myelogenous leukemia (K-562), acute T lymphoblastoid leukemia (CEM), human breast adenocarcinoma (MCF-7) cells. Besides, authors indicated that the carvacrol is the most cytotoxic monoterpene. Jin et al. (46) indicated geraniol inhibited the proliferation in BXPC-3 human pancreatic cancer cell. In 2017, geraniol was performed a significant reduction in cell viability of human hepatoma (HepG2) cell line and in peripheral blood mononuclear cells (2).

The mechanism of cytotoxicity of monoterpenes is currenlty unknown. However, some studies reported that many cancer cells are characterized by severe changes in energy metabolism, mitochondrial overproduction and permanent oxidative stress (3, 47). Because of essential oils capacity to interfere with mitochondrial functions, may add prooxidant effects and thus become genuine anticancer agents (3).

5. CONCLUSION

In the present study, the cytotoxic potential of monoterpenes Beta-Citronellol and (-)-Menthone were evaluated using MTT assay in MCF-7 cell line. In literature, this is the first study to determine the cytotoxic effects of Beta-Citronellol and (-)-Menthone on MCF-7 cells. Based on our findings, both monoterpenes exhibit cytotoxic effects on MCF-7 cells. Therefore, further investigations are needed in order to assess the cytotoxicity on human and ensure its safety.

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