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Antioxidant Effects of Myrtus communis L.'s Essential Oils in BEAS-2B Cells Induced by Oxidative Stress with Hydrogen Peroxide

Hidrojen Peroksit ile Okdisatif Stresin İndüklendiği BEAS-2B Hücrelerinde Myrtus communis L. Esansiyel Yağının Antioksidan Etkilerinin Araştırılması

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ABSTRACT

Aim: In this study, the effects of *Myrtus communis* L. essential oil on the human bronchial epithelial cell line (BEAS-2B) exposed to oxidative stress with hydrogen peroxide were investigated and their effects on apoptotic pathways.

Materials and Methods: The MTT 3- (4,5-Dimethylthiazol-2-yl) -2,5-Diphenyltetrazolium Bromide method was used to determine the appropriate doses of hydrogen peroxide (H_2O_2) and *M. communis* L.'s essential oil in BEAS-2B cells. Oxidative stress formation was determined by measuring malondialdehyde (MDA) level. The cells were divided into three groups: the group exposed to oxidative stress (group with H_2O_2), the treatment group (H_2O_2 + *M. communis* L.'s essential oil) and the control group. MDA levels were measured in all three groups and expression levels of Caspase 3, Caspase 8, Caspase 9 and p21 genes were determined by RT-PCR method in order to detect apoptotic effects.

Results: According to MTT test results, the appropriate doses were 40 µM for H_2O_2 and 15.625 µg/ml for *M. communis* L.'s essential oil. MDA levels were significantly increased in the group treated with 40 µM H_2O_2 when compared with the healthy cell group (p=0.0005). In the group treated with essential oil of *M. communis* L., MDA level was found similar to the control group (p>0.05). Expression levels of Caspase 3, Caspase 8 and p21 genes were significantly increased in cells where H_2O_2 was adminisrated at 40 µM concentrations compared to healthy cell group (p=0.001, p=0.017 and p=0.0003, respectively). However, Caspase 9 gene expression level did not change significantly (p=0.8). Compared to the group in which the oxidative stress model was established, it was found that Caspase 3 gene expression level decreased significantly in the cells treated with *M. communis* L.'s oil (p=0.00007).

Conclusion: In our study, it was shown that the essential oil of *M. communis* L. strongly decreased MDA levels and also had the potential to be a therapeutic agent due to its apoptotic inhibiting effect. *M. communis* L. has a strong antioxidant effect and is thought to be effective in stopping apoptosis caused by oxidative stress.

Keywords: Myrtus communis L, Apoptosis, BEAS-2B, Oxidative Stress

ÖZ

Amaç: Bu çalışmada, Myrtus communis L. esansiyel yağının, in vitro olarak hidrojen peroksit ile oksidatif strese uğratılmış insan bronşiyal epitel hücre hattında (BEAS-2B) antioksidan etkilerinin olup olmadığı ve apoptotik yolaklar üzerindeki etkileri araştırılmıştır.

Materyal ve Metod: BEAS-2B hücrelerinde H_2O_2 ile *M. communis* L. esansiyel yağının uygun dozlarının belirlenmesinde MTT yöntemi kullanılmıştır. Oksidatif stres oluşumu MDA düzeyi ölçülerek belirlenmiştir. Hücreler; oksidatif strese maruz bırakılan grup (H_2O_2), tedavili grup (H_2O_2+*M. communis* L) ve kontrol grubu olmak üzere üç gruba ayrılmıştır. Her üç grupta MDA düzeyleri ölçülmüş ve apoptotik etkilerin saptanması amacıyla Kaspaz 3, Kaspaz 8, Kaspaz 9 ile p21 genlerinin ekspresyon düzeyleri RT-PCR yöntemi ile saptanmıştır.

Bulgular: MTT testi ile uygun dozlar H_2O_2 için 40 µM, *M. communis* L. yağı için 15.625 µg/ml olarak saptanmıştır. Sağlıklı hücre grubuyla karşılaştırıldığında, 40 µM H_2O_2 maruziyeti uygulanan grupta MDA düzeyinde anlamlı artış gözlenirken (p=0,0005), *M. communis* L. esansiyel yağı ile tedavi edilen gruptaki MDA düzeyi kontrol grubu ile benzer düzeyde (p>0.05) bulunmuştur. 40 µM H_2O_2 uygulanan hücrelerde Kaspaz 3, Kaspaz 8 ve p21 genlerinin ekspresyon seviyelerinin sağlıklı hücre grubuna göre anlamlı bir şekilde arttığı (sırasıyla p=0.001, p=0.017 ve p=0,0003), Kaspaz 9 gen ekspresyon seviyesinin değişmediği (p=0.8) saptanmıştır. *M. communis* L. yağı ile tedavi edilen hücrelerde, oksidatif stres modelinin oluşturulduğu gruba göre Kaspaz 3 gen ekspresyon seviyesinin anlamlı şekilde azaldığı saptanmıştır (p=0.00007).

Sonuç: Çalışmamızda, *M. communis* L. esansiyel yağının MDA seviyesini güçlü şekilde azaltıcı etkisi olduğu, ayrıca apopitozu durdurucu bir etki göstererek, teröpatik ajan olarak potansiyeli olduğu gösterildi. Bu bağlamda *M. communis* L. yağının güçlü bir antioksidan etkiye sahip olduğu, oksidatif stresten kaynaklanan apoptozisin durdurulmasında etkili olduğu düşünülmektedir.

Anahtar Kelimeler: Myrtus communis L, Apopitozis, BEAS-2B, Oksidatif Stres

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INTRODUCTION

Myrtle (*Myrtus communis* L) is an evergreen plant of the Myrtaceae family. It grows naturally in the Mediterranean region and has been used as food and spices since ancient times. In addition, medical use is also common. Its leaves and fruits have traditionally been used as antiseptic, disinfectant and hypoglycemic agents [1]. Murten, sturgeon, and especially in Hatay "hambeles" also known as *M. communis* L., especially Turkey, Greece, Italy, Algeria, Tunisia and Morocco are grown widely in Mediterranean countries [2]. In folk medicine, the fruit of the plant is used in the treatment of various infectious diseases, including diarrhea and dysentery. The leaves of the plant are used as antiseptic and anti-inflammatory agent in wound healing and treatment of candidiasis [3]. Oil composition of the plant varies according to geographical location. According to numerous published scientific studies, essential oil has a strong antimicrobial activity and is widely used in the cosmetics, pharmaceutical and food industries [4,5].

Oxidative stress, defined as the imbalance between the levels of various oxidant molecules and antioxidants, can often lead to biochemical changes and thus serious diseases in many organisms [6]. Oxidative stress can cause cytotoxic and genotoxic effects, while damaging basic biomolecules such as lipids, proteins and DNA [7]. Nowadays, the damages of mutagenicity and reactive oxygen species have been shown in many diseases such as aging, atherosclerosis, cancer, diabetes and various neurodegenerative disorders. It is clear that it takes place. Over the past two decades, natural antioxidants have been highly emphasized [8,9]. Many essential oils and their components have recently been described as natural antioxidants, and have been proposed as a potential alternative to synthetic antioxidants.

In this study, it was investigated whether M.communis L.'s essential oil suppresses induced apoptosis due to its antioxidant effects and oxidative damage in human bronchial epithelial cells (BEAS-2B) exposed to oxidative stress with hydrogen peroxide.

MATERIAL and METHODS

Cell Culture

In the study, the BEAS-2B cells, a human bronchial epithelial cell line derived from healthy bronchial epithelial tissue, were used. The cell line was obtained from the cell culture laboratory of Mustafa Kemal University Medical Biology Department. The BEAS-2B cell line is a Human Bronchial Epithelial Cell line widely used in in-vitro studies. The BEAS-2B cell line was cultured in Dulbeco medium containing 10% Fetal Bovine Serum (FBS; Gibco, USA) and 1% penicillin/streptomycin (Gibco, USA) in 175 cm^2 culture flasks. Incubation of the cells was performed in a 95% humidified atmosphere and 5% CO_2 at 37 ° C. The medium was changed twice a week to maintain the cultures. Cell culture studies were performed when the cells covered 70-80% of the culture flasks.

Preparation of *M. communis* L.'s Essential Oil Doses

The essential oil we used in the present study was obtained from the Faculty of Agriculture at Hatay Mustafa Kemal University. Doses of 1000-500-250-125-62.5-31.25-15.625 µg/ml *M. communis* L. were prepared by serial dilution (dissolving the highest dose in 1% DMSO) in serum-free DMEM medium. The MTT test was performed to determine the appropriate dose.

Preparation of H_2O_2 Doses

In order to determine the dose that can be applied, the commercially available stock H_2O_2 was diluted in serum-free DMEM medium. MTT test was performed for doses diluted 0, 10, 20, 30, 40, 50, 75 and 100 µM.

Cell Viability

Cell viability was analyzed by MTT test. For the MTT assay, DMEM medium containing 10% FBS and 1% penicillin/streptomycin in 48-well plates was inoculated to $1x10^5$ cells/ml. Incubation of the cells was performed in a 95% humidified atmosphere and 5% CO_2 incubator at 37 °C. The cells were expected to coat the culture vessel surface at 70-80%. Serum-free medium was then added to the cells and incubated under the same

conditions for 24 hours. Two different MTT tests were performed on the prepared cells for different concentrations of H_2O_2 and essential oil of *M. communis* L.

To determine the non-cytotoxic concentrations of *M. communis* L.'s oil, the cells were exposed to essential oil of *M. communis* L. at concentrations of 1000-500-250-125-62.5-31.25-15.625 µg/ml for 24 hours. Similarly, to determine non-cytotoxic concentrations of H_2O_2, the cells were exposed to doses of H_2O_2 of 0, 10, 20, 30, 40, 50, 75, 100 µM for 24 hours. For both MTT experiments: MTT solution was prepared to be 1 mg/ml. The solution in the 48-well plates was removed, and 250 µl MTT solution was added to each well. The plate was then incubated for 1 hour at 37 °C. At the end of the incubation period, the MTT solution in the plate was discarded and inverted on a napkin for 2-3 min. Then, 250 µl of DMSO was added to each well and the plate was incubated at room temperature for 5 min. The color change was evaluated with a spectrophotometer at 590-670 nm wavelength.

Oxidative Stress

Cultivation of BEAS-2B cells was performed in 175 cm^2 cell culture flasks. The cell density was adjusted to $1x10^5$ cells/ml. DMEM (Dulbecco's Modified Eagle Medium, Gibco, UK) containing 10% FBS (Gibco, USA) and 1% antibiotic suspension (penicillin/streptomycin; Gibco, USA) was used as cell maintenance medium. The incubation of the cells was continued until the cells covered 70-80% of the surface of the culture flasks. At the end of incubation, serum-containing medium in cell culture flasks was discarded, and serum and pyruvate-free medium was placed as culture medium. The cells were treated with 0.40 µM H_2O_2 and *M. communis* L.'s essential oil (15.625 µg/ml) + 40 H_2O_2. And evaluation was performed after 24 hours incubation. The cells were harvested from the culture vessel surface and collected in a separate tube in cold HBSS. The collected samples were stored at -80 °C until the study was performed. For the tests, the cells were thawed at -80 ° C, and then with a homogenizer medium for 30 min. homogenized, and MDA and protein measurements were performed. MDA levels were determined by the method described by Sushil et al. previously [10].

MDA, the final product of lipid peroxidation, forms a pink complex when incubated with thiobarbituric acid at 95 °C and pH 3.5 under aerobic conditions. MDA amount was determined by spectrophotometric measurement of this complex at 532 nm wavelength. By measuring the amount of protein in each sample, the MDA was calculated as nmol/mg protein.

Gene Expression

The transcription levels of Caspase 3, Caspase 8, Caspase 9, p21 genes and β-actin gene as reference gene were determined by real-time PCR method. The primer sequences of these genes are given in Table 1.

Statistical Analysis

MTT analysis results of the study were calculated by applying GraphPad Prism Version 5.01 (GraphPad Software Inc., USA) program. The suitability of the data in the groups for normal distribution was determined by Shapiro-Wilk test. Kruskal-Wallis test was used to determine whether there was a difference between the groups. Dunn's Multiple Comparison Test was used to determine the significance between the groups. Each study group was compared with the control group and values $p < 0.05$ were considered statistically significant. Results are given as mean (mean) ± standard deviation. Analysis of gene expression data was performed with RT2 profiler PCR Array Data Analysis version 3.5. β-actin was used as the "housekeeping gene". The results were given as Fold change.

RESULTS

Assessment of Cell Viability

Different concentrations of H_2O_2 (0, 10, 20, 30, 40, 50 and 75 and 100 µM) were used to determine the appropriate doses to generate an oxidative stress model in BEAS-2B cells. The cells were exposed to H_2O_2 for 24 hours, and then cell viability analysis was performed by the MTT method. The effect of H_2O_2 was compared to the control group (group not containing H_2O_2) after 24 hours incubation in BEAS-2B cells. In the experiments, no significant difference was observed in the viability of cells

exposed to H_2O_2 doses of 30 µM and lower, whereas 40, 50, 75 and 100 µM doses of H_2O_2 significantly reduced cell viability (Figure 1.A). Cytotoxic effect was detected at the strongest level at a dose of 100 µM ($p<0.0001$) (Table 2).

Table 1. Primer sequences of genes whose expression levels were determined.

Gene	Forward Primer	Reverse Primer
Caspase 3	5'-CTTCTACAAC-GATCCCCTCTG-3'	5'-TGTGCTTCTGAG-CCATGGGTG-3'
Caspase 8	5'-GGGCTCAATTTCT-GCCTAC-3'	5'-GGCAC TGGCT-GTTTGCTT-3'
Caspase 9	5'-GTCACAAGACCTT-GACACCCG-3'	5'-ACCAGGTG-GTCTAGGGGTTT-3'
p21	5'-CCGAAGT-CAGTTCCTTGTGG-3'	5'-AGTACGGCCA-GAGGTGTACG-3'
β-aktin	5'-TCAACACCCCAGC-CATGTA-3'	5'-AGTACGGCCA-GAGGTGTACG-3'

Table 2. The mean optical density ± standard deviation values of the applied hydrogen peroxide doses.

H_2O_2 µM	Mean ± Standard Deviation	p value
100 µM	0.04073±0.005347***	< 0,0001
75 µM	0.07079±0.01433***	< 0,0001
50 µM	0.1919±0.04240***	< 0,0001
40 µM	0.2323±0.04196*	0,0202
30 µM	0.2639±0.04529	0.4769
20 µM	0.3031±0.04686	0,9996
10 µM	0.3170±0.04720	0,8715
Control (0)	0.2966±0.06281	-

Statistical Significance: *p<0.05, **p<0.01 ve ***p<0.001.

In BEAS-2B cells, different concentrations of essential oils were studied (15,625-31,25-62,5-125-250-500-100 µg/ml) to determine the appropriate doses of *M. communis* L. for essential oil.

After the exposure of the essential oil of *M. communis* L. to the cells for 24 hours, the cell viability analysis was performed by MTT method. At the end of the 24-hour incubation, no significant difference was detected between the control group and the cell viability of the essential oil concentrations of 62.5 µg/ml and lower (Figure 1.B.) ($p> 0.05$). However, at doses of 125-250-500-1000 µg/ml of essential oil, it was found to be significantly lower in cell viability compared to the control group (Table 3).

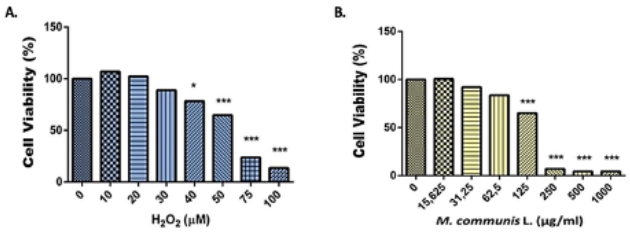

Figure 1. A. Cell viability graph of 24 hours H_2O_2 exposure at different doses in BEAS-2B cell line,
B. Cell viability graph of 24 hours M. communis L.'s essential oil exposure at different doses in BEAS-2B cell line (Statistical Significance: *p<0.05, **p<0.01 ve ***p<0.001).

In the following experiments, the lowest dose of H_2O_2 (40 µM) in which cell viability was suppressed and the lowest dose of essential oil of *M. communis* L. (15,625 µg/ml) in which the therapeutic effect was to be investigated were selected in order to detect oxidative stress stimulation. In all subsequent steps, the cells divided into 3 groups:

i) Control group (healthy cell group in which no substance was added)

ii) Oxidative stress-induced group (40 µM H_2O_2 added)

iii) Treatment group with *M. communis* L.'s oil (40 µM H_2O_2 + 15,625µg/ml essential oil of *M. communis* L. added).

Table 3. The mean optical density ± standard deviation values of applied *M. communis* L.'s essential oil doses.

M. communis L. µg/ml	Mean ± Standard Deviation	p-value
1000	0.01956±0.002562***	< 0,0001
500	0.01914±0.0008331***	< 0,0001
250	0.03076±0.006123***	< 0,0001
125	0.2848±0.1351***	0,0004
62.5	0.3670±0.08920	0,2151
31.25	0.4034±0.06874	0,8578
15.625	0.4416±0.06340	0,9999
Control (0)	0.4386±0.06989	-

Statistical Significance: *p<0.05, **p<0.01 ve ***p<0.001.

MDA Levels

BEAS-2B cells containing 40 µM H_2O_2 and 40 µM H_2O_2 + 15,625 µg/ml *M. communis* L.'s essential oil were incubated for 24 hours and MDA levels in the cells were evaluated. When the control group

(non-H_2O_2) was compared with the 40 µM H_2O_2 group there was a significant increase in MDA level at 40 µM H_2O_2 dose (p=0.0003), whereas there was no significant difference in MDA level between the control and the 40µM H_2O_2+15,625 µg/ml *M. communis* L. dose (p=0,2717) (Figure 2) (Table 4). The oxidative stress group treated with 40 µM H_2O_2 and the treatment group containing essential oil of *M. communis* L. were compared in terms of MDA levels. A significant decrease in MDA levels was detected in the treatment group (p = 0.0009), (Table 4).

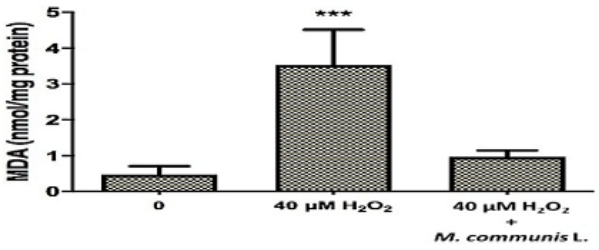

Figure 2. Comparison of MDA in the group of oxidative stress (40µM H_2O_2) and the group with *M. communis* L. (40µM H_2O_2+15,625 µg/ml *M. communis* L) with the control (Statistical Significance: *p<0.05, **p<0.01 ve ***p<0.001).

Gene Expression

In the 24th hour of incubation in BEAS-2B cells, the control group and the cell group containing 40 µM H_2O_2 were compared in terms of gene expression levels. In the group exposed to oxidative stress, expression levels of the three genes were found to be significantly increased [Caspase 3; 2.6 fold (p = 0.001), Caspase 8; 2.19 fold (p = 0.017), p21; 10.9 fold (p = 0.0003)], while no significant change was found in the expression level of the Caspase 9 (p = 0.86).

Table 4. MDA levels in BEAS-2B cells according to the groups

Groups	Mean ± Standard Deviation	p-value (0µM compared)	p-value (40µM compared)
0 µM (Control)	0.4638±0.2433	-	0,005
40µM H_2O_2	3.530±0.9798	0,0005	-
40µM H_2O_2 + 15,625µg/ml *M. communis* L.	0.9675±0.1825	0,4270	0,0009

Statistical Significance: *p<0.05, **p<0.01 ve ***p<0.001

In the cell group with essential oil of *M. communis* L., the expression level of the Caspase 3 gene was not statistically different to control group (p=0.3). However, there was a significant decrease in this group compared to the group exposed to oxidative stress (p=0.00071). The expression level of the Caspase 8 gene decreased slightly in the group treated with the essential oil of *M. communis* L. compared to the group exposed to oxidative stress (2.07 fold). However, this decrease was not statistically significant (p = 0.93). The expression level of p21 gene was increased by 12.71 fold in the group treated with essential oil of *M. communis* L. (p=0.0003) compared to the control group, and the expression levels in the group in which oxidative stress was induced were found to be similar (p=0.45). No statistically significant difference was found in the expression levels of the Caspase 9 gene in all three groups (Figure 3).

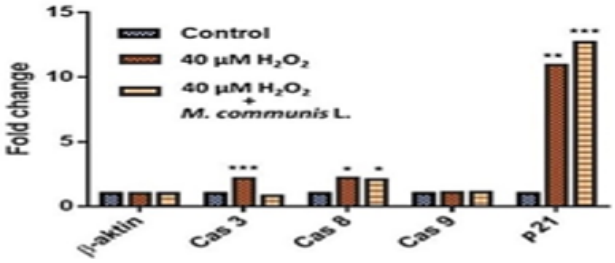

Figure 3. Expression levels of Caspase 3, Caspase 8, Caspase 9 and p21 genes in different groups (Statistical Significance: *p<0.05, **p<0.01 ve ***p<0.001)

DISCUSSION

In our study, oxidative stress was induced by exposure to hydrogen peroxide on BEAS-2B bronchial epithelial cells originating from human lung tissue, and the effects of *M. communis* L.'s essential oil on apoptotic pathway and malondialdehyde levels were investigated experimentally. The MTT method was performed to determine the non-cytotoxic concentrations of *M. communis* L.'s essential oil. Subsequently, oxidative stress status and Caspase 3, 8, 9, and p21 gene expressions involved in the apoptotic pathway were evaluated together. This study is valuable in that it demonstrates the beneficial effects of *M. communis* L.'s essential oil on lung cells at the cell level, both on oxidative damage and on apoptosis. As a result of this study, it is an important study that can contribute to the effectiveness of *M. communis* L.'s essential oil in medical applications.

Today, *M. communis* L.'s essential oil, which is rich

in bioactive components, is used in the treatment of various diseases among the population [11]. Kumar et al. reported that different extracts and compounds obtained from the leaves of *M. communis* L. and other parts of the plant have antioxidant activity [12]. To date, although in-vitro studies have been conducted using different forms of *M. communis* L.'s essential oil, studies in this area are very limited [13]. Recently, because of the carcinogenic effects of synthetic antioxidants, their use for human applications has been limited, so the interest of modern medicine in natural antioxidants is increasing [14].

M. communis L.'s essential oil is among the important plants for use in medical applications, as it is a natural source of antioxidants due to the activity of secondary metabolites such as phenylpropanoids and essential oils [15]. Gardeli et al. reported that the antioxidant activities of phenolic compounds are due to redox properties that enable them to act as reducing agents, hydrogen donors, and single oxygen reductants [5]. In our study, unlike these studies, the effects of *M. communis* L.'s essential oil on living cells were investigated not only with oxidative stress but also with apoptotic gene expression.

MTT test was performed to determine the non-cytotoxic doses of essential oil of *M. communis* L. The cells were incubated with different concentrations of the essential oil of *M. communis* L. (1000-500-250-125-62.5-31.25-15.625 µg/ml) for 24 hours. The MTT test showed that the dose of 15,625 µg/ml *M. communis* L.'s essential oil was not cytotoxic dose.

In studies, the scavenging ability of the hydroxyl radical is accepted as a common way to evaluate the potential of antioxidants. It is also believed that the formation of the hydroxyl radical can be achieved by clearing the free metal ions by chelating or converting H_2O_2 to other harmless compounds [16]. In our study, to demonstrate this effect, BEAS-2B cells were treated with different concentrations of H_2O_2 (0, 10, 20, 30, 40, 50 and 75, and 100 µM) cells for 24 hours to determine the appropriate dose and exposure time to form an oxidative stress model. At the end of incubation, cell viability analysis was performed by MTT method (Figure 1.A, Table 2). At the end of 24 hour incubation of H_2O_2 in BEAS-2B cells, no significant difference was observed at a dose of 30 µM compared with the control group. However, it was found that concentrations of 40, 50 and 75, 100 µM of H_2O_2 significantly reduced cell viability. This cytotoxic effect was detected at the strongest level at a dose of 100 µM. The first dose (40 µM dose of H_2O_2) in which cell viability was suppressed to create oxidative stress was chosen as the application dose.

Oxidative stress, which is defined as the imbalance between the levels of various oxidant molecules and antioxidants, causes biochemical changes in many organisms and thus serious diseases [17]. In particular, Park et al. [18] and Barrera et al. [19] reported that oxidative stress caused cytotoxic and genotoxic effects, causing damage to basic biomolecules such as lipids, proteins and DNA, and mediating irreversible damage at the cell level. Consequently, DNA damage resulting from the attack of reactive oxygen species is considered to be the main cause of mutagenesis and carcinogenesis [20]. Romani et al. [21] evaluated the effect of *M. communis* L. extracts on antioxidant activity in their study. In a different study, Ines et al. Reported that some compounds isolated from *M. Communis* L.'s leaf had antioxidant activity [22]. Antioxidant activity was determined in the K562 cell line with its ability to inhibit lipid peroxidation induced by hydrogen peroxide.

TBA test was used to measure MDA, which formed after lipid hydroperoxide decomposition, forming a pink chromophore with thiobarbituric acid. Turhan et al. observed lipid oxidation by determining the peroxide value, thiobarbituric acid reactive substance and oxidative compliance score [23]. In another study conducted in 2015, myrtle and rosemary extracts were shown to be very effective in slowing lipid oxidation, and in this study *M. communis* L. was shown to decrease the MDA value [24]. In our study, similar to these studies, the MDA level was measured to evaluate the effect of *M. communis* L.'s oil on living cells. BEAS-2B cells were incubated with 40 µM H_2O_2 and 40 µM H_2O_2+15.625 µg/ml *M. communis* L.'s essential oil for 24 hours and MDA levels in the cell were measured. Compared to the control group, a significant increase in MDA level was

observed at 40 μM H_2O_2 dose. However, in the group containing 40 mM H_2O_2+15.625 μg/ml *M. communis* L.'s essential oil, MDA levels were similar to the control group. (Figure 2, Table 4). *M. communis* L.'s oil has been able to reduce the oxidative stress caused by H_2O_2 in the cells, indicated by the increase in MDA, to the levels detected in the control cells.

In the study of Bajpai et al., *M. communis* extracts were reported to be a good scavenger of reactive oxygen species [25]. Sahreen et al. showed that hydrogen peroxide reacted with the main cell components, involved in lipid peroxidation, and also caused DNA damage [26]. In a study by Miguel et al., the capacity of H_2O_2 to inhibit the oxidative effect was reported to be directly proportional to the concentration of *M. communis* extracts. This observed H_2O_2 scavenging activity can be attributed to the presence of phenolic compounds that can readily transfer electrons to hydroxyl radicals [27]. Kumar et al. showed that myrtle extracts inhibited lipid peroxidation and thus decreased MDA levels [12].

In our study, the dose selected for H_2O_2 was chosen as the first dose (40 μM) to suppress cell viability in order to induce oxidative stress. In fact, the final non-cytotoxic dose, 30 μM H_2O_2, did not produce a significant increase in MDA levels in cells. However, H_2O_2 at a concentration of 40 μM caused an increase in MDA levels in the cells, and MTT test revealed the parallelism between MDA. In the cell group in which *M. communis* L.'s essential oil was used, MDA values were found to be similar to those of the control group. This shows the antioxidant activity of *M. communis* L.'s essential oil. These results are consistent with the studies of Kumar et al. [12] and Gonçalves [28].

In our study, it was found that the expression of Caspase 3, 8 and p21 genes were significantly increased in BEAS-2B cells at the 24th hour when the control group and the cell group exposed to oxidative stress (40μM H_2O_2) were compared (Figure 3). An increase in p21 expression, a Cdk inhibitor, suggested that the cell stopped the cell cycle in order to decide on repair or apoptosis. There was no significant change in the expression level of the Caspase 9 gene. This shows us that 40 μM H_2O_2 triggers apoptosis in the cell via the extrinsic pathway. Thus, we concluded that this dose, which we found high levels of MDA in the cell, caused apoptosis due to irreparable oxidative damage in the cells.

When the *M. communis* L. group (40μM H_2O_2+15,625μg/ml *M. communis* L.) was compared to the group exposing to oxidative stress (40μM H_2O_2), Caspase 3 expression was reduced in *M. communis* L. treated cells. Although we observed a small decrease in caspase 8 gene expression, this decrease was not statistically significant. However, Caspase 3 gene, which is responsible for the DNA fragmentation stage which is the last step of caspase cascade, decreased below the control levels in the last stage of apoptosis. It showed that *M. communis* L. was highly effective in preventing apoptosis due to oxidative damage.

Fernald and Kurokawa stated that besides targeting apoptosis stimulation, developing a therapeutic strategy to simultaneously reduce oxidative stress in the environment would be a very important approach [29]. The study of Tretiakova et al. in cancer cells is important in elucidating the activation of apoptosis via intrinsic and extrinsic pathways via *M. communis* L.'s essential oil [30]. It has been shown that *M. communis* L. in particular activates apoptosis by caspase 3, 8 and 9 in cancer cells. In our study, unlike these studies, the cell line studied was not a cancer cell line. In cancer therapies, directing apoptosis-resistant cancer cells to apoptosis is an important treatment strategy. In healthy cells, regression of apoptosis due to oxidative damage is extremely important in terms of cell survival.

Limitations: Our study has some budgetary limitations, such as the fact that apoptotic cells could not be demonstrated by more advanced methods such as TUNEL, and gene expression products could not be demonstrated at the protein level. We believe that by using these methods, the antioxidant activity of *M. communis* L.'s essential oil can be better understood by showing apoptotic cells and gene product proteins.

Conclusion: We believe that the essential oil of *M. communis* L. will provide medical benefit because it reduces oxidative stress induced by hydrogen peroxide and simultaneously suppresses apoptosis from oxidative stress. In our study, it

was shown that the essential oil of *M. communis* L. has a strong reducing effect on oxidative stress at a dose of 15.625 µg/ml based on MDA levels. Furthermore, the inhibitory activity on the mechanism of apoptosis induced by oxidative stress at the same dose was determined. As a result, *M. communis* L.'s oil showed a medically strong therapeutic effect both by reducing oxidative stress and by inhibiting oxidative stress induced apoptosis.

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