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Research Article/Özgün Araştırma

Evaluation of the effect of a natural monoterpenic phenol on the cytotoxicity of carfilzomib

Doğal bir monoterpenik fenolün carfilzomibin sitotoksisitesine etkisinin değerlendirilmesi

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Abstract

Aim: The aim of this study was to reveal whether carfilzomib, proteasome inhibitor, and carvacrol, a natural monoterpenic phenol, causes cytotoxic and apoptotic effects and oxidative stress on A-549 cells.

Materials and Methods: Lactate dehydrogenase (LDH) activity test was used. Changes in caspase 3 and glutathione peroxidase enzyme activities in cells were determined.

Results: It was determined that carfilzomib alone and together with carvacrol caused a raise in the activities of lactate dehydrogenase (LDH), glutathione peroxidase and apoptotic enzyme, caspase-3 activity, compared to the control.

Conclusion: Our study showing that carfilzomib alone and together with carvacrol gave different responses may be guiding in determining new strategies to be applied in lung cancer treatment.

Keywords: Lung cancer; Carvacrol; Carfilzomib; Apoptotic effect.

Öz

Amaç: Bu çalışmanın amacı, bir proteazom inhibitörü olan carfilzomib ile doğal bir monoterpenik fenol olan karvakrolün A-549 hücrelerinde sitotoksik ve apoptotik etkilere ve oksidatif strese neden olup olmadığını ortaya koymaktır.

Gereç ve Yöntem: Laktat dehidrogenaz (LDH) aktivite testi kullanılmıştır. Hücrelerdeki kaspaz 3 ve glutatyon peroksidaz enzim aktivitelerindeki değişiklikler belirlenmiştir.

Bulgular: Carfilzomib'in tek başına ve karvakrol ile birlikte laktat dehidrogenaz (LDH), glutatyon peroksidaz ve apoptotik enzim olan kaspaz-3 aktivitelerinde kontrole göre artışlara neden oldukları belirlenmiştir.

Sonuç: Carfilzomib'in tek başına ve karvakrol ile birlikte farklı yanıtlar verdiğini gösteren çalışmamız, akciğer kanseri tedavisinde uygulanacak yeni stratejilerin belirlenmesinde yol gösterici olabilir.

Anahtar Kelimeler: Akciğer kanseri; Karvakrol; Carfilzomib; Apoptotik etki.

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Introduction

While lung cancer was a rare disease in the early twentieth century, it has become one of the most important health problems today and is one of the leading causes of cancer-related deaths worldwide.[1,2] Lung cancer, one of the most important health problems today, is the most common type of cancer that causes death in both women and men.[1-3] More than one million people die every year in the world due to lung cancer. It has been determined that the number of lung cancer cases has increased by 44% in men and 76% in women since 1985 worldwide.[3] While deaths from other cancer types have decreased over the years, 3-fold increase has been detected in lung cancer-related deaths.[4] The prognosis of lung cancer is worse than other types of cancer, with a five-year survival rate of less than 15%.[5]

Surgical methods, radiotherapy, chemotherapy, hormone therapy and new treatment methods such as immunotherapy, gene therapy, angiogenesis inhibitors and signal transduction system inhibitors are used to reduce the death rate and increase survival in cancer treatment.[6,7]

The proteasomal system is involved in many cellular processes, and changes in the regulation of these cellular events are directly related to cancer development.[8] Studies on the use of proteasome inhibitors in cancer treatment have been continuing for more than 20 years. Carfilzomib is one of the most studied and clinically used proteasome inhibitors *in vitro* and *in vivo*. However, these proteasome inhibitors, which are used very effectively in various cancers, have side effects such as diarrhea, constipation, thrombocytopenia and most importantly, peripheral type neuropathy.[9] These side effects can limit treatment and even lead to discontinuation of treatment. As a result, dose limitation, treatment plan changes or chemotherapy must be abandoned.[10,11]

Carfilzomib, which we used in our study, is a second generation proteasome inhibitor, causes irreversible proteasome inhibition and has an epoxyketone structure. Carfilzomib is structurally different from Bortezomib but similarly but more specifically inhibits the β5 subunit of the proteasome and does not affect other proteases.[12] In studies conducted with Bortezomib and Carfilzomib in cell culture, it was determined that Carfilzomib had a more cytotoxic effect than Bortezomib. Hematological cancer cells are more sensitive to Carfilzomib exposure than solid tumors and non-transformed cells. Proteasome inhibition with Carfilzomib is longer lasting than Bortezomib due to Carfilzomib covalently binding to the target protein.[11,13]

Essential oils obtained from various *Origanum* species have been widely used to flavor foods, alcoholic beverages, and wounds and burns since ancient times.[14-16] Essential oils of *Origanum* genus, the main component of which is carvacrol, have been scientifically demonstrated to have many specific biological effects such as antioxidant, analgesic, antifungal, antibacterial, insecticidal, antimelanogenic, anti-inflammatory and wound healing effects.[17-29] Carvacrol, a monoterpenic phenolic essential oil component, is known to have antibacterial, antioxidant, analgesic, antifungal, insecticidal, phytotoxic, antiviral, antiparasital and anti-inflammatory effects in different organisms, parallel to the effects of the essential oil.[17,22,30-40]

There are studies on the anti-cancer effect of carvacrol in some types of cancer in *in vivo* and *in vitro* conditions. The growth and tumorigenesis of chronic myeloid leukemia cells, N-ras transformed mouse myoblast cells, murine melanoma cells, and human cervical, lung, and breast cancer cells are known to be inhibited by carvacrol.[41-49] Although there is not much information about the mechanism of action of carvacrol, studies have shown that carvacrol specifically changes the cytoplasmic membrane surface and permeability and thus affects cells.[38,50] Although many studies have been carried out at the molecular level on the effect of carvacrol, especially on cancer cells, there is still not enough information today, so it is very important to investigate its anti-cancer effect mechanism.

Carfilzomib, known to be a second-generation proteasome inhibitor, may cause many side effects when used alone in treatment. New strategies in cancer treatment

may emerge as a result of the combined application of targeted cancer drugs such as carfilzomib and natural compounds such as carvacrol. By increasing the effectiveness of targeted drugs at low doses, their side effects can be reduced and the desired success in treatment can be achieved.

The results of this study revealed the cytotoxic, membrane-damaging, oxidative stress-inducing and apoptotic effects of carfilzomib, a targeted drug, alone and in combination with carvacrol, on lung cancer cells (A-549), which is the most common type of cancer that causes death.

Materials and Methods

Chemicals and drugs

Carfilzomib was supplied under the trade name as Kyprolis. The drug used in the experiments was diluted in appropriate proportions using the medium. Carvacrol is commercially available with 99% purity (Sigma Chemical Co.). The kit used for caspase activity was obtained from Elabscience Biotechnology Co., Ltd, USA. The kit used to determine Lactate Dehydrogenase Activity was purchased commercially from Sigma-Aldrich (St. Louis, MO, USA).

Cell Lines and culture

The cell line we used in the experiments, A-549 (human non-small cell lung cancer (NSCLC)), was purchased from the American Type Culture Collection (ATCC) and reproduced under suitable conditions. Cells were grown using Roswell Park Memorial Institute 1640 medium (RPMI 1640) and other medium components in appropriate proportions. After the cells reach sufficient density (more than 75%) in the culture vessel, experimental groups were created and then proteasome inhibitör (carfilzomib) ($<IC_{50}$), and natural monoterpenic phenol (carvacrol) ($<IC_{50}$), were applied to the cells for 48 h.

Cytotoxicity assay

After the cells grown in the flask were trypsinized, they were counted and planted in 96-well plates at 10^4 cells per well. The cytotoxicity of carfilzomib (250-2000 µg/mL) and carvacrol (20-70 µg/mL) on A-549 cells was determined for 48 h. Moreover, cells were treated with carfilzomib ($<IC_{50}$) and carvacrol ($<IC_{50}$) together for 48 h. 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide (MTT) test was used to evaluate the cytotoxic effect after applications. In this test, tetrazolium salts such as MTT are metabolized by mitochondrial dehydrogenases to form a blue formazan dye that is used to measure cytotoxicity. Test reagents were added to the culture medium and incubated at 37 °C for 2 h. Then, solubilizing/stopping solutions (dimethyl sulfoxide) was added to each well for a 1 h incubation. The absorbance of all samples was measured at 490 nm.[51] Eight wells were replicated for each concentration.

Lower IC_{50} concentrations ($<IC_{50}$ values) were calculated separately for carfilzomib and carvacrol. Subsequent combination applications were continued using the calculated IC values ($<IC_{50}$).

According to the cytotoxicity results, the most effective combination concentration was determined after carfilzomib ($<IC_{50}$) and carvacrol ($<IC_{50}$) were applied together. The most effective combination concentrations were also used in other ongoing experiments. The combination index (CI) was calculated to determine whether the combined administration of carfilzomib and carvacrol in cells exhibited additive, synergistic or antagonistic effects.[52] Cells treated with only medium or 0.1% DMSO were considered as control cells.

Lactate dehydrogenase (LDH) assay

LDH activity were determined after the cells were exposed to carfilzomib alone (IC_{10}) and combination of carfilzomib (IC_{10}) and carvacrol concentrations (IC_{10}), showing the most effective cytotoxic effects, in A-549 cells for 48 h. Changes in LDH activity were determined to determine whether either application caused any membrane damages of lung cancer cells. LDH activity in each sample was determined by following the procedure included in the commercially available kit (MAK066, Sigma-Aldrich). The formula used to calculate LDH activity is given below.

LDH Activity = The amount of NADH that occurs between the first and last measurement (nmol) × Sample Dilution Factor/Reaction Time× Sample volume (mL)

Glutathione peroxidase (GPx) activity

After carfilzomib alone (IC_{10}) and combination of carfilzomib (IC_{10}) and carvacrol concentrations (IC_{10}), showing the most effective cytotoxic effects, were applied to the cells for 48 h, the cell supernatant to be used in GPx activity measurement was prepared. GSH-Px activity was determined according to the method using tert-butyl hydroperoxidase as the substrate. [53] The amount of protein was determined using the Bradford method, in which bovine serum was used as standard. [54] Tests were performed in triplicate.

Caspase-3 activity

Caspase-3 activity was determined after A-549 cells had been exposed to carfilzomib (IC_{10}) and combined with carvacrol (IC_{10}) (the most effective combination concentrations in cytotoxicity) for 48 h. Apoptotic enzyme activity were determined using commercially available colorimetric Caspase-3 Activity Assay Kit (Elabscience) according to kit protocol after application of carfilzomib alone and also together with carvacrol. The plates were read at 405 nm using the microplate reader. Tests were performed in triplicate. Results are given as Unit/mg protein.

Data analysis

The results of the replicates were pooled and expressed as mean ± standard deviation (SD). Analysis of variance (ANOVA) was carried out. The one-way ANOVA was used to determine whether there were any significant differences between the means of three or more independent (unrelated) groups on some variable. Tukey multiple comparisons tests were used. Statistical differences were considered significant at $p<0.05$.[55] Statistical analyses were performed using the Minitab program (http://www.minitab. com/products), release 13.0.

Results

Determination of cytotoxic effects of carfilzomib and carvacrol

The cytotoxic effect of both applications was measured using the MTT assay. IC_{10}, IC_{20}, IC_{30}, IC_{40} and IC_{50} concentrations (the concentration that kills 50% of cells) were determined for each of carfilzomib and carvacrol, which will be used in further experiments (Figure 1 and 2). After applying carfilzomib and carvacrol for 48 h, the cytotoxicity observed in A-549 cells was observed to be parallel to the increase in concentration (Figure 1 and 2).

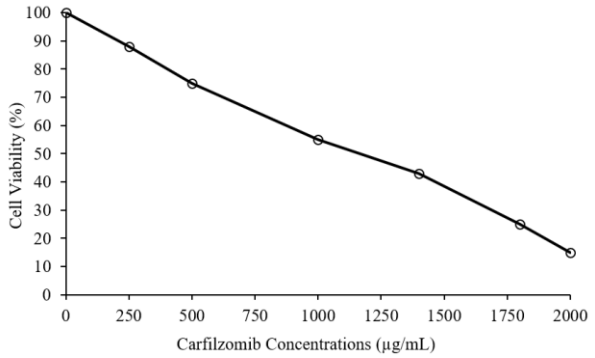

Figure 1. Dose-dependent cytotoxicity of carfilzomib.

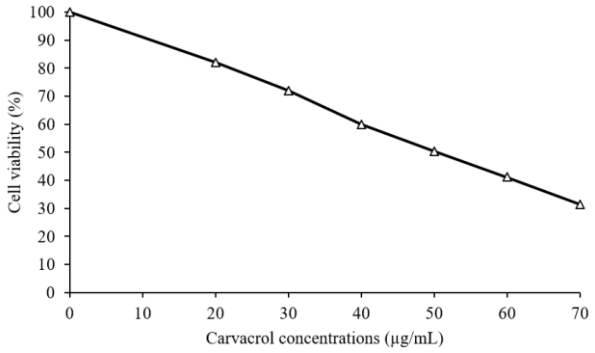

Figure 2. Dose-dependent cytotoxicity of carvacrol.

IC_{10}, IC_{20}, IC_{30}, IC_{40} and IC_{50} values of A-549 cells exposed to carfilzomib for 48 hours were calculated as 197, 440, 683, 927 and 1170 µg/mL, respectively. In this study, we also wanted to investigate whether carvacrol concentrations lower than IC_{50} increased the cytotoxic effect of carfilzomib ($<IC_{50}$). By applying carfilzomib and carvacrol together at concentrations lower than the IC_{50} concentration, it was determined at which combination concentrations they showed the most effective cytotoxic effect (Figure 3). When carvacrol (IC_{10}, IC_{20}, IC_{30}, IC_{40}) treated with carfilzomib ($<IC_{50}$) were ranked among

themselves, the concentrations showing the most effective cytotoxic effect were found to be IC_{10} carfilzomib + IC_{10} carvacrol. The synergistic effect of IC_{10} carfilzomib and IC_{10} carvacrol application in A-549 cells was also demonstrated by calculating the CI value of 1.75. Subsequent studies with cells continued using IC_{10} carfilzomib + IC_{10} carvacrol, which are the most effective cytotoxic combination concentrations.

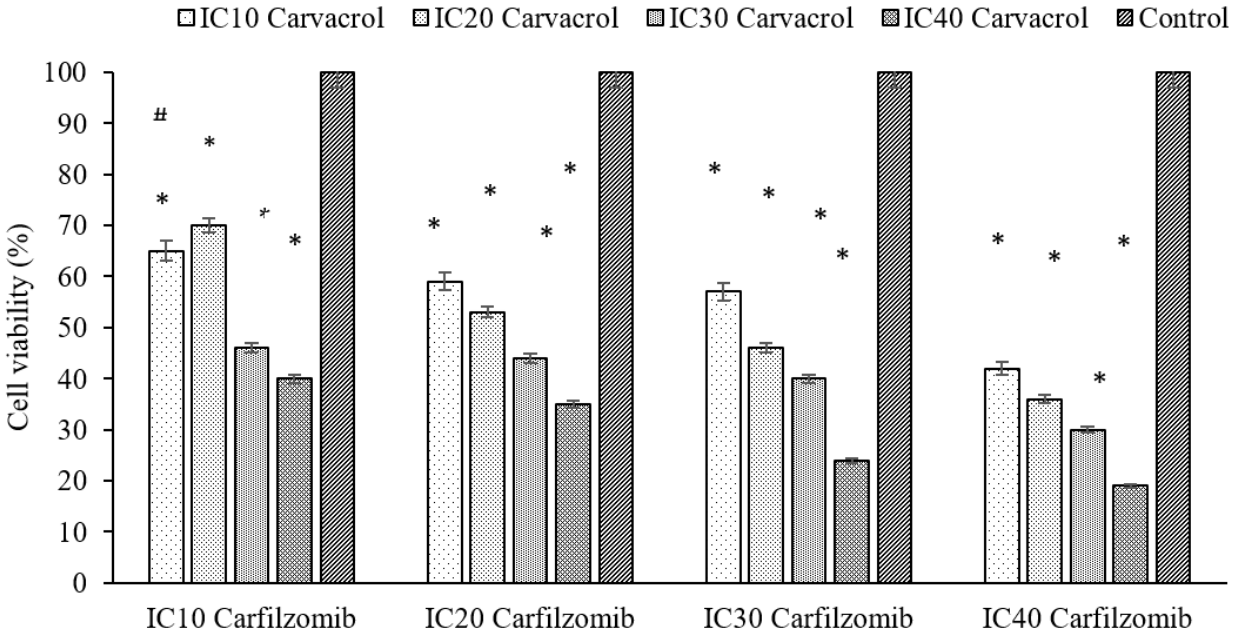

Figure 3. Combined cytotoxic effects of carfilzomib (IC_{10}, IC_{20}, IC_{30}, IC_{40}) and carvacrol (IC_{10}, IC_{20}, IC_{30}, IC_{40}) for 48 h on A-549 cells. Results are presented as viability ratio compared with the control group (treated with only the medium-untreated cells). Values are expressed as the mean of three separate trials with three replications ± standard deviation (SD). * Significantly different from control (untreated cells) (p<0.05). #Significantly different that IC_{10} carvacrol and IC_{10} carfilzomib combination treatment from all other combination treatments except IC_{10} carfilzomib + $IC_{2\ 0}$ carvacrol combination treatment in A-549 cells (p<0.05)

Lactate dehydrogenase (LDH) activity measurement

Since release of Lactate dehydrogenase (LDH) from the cells into the medium was measured, as an indicator of early cell apoptosis, we determined the changes in LDH activity to measure whether there was any damage to the membranes of A-549 cells when applied with carfilzomib alone (IC_{10}) and also combined with carvacrol (IC_{10} carfilzomib + IC_{10} carvacrol) (combination concentrations showing the most potent cytotoxic effect).[56]

After 48 h of applications, LDH enzyme activity rised approximately 2.1 times where combination concentrations (IC_{10} carfilzomib + IC_{10} carvacrol) were applied and approximately 1.6 fold in the cells where carfilzomib was applied only, relative to control group cells (cells present in medium containing only culture medium components). LDH enzyme activity in cells where combination concentrations and carfilzomib were applied alone was shown to be statistically different according to control (Figure 4).

Measurement of the effects of carfilzomib alone and combined with carvacrol on glutathione peroxidase activity in A-549 cells

Cells have a complex enzymatic and non-enzymatic antioxidant defense system. Antioxidant mechanisms develop a defense system against free radicals that have harmful effects on cells. Glutathione peroxidase (GPx) is one of the enzymes that constitute the basic line of defense against free radicals in the cell.

After treatment with carfilzomib alone (IC_{10}) and also with combination concentrations (IC_{10} carfilzomib + IC_{10} carvacrol) (the most potent cytotoxic effect) to A-549 cells for 48 h, changes in GPx activity, which is an important antioxidant that breaks down hydrogen peroxide into water in

mitochondria and sometimes in the cytosol, were determined (Figure 5).

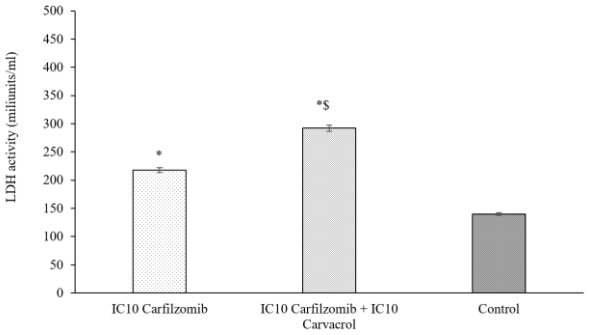

Figure 4. Changes in LDH activities after treated with carfilzomib alone and combine with carvacrol. *Significantly distinct from control (p<0.05). $Significantly distinct from carfilzomib alone treatment (p<0.05). One unit of LDH activity is defined as the amount of enzyme that catalyzes the conversion of lactate into pyruvate to generate 1.0 µmol of NADH per min at 37 °C.

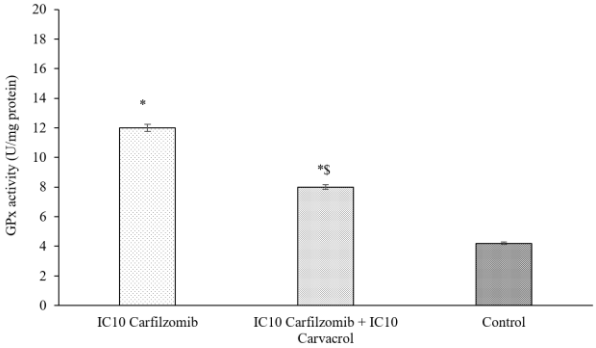

Figure 5. Effect of carfilzomib alone and combine with carvacrol on glutathione peroxidase (GPx) activity. *Significantly distinct from control (p<0.05). $Significantly distinct from carfilzomib alone treatment (p<0.05).

It was determined that GPx activity in A-549 cells applied only with carfilzomib (IC_{10}) was 90.5% higher than the glutathione peroxidase activity measured in the control group cells, and this excess was found to be statistically significant (p<0.05). It was found that GPx activity measured in the cells after the combination application (IC_{10} carfilzomib + IC_{10} carvacrol) was 2.9 fold higher than GPx activity measured in the control group cells. This difference was shown to be statistically significant. It was determined that the combination application was more effective in increasing GPx activity than the carfilzomib application alone, and the GPx activity measured in the cells after the combined application was 1.5 times higher than GPx activity measured after the application alone (Figure 5).

Measurement of the effect of carfilzomib alone and together with carvacrol on caspase-3 enzyme activity in A-549 cells

There are at least 12 caspases in mammals; It is divided into two groups: initiator (-8, -9, -10) and terminator (effector) (-3, -6, -7) caspases. Caspase-3, whose activity we determined through experiments after carfilzomib alone and also with carvacrol application, is also one of the effector caspases.

It was determined that after carfilzomib alone incubation, caspase-3 activity in A-549 cells was 1.8 fold higher compared to control, and after combined application, caspase-3 activity was increased 2.5 fold compared to control (Figure 6). While it was observed that the combined application was more effective in increasing caspase-3 activity than the application of carfilzomib alone, it was revealed that the combined application caused an increase in caspase-3 activity 1.4 fold more than the application alone. It was determined that caspase-3 activity in all applications was statistically different from each other and from the control (p<0.05).

Discussion

Approximately 2 million patients are diagnosed with lung cancer every year and 1.8 million people die due to lung cancer. Therefore, it is one of the cancer types with high cancer incidence and mortality in the world. It ranks second in frequency in women and men.[57] Survival rates after lung cancer treatment are very low. Although cancer survival rates are successful thanks to existing treatment methods (surgery, chemotherapy, radiotherapy), patients have begun to seek new methods other than existing methods. For this reason, cancer patients have turned to the use of complementary and alternative treatments.[58]

The development of new pharmacological strategies for cancer treatments is seen as a very important need today. In this context, there is an increasing interest in natural compounds day by day, thanks to the revealing of their promising therapeutic potential against cancer types such as lung, breast and colon.[59]

According to research conducted in recent years, new potential drugs have begun to be

produced for different pathologies that have important social impacts. For this purpose, studies have been conducted on biologically active species of medicinal plants.[60] Approximately 1/3 of the plants in Türkiye consist of medicinal and aromatic plants. The Lamiaceae family, which includes the thyme plant, has the widest distribution in the world and is a widespread plant family. Plants belonging to the Lamiaceae family; it is the source and main component of many essential oils used in medicine and perfumery. Its use for treatment and spice purposes shows that this family is important. One of the most important plants belonging to the Lamiaceae family is thyme. There are five species of thyme in Türkiye. These; *Thymus*, *Origanum*, *Satureja*, *Tymbra* and *Coridothymus*. With some exceptions, the main component of the essential oils of these genera is usually thymol or carvacrol or both.[61,62]

Carvacrol (2-methyl-5- (1-methylethyl)-phenol) is a monoterpenic phenol component that has an isomeric structure with thymol, an essential oil component found in many aromatic plants.[63] Carvacrol has antimicrobial, antioxidant, anticancer and anti-inflammatory effects.[64]

Oxidative stress occurs by detoxifying reactive intermediates with reactive oxygen species. Free radicals affect proteins, lipids and nucleic acids, causing oxidative damage to different molecules in cells. Essential oils, which can be obtained from many aromatic plants, can prevent or decrease oxidative damage by showing antioxidant effects. In addition, since essential oils have an antioxidant effect and scavenge radicals, they can prevent mutagenesis, carcinogenesis and aging, which are known to be effective in the formation mechanisms of radicals. It is known that different enzymatic antioxidants such as superoxide dismutase, catalase and glutathione peroxidase show activity within the cell. Carvacrol, which is an essential oil component and is known to show different biological activities, can also increase the activity of such antioxidant enzymes. It not only increases the activity of enzymatic antioxidants, but also increases the activity of non-enzymatic antioxidants such as vitamin C, vitamin E and reduced glutathione.[65] It has been demonstrated that carvacrol stimulates reactive oxygen-mediated apoptosis and arrests the cell cycle in human prostate cancer cells, and that it does this in the G0/G1 phase of the cell cycle.[65]

Since proteasome inhibition in multiple myeloma causes intracellular proteins to accumulate and cause cell death, the first generation Bortezomib has revolutionized the improvement of survival times of multiple myeloma patients. Second-generation carfilzomib provides a significant reduction in the risk of death by overcoming the resistance that occurs in bortezomib -resistant patients in the clinic.[66] Drug resistance developing under carfilzomib treatment currently limits therapeutic success in multiple myeloma, and furthermore, the mechanism of carfilzomib resistance is not fully understood to date.[67] Although carfilzomib is more effective than bortezomib, the desired treatment response rates are still not achieved from carfilzomib in patients. Additionally, drug resistance that develops in patients also affects the treatment response rates achieved by carfilzomib. Therefore, new combined treatment strategies are needed in which the drug doses used in treatment can be reduced. In this context, the combined application of existing proteasome inhibitors such as carfilzomib with naturally occurring essential oil components such as carvacrol may be a new strategy.

In one of the studies, carfilzomib was loaded into new nickel-based metal-organic frameworks (Ni-MOFs) and a drug delivery system that could be evaluated in targeted cancer treatment was created. The effects of drug delivery systems loaded with carfilzomib were investigated *in vitro* and *in vivo* by comparing them with standard drugs. After experiments, it has been shown that the drug delivery system releases the drug in a controlled manner and has a high loading efficiency. According to cytotoxicity results, it has been reported that carfilzomib-loaded drug delivery systems are more cytotoxic than free carfilzomib and show this effect more effectively on A-549 lung cancer cells. It has been shown that drug delivery systems not only increase cytotoxicity more effectively,

but also affect the mRNA level of TP53 and are more effective in increasing the level. It has been reported that when free and loaded carfilzomib was applied to rats, it affected various biochemical parameters and significantly increased serum alanine aminotransferase (ALT), serum creatinine, blood urea nitrogen (BUN), aspartate aminotransferase (AST) and liver malondialdehyde (MDA). Ni-MOFs loaded with high doses of carfilzomib were also found to cause serious histopathological changes.[68] In another study, researchers investigated whether carfilzomib (proteasome inhibitor) and vorinostat (histone deacetylase inhibitor) had higher antitumor effects after co-administration in non-small cell lung cancer (NSCLC) cell lines by increasing endoplasmic reticulum stress. It has been reported that the co-administration of the two inhibitors showed a synergistic effect at the end of 48 and 72 h in all cell lines used. H520 and A-549 cell lines were used in this study to evaluate cell viability and apoptosis. It was found that co-administration of the two inhibitors caused more death and caspase-3 cleavage in both cell lines studied than the application of each inhibitor alone. Co-administration of the two inhibitors was observed to cause upregulation of endoplasmic reticulum stress-regulating proteins, activating transcription factor 4, GRP78/BiP and C/EBP homologous protein. After the application of the two inhibitors together, it was observed that there was an increase in the amount of reactive oxygen species in the cell and the level of oxidative stress-related proteins such as heat shock protein 70 in both cell lines.[69] In a different study, the anti-proliferative and cytotoxic effects of carfilzomib were evaluated using different lung cancer cell lines, and after the experiments, it was found that carfilzomib had strong anti-proliferative and cytotoxic activity. Carfilzomib-resistant cells were obtained by exposing A-549 and H520 non-small cell lung cancer (NSCLC) cells to increasing concentrations of carfilzomib. When IC_{50} value calculated for drug-resistant cells and parental cells was compared, it was shown that IC_{50} value in drug-resistant A-549 cells increased 2.5 times compared to the parental cells, and IC_{50} value in the drug-resistant H520 cells increased 122 times compared to the parental cells. It was observed that after carfilzomib application in resistant cells, cell deaths decreased compared to parental cells, and there was also a decrease in the expression of apoptotic and autophagy markers. When both resistant cells were compared with the parental cells, higher P-glycoprotein (Pgp) gene expression was observed to increase 1.2 times in A-549 drug-resistant cells and more than 9000 times in H520 drug-resistant cells. It has been observed that in drug-resistant cells, doxorubicin accumulates less intracellularly and cross-resistance develops against drugs such as bortezomib, doxorubicin and paclitaxel, except cisplatin (P-glycoprotein client drugs).[70]

In one of the studies, it was investigated whether fractional distillation had any effect on the physicochemical and biological properties of oregano essential oil obtained from *Lippia graveolens* H.B.K. It was observed that oregano essential oil was separated into two different fractions by dry vacuum fractional distillation.

When these two fractions were compared in terms of their content, it was determined that they consisted of different main components and the ratios of these components were different. According to the content analysis results, it was found that 45.32% of the first fraction was ρ-cymene and 19.14% was γ-terpinene, while 47.63% of the second fraction was phenolic thymol and 35.56% was carvacrol, approximately 83% of the content of the fraction was determined. It has also been shown that obtaining different fractions affects the biological properties of the essential oil. When the antioxidant activity of the fraction containing thymol and carvacrol as the main components was evaluated using the DPPH method, it was determined that it showed more antioxidant activity than the whole oregano essential oil and also the fraction containing ρ-cymene and γ-terpinene as the main components. It has been observed that the phenolic fraction has a cytotoxic effect on HeLa, Hep2 and A-549 cancer cell lines, and this effect increases with increasing concentration. When the cytotoxic activities of different fractions (phenolic and terpenic

fractions) and whole oregano essential oil were compared, it was determined that the phenolic fraction showed the highest cytotoxic activity.[71]

The results obtained from this study, in which carfilzomib was treated alone and together with carvacrol to human lung cancer A-549 cells for the first time, may may serve as a precursor to future clinical trials of combination therapies using carfilzomib.

Conclusion

The results obtained from our study may provide justification for future combined treatments with carfilzomib. Thus, lower doses of carfilzomib can be used in combined application compared to carfilzomib alone, and side effects caused by carfilzomib can be reduced or eliminated. In addition, greater response to treatment can be achieved, thus easing the economic burden of patients. Indirectly, profits can be made for the country's economy. Our study showing that carfilzomib alone and together with carvacrol gave different responses may be guiding in determining new strategies to be applied in lung cancer treatment.

Ethics Committee Approval

The study does not require ethics committee approval since it does not involve any human or animal subject.

Author Contributions

Concept – A.E.; Design – A.E.; Supervision – A.E.; Resources – A.E.; Materials – A.E; Data Collection and/or Processing – A.E.; Analysis and/or Interpretation – A.E.; Literature Search – A.E.; Writing – A.E.; Critical Reviews – A.E.

Conflict of Interest

There is no conflict of interest to declare.

Financial Disclosure

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Peer-review

Externally peer-reviewed.

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