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***Research Article/Araştırma Makalesi***

# Evaluation of the Effect of Thymol on the Cytotoxicity of Cetuximab in Lung Cancer Cells

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**Abstract:** The treatment of lung cancer continues to be a significant challenge for many oncologists and their patients. Treatment using epidermal growth factor receptor inhibitors is connected to a positive outcome. Cetuximab, a chimeric monoclonal antibody targeting the epidermal growth factor receptor (EGFR), in conjunction with monoterpene phenol thymol, is recommended for the treatment of lung cancer. While a mild acne-like skin rash is quite frequent in patients using cetuximab, a severe rash is rare. The goal of the current study was to assess whether thymol could enhance the anticancer effectiveness of cetuximab in A-549, non-small cell lung cancer (NSCLC) cell line. We found that the combination of cetuximab and thymol synergistically suppressed cell proliferation by inducing membrane damaging, oxidative stress, and apoptosis in A-549 cells. Taken together, our results indicate that the combination of thymol and cetuximab could improve anticancer responses and may notably enhance treatment outcomes in NSCLC.

**Keywords:** Cetuximab, carvacrol, lung cancer, synergistic anticancer effect, apoptosis.

## Timol'ün Akciğer Kanseri Hücrelerinde Cetuximab'ın Sitotoksisitesi Üzerindeki Etkisinin Değerlendirilmesi

**Öz:** Akciğer kanserinin tedavisi, birçok onkolog ve hasta için önemli bir zorluk olmaya devam etmektedir. Epidermal büyüme faktörü reseptörü inhibitörleri kullanarak yapılan tedaviden olumlu bir sonuçlar alınmaktadır. Epidermal büyüme faktörü reseptörünü (EGFR) hedefleyen bir kimerik monoklonal antikor olan cetuximab, monoterpen fenol timol ile birlikte, akciğer kanserinin tedavisi için önerilmektedir. Cetuximab kullanan hastalarda hafif akne benzeri cilt döküntüsü oldukça sık görülürken, şiddetli döküntü nadir görülmektedir. Mevcut çalışmanın amacı, timolün cetuximabın A-549, küçük hücreli olmayan akciğer kanseri (NSCLC) hücre dizisindeki antikanser etkinliğini artırıp artırmayacağını değerlendirmektir. Cetuximab ve timol kombinasyonunun A-549 hücrelerinde zar hasarını, oksidatif stresi ve apoptozu uyararak hücre proliferasyonunu sinerjik bir şekilde baskıladığını bulduk. Bir arada değerlendirildiğinde, bulgularımız timol ve cetuximab kombinasyonunun antikanser yanıtların iyileştirilebileceğini ve NSCLC'deki tedavi sonuçlarının önemli ölçüde arttırılabileceğini göstermektedir.

**Anahtar kelimeler:** Cetuximab, karvakrol, akciğer kanseri, sinerjik antikanser etki, apoptoz.

## 1. Introduction

Lung cancer has the highest incidence rate among men worldwide, resulting in approximately 1.2 million deaths annually. Lung and prostate cancer are among the most common cancers in men in terms of incidence (Parkin et al., 2005). Lung cancer, originating from bronchial epithelium, is a type of cancer that has a very high incidence rate in men and women between the ages of 60 and 70. The incidence rate in young adults (under 50 years old) is quite low, ranging from 5-10%. Because it affects both men and women, it is recognized as the leading cause of cancer-related deaths worldwide. Smoking is the primary cause of lung cancer (Alar & Şahin, 2012; Ergelen & Çimşit, 2000). Due to existing medical treatments, the average 5-year survival rate for patients is 16% (Üstüner & Entok, 2019). Lung cancer is divided into two primary categories. These two groups are named non-small cell lung cancer and small cell lung cancer (Işıtmangil, 2013). Non-small cell lung cancer (NSCLC) makes up 80% of all lung cancer cases. Choosing the appropriate treatment method for NSCLC is of great importance. Surgical intervention is applied to the disease if diagnosed in early stages. In advanced stages, chemotherapy and radiotherapy treatments are applied. The survival rate in these patients is very low and their condition is poor (Balta et al., 2013; Ceylan et al., 2009). A-549 cells used in our study are non-small cell lung cells. A-549 cells are a monolayer of cells that adhere to the culture flask and can be propagated in these flasks. A-549 cells have a hypotriploid structure and contain the tumor suppressor P53 gene (Ding et al., 2008).

Cetuximab is a drug that belongs to a group of medications called monoclonal antibodies. Monoclonal antibodies are proteins that selectively recognize and bind to substances referred to as antigens. Cetuximab is a chimeric monoclonal IgG1 antibody, produced in mammalian cells, that attaches to the extracellular active site of EGFR (Cai et al., 2020). Thus, cetuximab blocks the ligand-mediated (RAS) activation of EGFR and induces antibody-dependent cellular cytotoxicity. When

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cetuximab binds to EGFR, it prevents the phosphorylation and activation of kinases associated with the receptor, such as MAPK and PI3K/Akt. In this way, it inhibits cell growth and induces apoptosis, thereby preventing the development of cancer invasion and metastasis. Cetuximab is commonly associated with skin toxicity, occurring in 80% of cases. It appears on the face, neck, scalp, chest, and upper back. Patients usually experience mild to moderate rashes, with severe rashes being uncommon. This situation emphasizes the importance of developing alternative treatment approaches to minimize the side effects associated with cetuximab-based therapies.

Thymol, chemically referred to as 2-isopropyl-5-methylphenol, is a transparent crystalline monoterpene phenol. It is a secondary metabolite present in species of thyme. Used in traditional medicine for centuries, it has demonstrated a range of pharmacological effects, such as antioxidant, free radical neutralizing, anti-inflammatory, pain-relieving, antispasmodic, antibacterial, antifungal, disinfectant, and anticancer properties (Nagoor Meeran et al., 2017). Thymol has also lowered the levels of byproducts of lipid degradation in plasma, including thiobarbituric acid reactive substances (TBARS), lipid hydroperoxides (LOOH), and conjugated dienes (CDs). It has also been reported that thymol, due to its potent antioxidant properties, boosts the levels of non-enzymatic antioxidants like vitamin C, vitamin E, and GSH in plasma (Nagoor Meeran & Prince, 2012; Javed et al., 2019).

This study aimed to identify the most effective combination concentrations by combining different concentrations of cetuximab, a drug used in targeted therapy for A-549, NSCLC cell line, with non-cytotoxic concentrations of thymol to minimize the side effects of cetuximab.

## 2. Material and Method

### 2.1. Chemicals and drugs

Cetuximab used in the experiments was mixed with the medium in suitable ratios for dilution. Thymol is available for purchase with a purity of 99% (CDH, CAS-No: 89-83-8). The caspase activity kit was sourced from Elabscience Biotechnology Co., Ltd, USA. The kit used to measure Lactate Dehydrogenase Activity was acquired commercially from Sigma-Aldrich (St. Louis, MO, USA).

### 2.2 .Cell Lines and cultivation

A-549 cell line (human non-small cell lung cancer (NSCLC)) used in our experiments was obtained from the American Type Culture Collection (ATCC) and cultured under appropriate conditions. The cells were cultured in Roswell Park Memorial Institute 1640 medium (RPMI 1640) along with other components in appropriate proportions. Once the cells reached an adequate density (over 75%) in the culture vessel, experimental groups were established, and then cetuximab (<$IC_{50}$) and the natural monoterpenic phenol (thymol) (<$IC_{50}$) were administered to the cells for 48 h.

### 2.3. Cell viability test

After the cells in the flask were treated with trypsin, they were quantified and transferred into 96-well plates, with 10,000 cells per well. The cytotoxic effects of cetuximab (500-4000 µg/mL) and thymol (25-400 µg/mL) on A-549 cells were assessed over a 48 h period. Additionally, the cells were exposed to cetuximab (<$IC_{50}$) and thymol (<$IC_{50}$) simultaneously for 48 h. The cytotoxic effects of the treatments were assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. In this assay, mitochondrial dehydrogenases metabolize tetrazolium salts like MTT to produce a blue formazan dye, which is then used to assess cytotoxicity. The test reagents were introduced into the culture medium and the samples were then incubated at 37°C for 2 h. The plates were incubated for 1 h after adding solubilizing/stop solutions (dimethyl sulfoxide) to each well.

The optical density of each sample was recorded at 490 nm (Mosmann, 1983). Each concentration was replicated in eight wells.

The concentrations below $IC_{50}$ (<$IC_{50}$) were determined separately for cetuximab and thymol. The combination treatments were then carried out using the calculated IC values (<$IC_{50}$). Based on the cytotoxicity results, the optimal combination concentration was identified after applying cetuximab (<$IC_{50}$) and thymol (<$IC_{50}$) together. The optimal combination concentrations were also applied in other ongoing experiments. The combination index (CI) was calculated to assess whether the co-treatment of cetuximab and thymol in cells produced additive, synergistic, or antagonistic effects (Huang et al., 2014). Cells treated with only the medium or 0.1% DMSO were regarded as the control group.

### 2.4. Lactate dehydrogenase (LDH) activity test

LDH activity was measured after exposing A-549 cells to cetuximab alone ($IC_{30}$) and combination of cetuximab ($IC_{30}$) with thymol ($IC_{10}$) concentrations, which demonstrated the most significant cytotoxic effects, for 48 h. LDH activity changes were assessed to evaluate whether either treatment induced membrane damage in lung cancer cells. LDH activity in each sample was analyzed using the protocol provided in the commercially available kit (MAK066, Sigma-Aldrich). The equation used to determine LDH activity is provided below.

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

### 2.5. Glutathione peroxidase (GPx) enzyme activity

After exposing the cells treated with cetuximab only ($IC_{30}$) and the combination of cetuximab ($IC_{30}$) and thymol ($IC_{10}$), which demonstrated the strongest cytotoxic effects, for a duration of 48 h, the supernatant from the cell culture was collected for assessment of GPx activity. GPx activity was evaluated using tert-butyl hydroperoxide as the substrate, following the specified method (Flohe & Gunzler, 1984). Protein concentration was measured using Bradford assay, with bovine serum as the standard (Bradford, 1976). The tests were conducted in three replicates.

### 2.6. Caspase-3 enzyme activity

Caspase-3 activity was evaluated after A-549 cells were exposed to cetuximab ($IC_{30}$) individually and together with thymol ($IC_{10}$), the concentrations that showed the highest cytotoxic effects, for 48 h. Apoptotic enzyme performance was assessed by utilizing the colorimetric Caspase-3 Activity Assay Kit (Elabscience), following kit's

instructions, after treating the cells with cetuximab alone and in combination with thymol. The plates were examined at 405 nm using a well plate spectrophotometer. The experiments were conducted in triplicate and the results are presented as units per milligram of protein.

### 2.7. Analysis of data

The findings from the replicates were pooled and presented as the mean ± standard deviation (SD). A statistical analysis using ANOVA was conducted. One-way ANOVA was employed to evaluate if there were notable differences between the averages of three or more independent groups for a particular variable. Tukey multiple comparisons tests were applied. Quantitative importance was accepted at $p<0.05$. Statistical assessments were carried out using Minitab software (http://www.minitab.com/products), version 13.0.

## 3. Results

### 3.1. Evaluation of the cell-damaging effects of cetuximab solitary and in partnership with thymol

Cytotoxic effects can be measured by analyzing the suppression of cancer cell growth and the triggering of cell death through different pathways. For this purpose, we used the MTT test, which is among the widely used cell viability tests in our experiments. In this research we explored the combination of cetuximab and thymol, the possible synergistic impact of both compounds on cancer cell survival was assessed.

A reduction in cell viability was noted in A-549 cells handled with several doses of cetuximab and thymol for 48 h, with a corresponding increase in concentration. When A-549 cells were put through diverse doses of thymol, changing from 25 to 400 µg/mL, $IC_{10}$, $IC_{20}$, $IC_{30}$, $IC_{40}$, and $IC_{50}$ values were determined to be 27 µg/mL, 77 µg/mL, 127 µg/mL, 177 µg/mL, and 227 µg/mL, sequentially (Fig. 1).

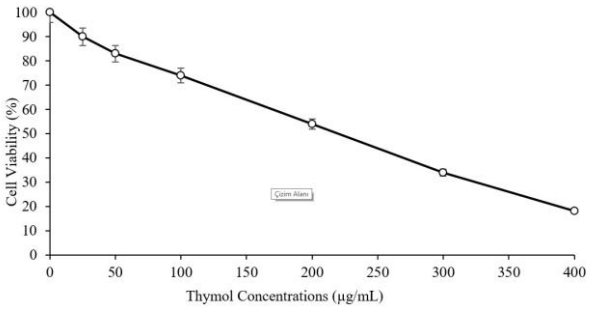


Figure 1. The cytotoxic influence of thymol on A-549 cells

When A-549 cells were subjected to multiple concentrations of cetuximab, starting at 500 and going up to 4000 µg/mL, $IC_{10}$, $IC_{20}$, $IC_{30}$, $IC_{40}$, and $IC_{50}$ values were assessed as 174 µg/mL, 472 µg/mL, 770 µg/mL, 1067 µg/mL, and 1365 µg/mL, in the same order (Fig. 2).

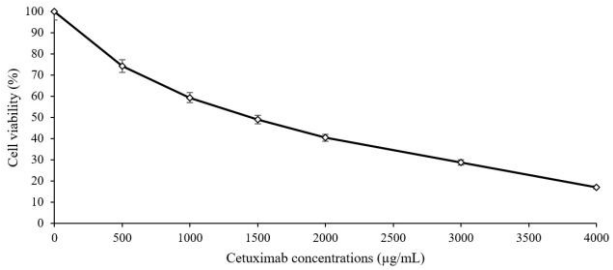


Figure 2. The cytotoxic effect of cetuximab on A-549 cells

By evaluating the cell viability in cells treated in conjunction alongside cetuximab ($<IC_{50}$) and thymol ($<IC_{50}$) and calculating the combination index (CI) value, it was determined that the most potent combination doses were $IC_{30}$ for cetuximab and $IC_{10}$ for thymol (Fig. 3). The combined effect of $IC_{30}$ cetuximab and $IC_{10}$ thymol treatment on A-549 cells was proven to be synergistic through CI value of 1.38 that was calculated.

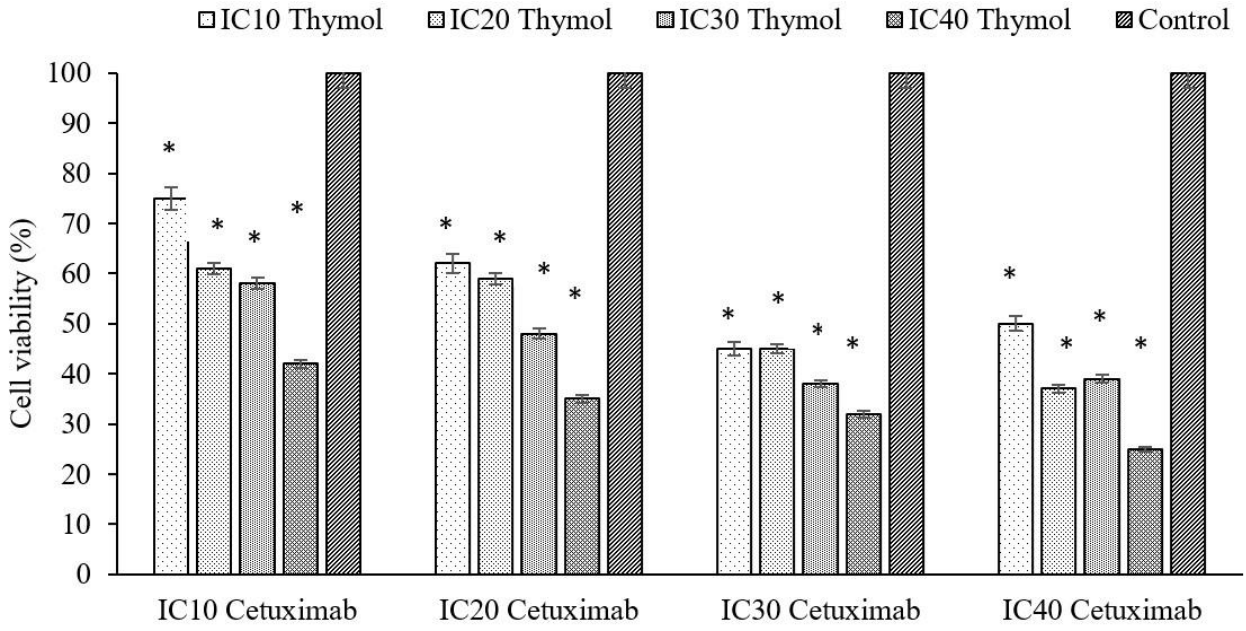


Figure 3. The joint cetuximab's cell-damaging effects (IC10, IC20, IC30, IC40) and thymol (IC10, IC20, IC30, IC40) on A-549 cells for 48 h were assessed. The results are displayed as the survival ratio relative to the reference group (cells exposed solely to the medium, devoid of treatment). The findings are shown as the average of three standalone experiments, each alongside three replicates, ± standard deviation (SD). *Notably different from the control (untreated cells) ($p<0.05$).

### 3.2. Assessment of the membrane disrupting effect of cetuximab alone and in combination with thymol

Lactate dehydrogenase (LDH) is essential for converting lactate to pyruvate in anaerobic metabolism. LDH is often used as a biomarker in medical diagnostics, particularly to assess tissue damage or cell injury. LDH activity is typically measured using a spectrophotometric method, which monitors changes in absorbance as the enzyme catalyzes the conversion of lactate to pyruvate (or vice versa). This method relies on detecting the changes in light absorbance at specific wavelengths, which is proportional to the enzyme's activity (Kaja et al., 2017).

LDH release is commonly applied in cell culture studies to assess cell viability and cytotoxicity. When cells are damaged or killed, LDH leaks out of the cells and into the culture medium. Measuring LDH levels in the medium can indicate the degree of cell damage. In summary, LDH activity measurement is a versatile and widely used tool in both clinical and research settings to assess cell viability, tissue damage, and disease progression (Parhamifar et al., 2019).

After A-549 cells were exposed to cetuximab at $IC_{30}$ concentration and to combination of $IC_{30}$ cetuximab + $IC_{10}$ thymol (the most potent cytotoxic concentration combinations) for 48 h, LDH enzyme activity changes were assessed. Cetuximab and thymol were applied to the cells for a duration of 48 h and it was observed that LDH enzyme activity was elevated in comparison to the control. In A-549 cells administered with cetuximab at $IC_{30}$ level, LDH enzyme activity was observed to increase approximately 2.1 times in relation to the control group. When cetuximab and thymol were implemented simultaneously, LDH activity rose by roughly 3.3 times in comparison to the control (Fig.4).

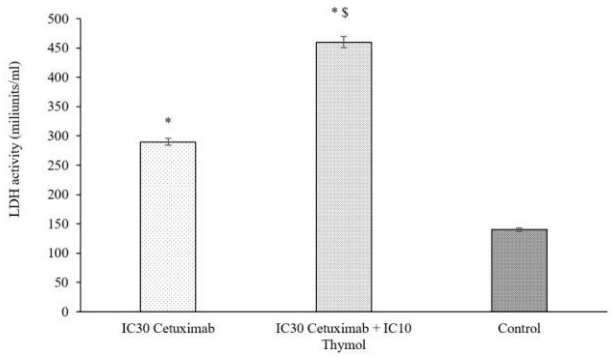


Figure 4. Alterations in LDH activity following treatment with cetuximab alone and in combination with thymol. *Markedly disparate from the control ($p<0.05$). $Significantly distinct from cetuximab solitary treatment ($p<0.05$). One unit of LDH activity is specified as the quantity of enzyme that accelerates the reduction of lactate to pyruvate, generating 1.0 µmol of NADH in a minute at 37 °C.

### 3.3. Examination of the influence of cetuximab alone and alongside thymol on glutathione peroxidase activity

Glutathione peroxidase (GPx) plays a key role in defending cells against oxidative harm by breaking down hydrogen peroxide ($H_2O_2$) and organic peroxides into benign substances, requiring glutathione (GSH) as a cofactor. Evaluating GPx activity is an essential method for assessing oxidative stress and the antioxidant potential within cells (Pei et al., 2023).

After A-549 cells underwent treatment with cetuximab alone and in conjunction with thymol for 48 hours, GPx activity changes were measured to evaluate the potential of the treatments to trigger oxidative stress. Approximately 2.8 -fold increase in GPx activity was observed with cetuximab treatment alone, relative to the controls. The combined treatment ($IC_{30}$ cetuximab + $IC_{10}$ thymol) was found to induce 4-fold greater boost in GPx activity compared to the controls. The rise in GPx activity observed after both cetuximab treatment alone and the combined treatment, relative to the controls, was established as statistically significant ($p<0.05$) (Fig. 5). Induction of oxidative stress was more pronounced with the combined treatment than with cetuximab treatment alone.

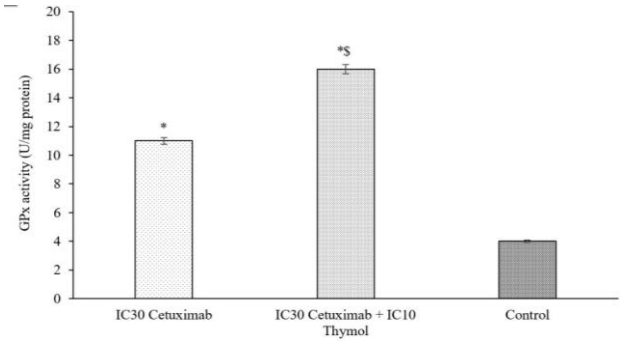


Figure 5. The influence of methotrexate treatment alone and in combination with carvacrol on glutathione peroxidase (GPx) activity. *Significantly distinct from control ($p<0.05$). $Markedly different from cetuximab monotherapy ($p<0.05$).

### 3.4. Investigation of the apoptotic response to cetuximab alone and along with thymol

Caspase-3 is a critical enzyme involved in the process of apoptosis or programmed cell death.

It is classified under the caspase family, a collection of cysteine-aspartic proteases that play essential roles in controlling cell death and inflammation. Specifically, caspase-3 is categorized as an "effector caspase," meaning it executes the final steps of apoptosis by breaking down key cellular components (McComb et al., 2019).

In cancer research, caspase-3 activity can indicate the effectiveness of chemotherapy or targeted therapies that induce apoptosis in cancer cells.

Our study involved measuring caspase-3 enzyme activity after 48 h of incubation with cetuximab alone and combined with thymol to assess the apoptotic impact of both treatments. It was revealed that the combination of $IC_{30}$ cetuximab and $IC_{10}$ thymol (the most effective cytotoxic concentrations) led to a more pronounced apoptotic response than cetuximab treatment alone. The caspase-3 activity recorded after the combined treatment was approximately 1.65 times more than the caspase-3 activity recorded after cetuximab treatment alone (Fig. 6). In addition, the augmentation in caspase-3 activity following the combination therapy was markedly different from the augmentation in caspase-3 activity following cetuximab treatment alone ($p<0.05$). Both treatments induced a statistically significant boost in caspase-3 activity versus the control group.

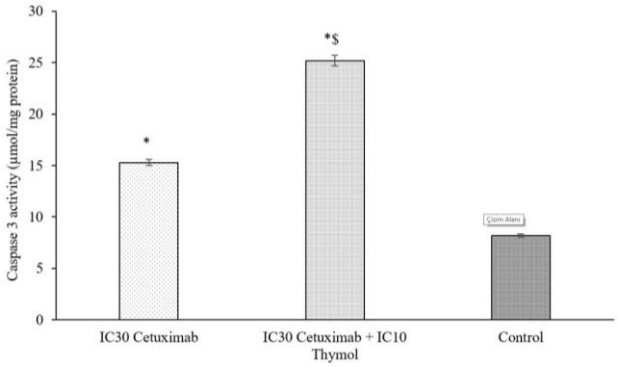


Figure 6. Impact of cetuximab alone and in combination with thymol on caspase-3 enzyme activity. *Significantly distinct from control ($p<0.05$). $ Notably distinct from cetuximab single care ($p<0.05$).

**4. Discussion**

EGFR inhibitors, particularly cetuximab, are becoming more widely used due to their lower systemic side effects, enhanced survival rates, and improved overall well-being for individuals with cancer. Cetuximab is employed in the treatment of colorectal, head and neck, pancreatic, and lung cancers. Hence, it is essential to gain a clearer understanding of the processes behind EGFRI-associated skin toxicities and their management (Peréz-Soler & Saltz, 2005; Lacouture, 2006; Albanell et al., 2002). Cetuximab is a composite monoclonal antibody composed of human and mouse components that selectively bind to the epidermal growth factor receptor (EGFR), blocking the attachment of epidermal growth factor and other signaling molecules through competition. Interacting with EGFR prevents phosphorylation and activation process of kinases tied to the receptor, leading to cell growth inhibition and programmed cell death. At present, there is an absence of definitive instructions for preventing and managing the cutaneous toxicities associated with epidermal growth factor receptor blockers. Conversely, the utilization of EGFR inhibitors is rising not just in head and neck cancer but also in colon and lung cancers (Bonner et al., 2006; Lacouture, 2006; Jacot et al., 2004; Lacouture et al., 2006; Chung et al., 2005).

At present, combination therapies are gaining increasing attention in various cases and have been designed to enhance effectiveness, minimize side effects, and overcome drug resistance in the treatment of cancer patients. Recent developments show that combination therapy offers several benefits over traditional treatments and presents a promising strategy to mitigate harmful effects by lowering the drug dosage (Anitha et al., 2016). Thus, it is crucial to prioritize the search for more targeted combination therapies that cause fewer harmful effects on normal cells. Among these, the incorporation of natural products is on the rise. When used alongside various cancer-fighting agents derived from plant-based sources, cetuximab has been found to enhance the occurrence of apoptosis in human cancer cells across several experimental models. Examining the combined impact of thymol and cetuximab on cancer cells is crucial to understand whether thymol has an effect on EGFR-targeted treatments. Although research on this combination is scarce, some studies propose that thymol could affect cell cycle regulation or boost immune responses.

No studies have been conducted on the combined cytotoxic and apoptotic effects of cetuximab and thymol treatment on A-549 cells. In this study, lung cancer cells treated with both cetuximab and thymol exhibited more extensive membrane damage and enhanced apoptotic effects compared to those treated with cetuximab alone. Our study is the first to show that thymol increases the cytotoxic impact of cetuximab at low concentrations ($<IC_{50}$) in lung cancer cells. In our study, higher concentrations of cetuximab and thymol led to a reduction in cancer cell viability. When cetuximab ($<IC_{50}$) was combined with different concentrations of thymol ($<IC_{50}$), we identified the optimal combined concentration that amplified cytotoxicity in A-549 cells. As a result, the co-administration of cetuximab and thymol demonstrated a stronger cytotoxic effect on A-549 cells compared to cetuximab alone. Lower concentrations of thymol may have exerted stronger cytotoxic effects on A-549 cells when combined with cetuximab, potentially due to their ability to more efficiently activate the cell's antioxidant mechanisms. LDH enzyme activity was measured following the treatment of A-549 cells with $IC_{30}$ cetuximab alone and in combination with $IC_{10}$ thymol, which demonstrated the highest cytotoxic effect over 48 h period. The results indicated that LDH enzyme activity was higher in A-549 cells exposed to the combination treatment compared to those treated with cetuximab alone. Our LDH activity results are consistent with the cell viability findings. In our study, both cetuximab alone and in combination with thymol were observed to increase GPx activity in A-549 cells. This effect is attributed to cetuximab, either by itself or in combination with thymol, inducing oxidative stress through the production of reactive oxygen species. Additionally, cetuximab treatment, both individually and alongside thymol, led to an increase in caspase-3 activity, resulting in a more pronounced apoptotic response. Based on our results, the combination of cetuximab and thymol triggered a higher level of apoptosis in the cells compared to cetuximab alone.

Park and colleagues (2010) noted significant anti-tumor effects when cetuximab was combined with genistein, a naturally occurring isoflavonoid in soybeans, in mouse models with an oral squamous cell carcinoma (OSCC) xenograft. Cetuximab when combined alongside docetaxel, a taxoid sourced from the foliage of the European yellow tree, was able to suppress the growth of non-small cell lung cancer PC9/G2 cells both *in vitro* and *in vivo* (Zhang et al., 2014).

Leeman-Neill and colleagues (2009) highlighted that guggulsterone, a natural compound used in traditional Indian medicine, enhances the inhibitory effects of cetuximab on cell proliferation in head and neck squamous cell carcinoma (HNSCC). The combination of cetuximab with oridonin, a potent compound derived from *Rabdosia rubescens*, is an effective strategy for inducing a joint antitumor effect against laryngeal squamous cell carcinoma (LSCC), both *in vitro* and *in vivo*. This study also explored the mechanisms behind the observed synergy between the two treatments. Throughout the entire experimental phase, no significant side effects were

observed in the treatment groups. The findings suggest that the combined use of cetuximab and oridonin has a strong anticancer effect on LSCC, primarily through the reduction of p-EGFR levels. The concurrent treatment using oridonin and cetuximab inhibited EGFR phosphorylation, leading to significant apoptosis both *in vitro* and *in vivo*. LSCC cells treated alongside oridonin and cetuximab exhibited superior levels of Fas, FasL, and engaged caspase-8 compared to cells treated with oridonin alone (Cao et al., 2016). Natural products, due to their minimal side effects on normal cells, offer an optimal strategy when used alongside the approved drug 5-fluorouracil to more precisely target colorectal cancer cells. This combination helps improve therapeutic outcomes and enhances patients' quality of life. In particular, the promising anticancer natural compound withaferin-A, when combined with 5-fluorouracil, is being evaluated for its synergistic anti-tumor effects in colorectal cancer cells. The goal is to improve the drug combination's efficacy, reduce the required dosage, and minimize side effects on normal cells. In this study, detailed analysis revealed that the combination treatment significantly reduces cell viability in colorectal cancer cells and exhibits a robust combination index. This not only lowers the required dosage but also shows strong effectiveness and a safe toxicity profile in normal colon cells. The combination treatment reduced cell viability at lower doses compared to when withaferin-A or 5-fluorouracil was used alone. Drug index analysis further confirmed that the combination produces a highly potent synergistic effect, enhances drug efficacy, and demonstrates a safe toxicity profile in normal colon cells (Alnuqaydan et al., 2020). Ambrož and colleagues (2017) showed that a sesquiterpene extracted from *Myrica rubra* enhances the effectiveness of doxorubicin in both sensitive and resistant cancer cells. In another study, it was shown that the coalition of cetuximab and honokiol manifested a strong antiproliferative impression on cetuximab-resistant clones both *in vitro* and *in vivo* (Alnuqaydan et al., 2020).

## 5. Conclusions

The current study demonstrates that the combination of thymol and cetuximab produces synergistic effects on inhibiting cell growth by inducing membrane damage, leading increase in GPx activity, and apoptotic effect. In conclusion, while cetuximab has a well-established role in cancer treatment, research on the combined use of cetuximab and timol is limited. However, there is potential for timol to enhance the effects of cetuximab. Further preclinical and clinical studies are necessary to determine whether timol provides a synergistic effect when combined with cetuximab in cancer treatment.

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**Ethics committee approval:** Ethics committee approval is not required for this study.

**Conflict of interest:** The authors declare that there is no conflict of interest.

**Author Contributions:** Conception – A.E.; Design – A.E.; Supervision – A.E.; Fund – Alanya Alaaddin Keykubat University; Materials – A.E.; Data Collection and Processing – A.E., E.H., M.B.; Analysis Interpretation – A.E.; Literature Review – A.E.; Writing – A.E.; Critical Review – A.E.

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