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ORIGINAL ARTICLE

The Anticancer Effect of Curcumin and Piperine Combination in Non-Small Cell Lung Cancer Cells

Küçük Hücreli Dışı Akciğer Kanseri Hücrelerinde Kurkumin ve Piperin Kombinasyonunun Antikanser Etkisi

[1]Nilay İŞİTEZ, [2]İbrahim Hakkı CİĞERCİ, [3]Ömer HAZMAN, [4]Nurullah OKUMUŞ

[1]Department of Basic Pharmaceutical Sciences, Faculty of Pharmacy, Afyonkarahisar Health Sciences University, Afyonkarahisar, Türkiye
[2]Department of Molecular Biology and Genetics, Faculty of Arts and Sciences, Afyon Kocatepe University, Afyonkarahisar, Türkiye
[3]Department of Chemistry, Faculty of Arts and Sciences, Afyon Kocatepe University, Afyonkarahisar, Türkiye
[4]Department of Neonatology, Faculty of Medicine, University of Health Sciences, Ankara, Türkiye

Correspondence

Nilay İşitez
Temel Eczacılık Bilimleri Bölümü, Eczacılık Fakültesi, Afyonkarahisar Sağlık Bilimleri Üniversitesi, Afyonkarahisar, Türkiye

E-Mail: nilay.isitez@afsu.edu.tr

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ABSTRACT

Aim: Curcumin is known to have anticancer effects in different types of cancer, but its bioavailability is low due to its poor absorption and rapid metabolism. Piperine is known to increase the bioavailability of numerous therapeutic agents such as curcumin. This study aimed to investigate the possible synergistic effect of combined curcumin and piperine treatment in the A549 non-small cell lung cancer cell line.
Materials and Methods: After IC_{50} doses were determined using the MTT technique, the mRNA expression levels of genes involved in apoptosis and the cell cycle *(p53, TRAIL-1, TRAIL-2, NF-κB, IKKB, Bax, and Bcl-2)*, as well as the effects of oxidative stress, DNA damage, and inflammation (TNF-α, TGF-β, IL-1β and DEF-β2) of the active substances were determined in the experimental groups.
Results: The results showed that curcumin and piperine dose-dependently reduced cell viability. DNA damage levels were the same as in the control group when curcumin and piperine were administered together. TAS level was found to be high compared to the control group, while TOS and OSI levels were low. It was also observed that inflammatory cytokines (TNF-α, TGF-β, IL-1β, and DEF-β2) decreased. Molecular analysis results also showed that the combination of curcumin and piperine up-regulated *p53, Bax, TRAIL-2, NF-κB* mRNA expression levels, while down-regulated *TRAIL-1, IKKB*, and *Bcl-2* mRNA expression levels.
Conclusions: These results suggest that the combination of curcumin and piperine could be considered as an adjuvant treatment approach to current therapies in the treatment of non-small cell lung cancer.

Keywords: A549 cells, bioavailability, combination therapy, curcumin, piperine

ÖZ

Amaç: Kurkuminin farklı kanser türlerinde antikanser etkileri olduğu bilinmektedir, ancak zayıf emilimi ve hızlı metabolizması nedeniyle biyoyararlanımı düşüktür. Piperinin, kurkumin gibi birçok terapötik ajanın biyoyararlanımını artırdığı bilinmektedir. Bu çalışmada, A549 küçük hücreli dışı akciğer kanseri hücre hattında kurkumin ve piperin kombinasyon tedavisinin olası sinerjik etkisinin araştırılması amaçlandı.
Gereç ve Yöntemler: IC_{50} dozları MTT tekniği ile belirlendikten sonra, deney gruplarında apoptoz ve hücre döngüsünde rol oynayan genlerin *(p53, TRAIL-1, TRAIL-2, NF-κB, IKKB, Bax ve Bcl-2)* mRNA ekspresyon düzeyleri ile oksidatif stres, DNA hasarı ve aktif maddelerin inflamasyon (TNF-α, TGF-β, IL-1β ve DEF-β2) üzerindeki etkileri belirlendi.
Bulgular: Sonuçlar, kurkumin ve piperinin doza bağımlı olarak hücre canlılığını azalttığını gösterdi. Kurkumin ve piperinin birlikte uygulanması sonucunda DNA hasarı düzeylerinin kontrol grubu ile aynı olduğu; oksidatif stres parametrelerinden, TAS düzeyinin kontrol grubuna göre yüksek olduğu, TOS ve OSI düzeylerinin ise düşük olduğu belirlendi. Ayrıca, inflamatuvar sitokinlerin (TNF-α, TGF-β, IL-1β ve DEF-β2) düzeylerinin de azaldığı görüldü. Moleküler analiz sonuçlarında da, kurkumin ve piperin kombinasyonunun *p53, Bax, TRAIL-2, NF-kB* mRNA ekspresyon düzeylerini indüklediği, *TRAIL-1, IKKB* ve *Bcl-2* mRNA ekspresyon düzeylerini ise baskıladığı görüldü.
Sonuçlar: Bu sonuçlar, kurkumin ve piperin kombinasyonunun küçük hücreli dışı akciğer kanseri tedavisinde mevcut tedavilere adjuvan tedavi yaklaşımı olarak değerlendirilebileceğini düşündürmektedir.

Anahtar Kelimeler: A549 hücreleri, biyoyararlanım, kombinasyon tedavisi, kurkumin, piperin

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Introduction

Lung cancer is one of the leading causes of cancer-related deaths worldwide. Non-small cell lung cancer (NSCLC) subtype accounts for approximately 85% of cases [1]. The development of lung cancer involves multiple carcinogenic stages involving genetic, epigenetic, and environmental factors, leading to activation of cancer-related signalling pathways due to dysregulated interactions between key oncogenes and tumour suppressor genes [2]. The understanding of the molecular changes that contribute to the occurrence and progression of cancer may be a key factor in the prevention and treatment of cancer. In the prevention and treatment of cancer, besides chemotherapeutic drugs, the interest in herbal compounds and their active ingredients is increasing day by day. Therefore, it is important to investigate the anticarcinogenic effects of plant-derived compounds.

Curcumin is an important bioactive compound obtained from the rhizomes of the Turmeric *(Curcuma longa)* plant [3]. It has been reported that curcumin exhibits important properties, including anti-inflammatory [4], anticarcinogenic [5-7], antioxidant [7, 8], antiallergic, antiviral, antifungal, antibacterial [9], free-radical-scavenging [10-13], and anti-ageing [14] effects. Anticarcinogenic properties of curcumin have been shown in studies on cancer types such as lung cancer, breast cancer, head and neck cancers, prostate cancer, and brain tumours [4]. Since curcumin is poorly soluble in water, its oral absorption is limited. In addition, curcumin is poorly permeable through the cell membrane. Therefore, it is emphasised that it should be used in combination with appropriate substances to increase its access into the cell [5-7]. It is stated that the bioavailability of curcumin increases, and it may be a potential therapeutic agent as a result of its use with auxiliary molecules such as Piperine, liposomal curcumin, curcumin nanoparticles, and curcumin phospholipid complexes [8].

Piperine is a chemically alkaloid compound obtained from *Piper nigrum, Piper longum L, Piper chaba, Piper guineense,* and *Piper sarmentosum* plants, which are different Piper species belonging to the Piperaceae family [9]. This compound attracts the attention of researchers because it offers multiple benefits, such as antioxidant, antitumour, antihypertensive, analgesic, anti-inflammatory, hepatoprotective, antidepressant, antibacterial, antifungal, antimutagenic, antispermatogenic, and antimetastatic [10]. Piperine is known to increase the bioavailability of numerous therapeutic agents [11]. The most important common feature of Curcumin and Piperine is their anticarcinogenic properties.

Curcumin prevents the metastasis of cancer cells to other organs by preventing the formation of blood vessels formed as its own special network, while Piperine prevents the maturation of cancer cells by reducing the risk of tumour formation [12]. Curcumin is known to regulate signalling pathways associated with the cell cycle, apoptosis, and inflammation, such as *p53, NF-κB, Bcl-2, Bcl-xL, TNF,* and *IL-1β* [3, 13, 14]. This study examined the anticancer effects of curcumin and piperine on A549 by assessing cell viability, apoptosis, and the expression of genes associated with the cell cycle, oxidative stress, DNA damage, and inflammation.

Materials and Methods

Cell line and culture treatments

A549 (ATCC, CCL-185) cells were used to determine the effects of curcumin and piperine administration on genotoxicity, oxidative stress, and the molecular level in non-small cell lung cancer. The medium used for the growth of A549 cells was prepared to contain 10% (v/v) fetal bovine serum (Capricorn), 1% (v/v) penicillin-streptomycin (Sigma), 1% glutamine (Sigma), and 88% high glucose DMEM (Sigma). The cells were cultured at 37 °C in an incubator containing 5% CO_2 and 95% relative humidity.

Cytotoxicity analysis

The cytotoxicity levels of curcumin, piperine, and docetaxel in A549 cells were determined by the MTT method. Cells were seeded in 96-well plates at 2×10^4 cells/well and allowed to adhere for 24 hours. To determine the cytotoxic effect of curcumin, piperine, and docetaxel on A549 cells, doses of 100, 50, 20, 10, 2, 1, and 0,2 µg/mL were used. Docetaxel, used in the treatment of certain types of cancer, including non-small cell lung cancer, was evaluated as a separate group in addition to curcumin and piperine applications. The control group was used with 1% DMSO. Following the treatments, 96-well plates were left for 24 incubations at 37°C and 5% CO_2. At the end of the incubation period, 20 µl MTT (Sigma) solution was added to each well. At the end of 4 hours, the absorbance of the samples was measured at 540 nm in an ELISA microplate reader (Biotek, ELx800). The percentage cell viability (% viability) was calculated by dividing the absorbance values of curcumin, piperine, and docetaxel-treated cells by the absorbance value of the control group and multiplying by 100. According to the doses determined by MTT analysis, the experimental groups and applications are presented in Table 1.

Table 1. Experimental groups and cellular applications

Groups	Cellular applications
Group 1: Control (Cnt)	The medium used to dissolve Cur, Pip, and Dtx was the same concentration and volume.
Group 2: Docetaxel (Dtx)	Dtx was administered to cell in IC_{50} (1 µg/mL) dose
Group 3: Curcumin (Cur)	Cur was administered to cell in an IC_{50} (2 µg/mL) dose.
Group 4: Piperine (Pip)	Pip was administered to a cell in IC_{50} (2 µg/mL) dose
Group 5: Curcumin+Piperine	The group that is a combination of Cur and Pip

Genotoxicity analysis

DNA damage levels induced by curcumin, piperine, and docetaxel in A549 cells were determined using the Comet assay (single-cell gel electrophoresis). The cells in the experimental group were added 200 µL PBS and centrifuged at 800 rpm for 5 min 12 µL of the cell suspension was mixed with 1.5% low-melting-point agarose (A4718, Sigma-Aldrich). The

cell-LMA mixture was prepared on 1% normal melting agarose (A9539, Sigma-Aldrich) slides prepared one day before. Slides were incubated for 20 min at 4 °C in an alkaline buffer (1 mM EDTA, 300 mM NaOH, pH > 13) and subsequently subjected to electrophoresis at 25 V and 300 mA for 20 min at 4 °C. After lysis and electrophoresis, each preparation was stained with ethidium bromide (20 µg/mL). The stained preparations were counted as 100 cells under a fluorescence microscope [15]. DNA damage levels were analysed by dividing them into five different classes using visual scoring (0-200 arbitrary unit scale). A score of 0 is represented by no damage (less than 5%), 1 by minor damage (5%–20%), 2 by moderate damage (20%–40%), 3 by severe damage (40%–85%), and 4 by complete DNA damage (>85%) [16].

Biochemical analyses

The biochemical analyses were performed on cell lysates. The experimental groups were treated as indicated in Table 1 and incubated for 24 hours. At the end of the incubation period, trypsin was added to the cells, and the cell suspension was centrifuged at 25 °C, 800 rpm for 5 min Subsequently, PBS was added to the cell pellet, and washing was performed. Then, 500 µL of lysis buffer was added to the cells after washing, and the cells were lysed using a sonicator (Binder). The procedure was repeated 10 times, and the intracellular fluids were allowed to pass into the cell lysate. Prepared cell lysates were centrifuged at +4 °C, 8500 rpm for 10 min. Following centrifugation, total protein levels, total antioxidant status (TAS), total oxidant status (TOS), and oxidative stress index (OSI), and cytokine (TNF-α, TGF-β, IL-1β, and DEF-β2) levels were determined in the supernatants [17].

TAS and TOS levels were measured using a commercial kit (Rel assay, Turkey) based on the spectrophotometric method. OSI levels were calculated according to the formula (OSI = [(TOS/TAS) x 100]) given in the kit protocol.

TNF-α, TGF-β, IL-1β, and DEF-β2 levels were determined using specific human ELISA kits (Fluka, USA) using a multiplate reader (Biotek, ELx800). The results obtained from each sample were normalised by dividing by the total protein levels in each sample, and cytokine levels were given as pg/mg-protein.

Molecular analysis (RNA isolation, cDNA synthesis, and mRNA expression levels)

mRNA expression levels of p53, *TRAIL-1, TRAIL-2, NF-κB, IKKB, Bax*, and *Bcl-2* genes were analysed by the qRT-PCR method. The total RNA used in these analyses was isolated using a commercial kit (GenMatrix, EURx, Poland). The concentrations of isolated total RNAs were determined on a nanodrop (Epoch 2, Biotek, USA).

The cDNA was synthesised from the isolated total RNA samples using the cDNA synthesis kit (Biorad). The qRT-PCR reaction mixture was prepared by using 1.5 µL of the synthesised cDNA of each sample and adding 12.5 µL of SYBR Green PCR Master Mix and forward and reverse primer pairs to a total volume of 20 µL. The primer pairs were specific for each gene region and were determined in light of studies in the literature [18-20]. Primers and qRT-PCR protocols used in qRT-PCR analysis are shown in Table 2. The mRNA expression levels of the target genes were calculated by the $2^{-\Delta\Delta Ct}$ Ct method [21]. The beta actin gene was used as an endogenous control. The expression levels of other genes were normalised according to the beta actin gene level of each sample. The mRNA expression levels of the analyzed genes were calculated relative to the control group. Expression levels above one were considered induction, and expression levels below one were considered repression.

Table 2. Oligonucleotide primer sequences and qRT-PCR programmes

Gene	Primer sequences	qRT-PCR protocol	
		Tm (°C)	Cycle
p53	F-5′CGGAGGTCGTGAGACGCTG′3 R-5′CACATGTACTTGTAGTGGATGGTGG′3	59	40
TRAIL-1	F-5′GAGAAGTCCCTGCACCACGAC′3 R-5′CCGGAAAGTTCCTGGTTTGCAC′3	59	35
TRAIL-2	F-5′TCCTTACCTGAAAGGCATCTGC′3 R-5′GTCGTTGTGAGCTTCTGTCCA′3	57	35
NF-κB	F-5′TCCCCAAGCCAGCACCCCAGC′3 R-5′GGCCCCCAAGTCTTCATCAGC′3	60	35
IKBB	F-5′GCTACAGAAGAGCCCCTATGGA′3 R-5′AGATCAATGGCACGCTGTTCC′3	57	35
Bax	F-5′AGAAGCTGAGCGAGTGTCTCC′3 R-5′GCCTTGAGCACCAGTTTGCTA′3	58	40
Bcl-2	F-5′CAGCTGCACCTGACGCCCTT′3 R-5′CCCAGCCTCCGTTATTCTGGA′3	58	40
Beta actin	F-5′CATCGTCACCAACTGGGACGAC′3 R-5′CGTGGCCATCTCTTGCTCGAAG′3	55	35

Statistical Analyses

Statistical analyses were made using SPSS 20.0 package programme, and results were presented as ‘mean ± standard deviation’ (X ± SD). ANOVA test, one of the parametric tests, and Duncan test as a post-test were applied to biochemical and genotoxicity analysis data. The statistical differences between the groups were presented as superscripts.

Results

The cytotoxic effects of curcumin, piperine, and docetaxel on A549 cells

The cytotoxic effects of Curcumin, Piperine, and Docetaxel on the viability of A549 cells were shown in Figure 1. Cells were treated with different concentrations of curcumin, piperine, and docetaxel for 24 hours, and cell viability was determined as % cell viability. In A549 cells, both Piperine and Docetaxel showed a dose-dependent decrease. Piperine, and docetaxel exhibited significant decreases in viability were determined at concentrations of 10 µg/mL and higher. The cytotoxic activity of curcumin was shown at doses of 2 µg/mL and 10 µg/mL, with the highest cytotoxic activiy at a concentration of 2 µg/

mL (Figure 1).

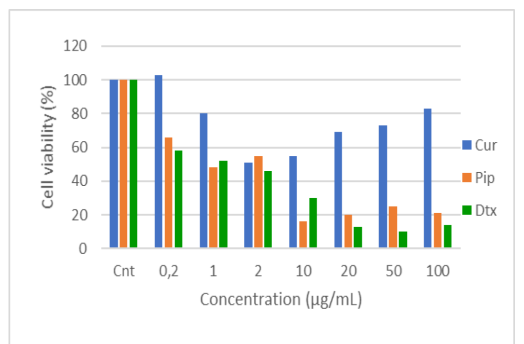


Figure 1. Cytotoxicity levels of Cur, Pip, and Dtx in different concentrations in A549 cells.

Abbreviations: Cnt; Control, Cur; Curcumin, Pip; Piperine, and Dtx; Docetaxel.

The effects of curcumin, piperine, and docetaxel on genotoxicity in A549 cells

The highest DNA damage in the A549 cell line was observed in the docetaxel group, while the lowest DNA damage was observed in the Cur+Pip group. Cur+Pip group were found to be statistically insignificant compared to the control group (Figure 2). Dtx group was found to be statistically significant compared to the control group (Figure 2).

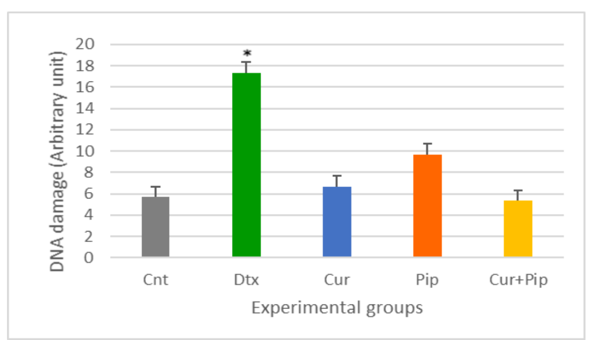
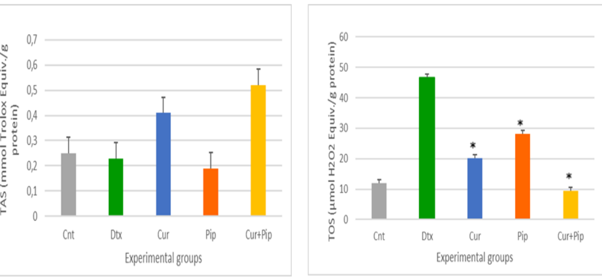


Figure 2. The effects of Cur, Pip, and Dtx on genotoxicity in A549 cells. The data are presented as X±SD. *The differences between the control and treatment groups were analyzed using the Duncan test. p<0.05 was accepted as significant. Abbreviations: Cnt; Control, Cur; Curcumin, Pip; Piperine, and Dtx; Docetaxel.

The effects of curcumin, piperine, and docetaxel on oxidative stress and inflammation in A549 cells

The effects of curcumin and piperine on oxidative stress and inflammation in A549 cells were determined. The data obtained from TAS analyses showed that the highest group was the Cur+Pip group (0.52±00.1) and the lowest group was the Dtx group (0.23±0.01). The combination of curcumin with piperine at the IC50 dose in the experimental groups increased the TAS level (Figure 3).

The Dtx group was found to have the highest TOS level. The Cur+Pip group also showed a decrease in TOS levels ($p<0.05$). One of the important parameters in the evaluation of oxidative stress, the OSI value decreased as a result of the combination of curcumin with piperine (Figure 3).



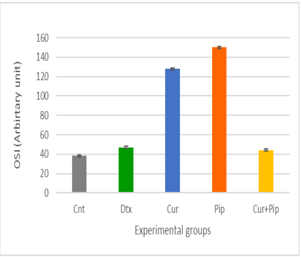


Figure 3. The effects of Cur, Pip, and Dtx on oxidative stress in A549 cells.

The data are presented as X±SD.

* The difference between the mean figures shown in the same column is statistically significant ($p<0.05$). Abbreviations: Cnt; Control, Cur; Curcumin, Pip; Piperine, and Dtx; Docetaxel.

The levels of some inflammatory cytokines (TNF-α, TGF-β, IL-β, and DEF-β2) were determined in A549 cells. The results of the analyses showed that the cytokine (TNF-α, TGF-β, IL-β, and DEF-β2) levels of the docetaxel group were higher than the control group, and that this difference was statistically significant ($p<0.05$). Piperine-treated groups had lower cytokine levels compared to the control group (Figure 4).

Cytokine levels in the Cur+Pip group were found to be lower than in the control group, but this level of reduction was not statistically significant ($p>0.05$).

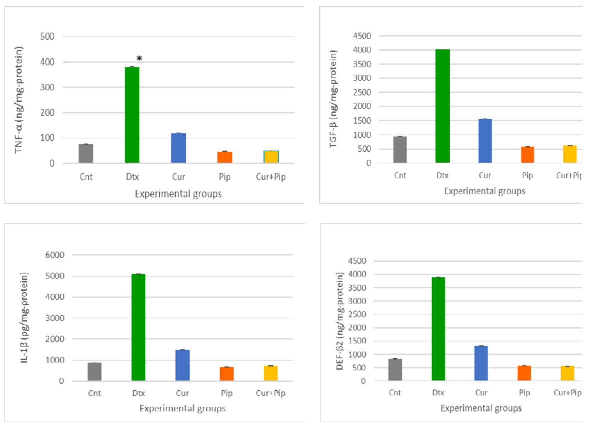


Figure 4. The effects of Cur, Pip, and Dtx on inflammation in A549 cells.

The data are presented as X±SD.

* The difference between the mean figures shown in the same column is statistically significant ($p<0.05$). Abbreviations: Cnt; Control, Cur; Curcumin, Pip; Piperine, and Dtx; Docetaxel.

The Effects of Curcumin and Piperine on mRNA expression in A549 cells

mRNA expression levels of *p53, TRAIL-1, TRAIL-2, NF-κB, IKKB, Bax,* and *Bcl-2*, which are genes involved in apoptosis and cell cycle, were determined in A549 cells.

The results of mRNA expression analyses showed that *p53, Bax, TRAIL-2,* and *NF-κB* expression were up-regulated, while *TRAIL-1, IKKB,* and *Bcl-2* levels were down-regulated in the Cur+Pip group (Table 3).

Table 3. Analyzed genes and relative gene expression levels

Genes	Experimental groups		
	Cur	Pip	Cur+Pip
p53	1.06±0.04	1.24±0.07	11.78±1.84
TRAIL-1	0.16±0.29	0.13±0.16	0.65±0.06
TRAIL-2	0.02±0.03	0.46±0.22	1.70±0.78
NF-κB	0.05±0.04	0.17±0.04	3.21±0.04
IKBB	0.03±0.01	0.37±0.39	0.71±0.10
Bax	0.03±0.04	0.07±0.10	1.80±1.21
Bcl-2	0.01±0.01	0.02±0.01	0.36±0.02

The data are presented as X±SD (n=3). The mRNA expression levels of the analyzed genes were calculated relative to the control group. Expression levels above one were considered induction, and expression levels below one were considered repression. Abbreviations: Cnt; Control, Cur; Curcumin, Pip; Piperine, and Dtx; Docetaxel.

Discussion

Natural bioactive compounds such as curcumin and piperine have been intensively investigated in the field of cancer biology in recent years. The A549 cancer cell line is a frequently used model for in vitro studies on lung cancer therapy. It has been reported that curcumin suppresses proliferation, induces apoptosis and regulates oxidative stress responses in A549 cancer cells [22, 23]. Besides, piperine has both anticancer effects alone and enhances the bioavailability of curcumin and strengthens its intracellular activity [24, 25]. The combined application of the curcumin, and piperine allows simultaneous targeting of both cytotoxic and anti-inflammatory mechanisms. Accordingly, it may lead to multifaceted results such as reduction of DNA damage, stabilisation of oxidative stress, and induction of apoptotic pathways in A549 cell line.

In the present study, curcumin decreased cell viability in the A549 cell line at concentrations of 1-50 μg/mL; piperine alone decreased viability in a similar manner. It has been reported that curcumin shows dose-dependent cytotoxic effect in A549 cells, and other cancer cell lines. Significant cell inhibition and loss of viability in the range of 2.7-54.3 μM was reported in A549 cells [26]. It is also reported that Piperine showed dose-dependent cytotoxic effect in A549 cells [27]. It has been reported that curcumin and piperine-loaded arginine, glycine, aspartic acid (IRGD) exhibited a synergistic antitumour effect in A549 cells [28].

Curcumin, and piperine combination as a result of DNA damage, degree of decreased in A549 cells. While curcumin has been reported to induce DNA breaks in some cell lines, it has also been reported that it may have a protective effect by suppressing DNA breaks and oxidative stress caused by environmental toxins [29, 30]. It has been reported that the combination of curcumin and piperine (5 μg/mL) protects human normal lymphocytes from genotoxicity caused by ionising radiation [31]. Curcumin and piperine together have been found to alleviate genotoxicity and oxidative stress increases in benzo(a) pyrene-induced toxicity in mice [32]. Plant-based products exhibit protective effects by eliminating free radicals and regulating carcinogen detoxification and the antioxidant defence system. This study also shows that curcumin and piperine together protect genetic material.

In the present study, the combination of curcumin, and piperine increased TAS levels, and TOS and OSI levels decreased. These results are consistent with numerous studies supporting the strong antioxidant properties of curcumin. Piperine is also reported to contribute to the strengthening of this antioxidant effect by increasing the bioavailability of curcumin [22]. Oxidative stress is known to be associated with cell apoptosis, and reactive oxygen species (ROS) levels are known to play a role in regulating cell proliferation. ROS levels above a certain threshold inhibit the cell cycle, leading to DNA breakage, cell apoptosis, and necrosis [33]. Curcumin has also been reported to induce apoptosis in A549 cells via oxidative stress and MAPK signalling pathways, depending on the dose and time [33]. Curcumin has been found to reduce ROS and increase superoxide dismutase (SOD) activity, further decreasing malondialdehyde (MDA) and 4-HNE production, and it has been noted that this also triggers the intrinsic apoptotic pathway [34]. A combination of curcumin and piperine increases antioxidant capacity and reduces oxidative stress markers in clinical studies [35, 36]. Thus, it is thought that piperine may reduce oxidative stress and increase antioxidant capacity by enhancing curcumin bioavailability.

According to the evaluation of inflammatory responses in our study, the combination was found to decrease TNF-α, IL-1β, TGF-β, and DEF-β2 levels. It has similarly been reported in the literature that curcumin reduces inflammation by suppressing proinflammatory cytokines and significantly decreases TNF-α and IL-1β levels [37, 38]. Piperine was also reported to have anti-inflammatory effects by reducing levels of inflammatory mediators in an animal model of arthritis [25]. Curcumin's anti-inflammatory effect has been reported to reduce cytokines and interleukins that play a role in the inflammation process [39]. These results are consistent with the literature, and it is thought that the combination of curcumin and piperine has a synergistic anti-inflammatory effect.

The p53 protein triggers apoptosis and cell cycle arrest in response to various cellular events, including oxidative stress, DNA damage, and oncogene activation [40]. It has been found that curcumin induces Bax expression, but suppresses *Bcl-2* expression in A549 cells [41]. Piperine has been found to decrease Bcl-2 protein expression and increase Bax protein expression in A549 cells. It is suggested that these results may trigger p53-mediated cell cycle arrest and apoptosis via activation of the caspase-3 and caspase-9 cascades and may increase the Bax/ Bcl-2 ratio [27]. Curcumin and cisplatin combinati-

on has been reported to inhibit Bcl-2 expression in A549 cells, promote caspase-3 expression, and thus support cell apoptosis by inhibiting cell proliferation [42]. Curcumin and cisplatin combination has been reported to inhibit *Bcl-2* expression in A549 cells, promote caspase-3 expression, and thus support cell apoptosis by inhibiting cell proliferation [42]. It has been reported that the combination of curcumin and carboplatin treatment increases the mRNA and protein expression of caspase-3, caspase-9, and proapoptotic, while suppressing the expression of the antiapoptotic *Bcl-2* gene [43]. It was reported that curcumin increases tumour suppressor genes and suppresses anti-apoptotic genes by activating apoptotic signalling pathways [22, 44]. It was found that pro-apoptotic and cell cycle regulatory genes *(p53, Bax, TRAIL-2, NF-κB)* were induced, while anti-apoptotic genes *(TRAIL-1, IKKB, and Bcl-2)* were suppressed in this study. Additionally, the combination treatment resulted in a reduction in DNA damage, oxidative stress levels, and pro-inflammatory cytokines. These results demonstrate that the Curcumin and piperine combination possesses a dual-action mechanism that both activates apoptotic processes and suppresses cellular stress responses.

Conclusion

This study demonstrates the promising potential of curcumin and piperine as multifaceted anticancer agents in A549 cells. The combination of curcumin and piperine was found to have a reductive effect on cell viability and to decrease DNA damage and oxidative stress. Furthermore, it was observed that it suppressed inflammatory cytokines and played a regulatory role in genes related to apoptosis and the cell cycle. These results indicate that the combination of curcumin and piperine may exhibit both cytotoxic and protective properties. Curcumin and piperine may form an important basis for evaluating their combined use as a complementary or supportive strategy in lung cancer treatment. However, studies need to be conducted on different cell lines as well as in vivo and in clinical trials.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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