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European Journal of Biology

European Journal of Biology,
EJB 2025

https://doi.org/10.26650/EurJBiol.2025.1612786
Submitted: 03.01.2025
Revision Requested: 16.04.2025
Last Revision Received: 18.06.2025
Accepted: 19.06.2025
Published Online 12.09.2025

Research Article

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Comparison of Catechol-Induced Expression Levels of *ATM*, *ATR*, *BAX*, *BCL2*, and *CCND1* Genes Between Parental and Epirubicin-Resistant NSCLC Cells

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Abstract

Objective: This study investigated the apoptotic effects of catechol on lung cancer by comparing gene expression levels in parental lung cancer cells (P-H1299), Epirubicin-HCl resistant lung cancer cells (R-H1299), and healthy fibroblast cells (Bj).

Materials and Methods: The cytotoxic effects of catechol on cells were determined using a Cell Titer-Blue Cell viability assay. To understand the apoptotic effect of catechol, caspase-3/7 activity was measured using a kit. RT-PCR was used to measure the mRNA expression of *the ATM*, *ATR*, *BAX*, *BCL2, CCND1*, and *PCNA* genes.

Results: The IC_{50} values for catechol were determined to be 58 µM for P-H1299, 90 µM for R-H1299, and 207 µM for BJ cells, indicating selective cytotoxicity towards cancerous cells. Caspase 3 activity and Bax/Bcl-2 ratio analyses revealed increased apoptosis in cancer cells, especially in drug-resistant R-H1299 cells. Gene expression analysis revealed increased ATM and ATR mRNA levels in P-H1299 cells, indicating the most DNA damage. Molecular docking studies have suggested that catechol's lower DNA binding affinity compared with known intercalating agents, such as Epirubicin and Doxorubicin, implies a mechanism of action reliant on reactive oxygen species rather than direct DNA intercalation. Efficient antioxidant mechanisms facilitate high survival rates of healthy cells.

Conclusion: Results indicate that catechol cytotoxicity is mainly caused by ROS-induced DNA damage, offering a novel strategy for targeting both parental and drug-resistant lung cancer cells. This study proposes a promising direction for the development of safer and more effective chemotherapy drugs that spare healthy cells and potential clinical applications for catechol.

Keywords Apoptosis · Catechol · Drug resistance · Lung cancer · Docking

Citation: Demir, Z, Ozkan, A & Sungur, B. Comparison of Catechol-Induced Expression Levels of *ATM*, *ATR*, *BAX*, *BCL2*, and *CCND1* Genes between Parental and Epirubicin-Resistant NSCLC Cells. Eur J Biol. 2025; Advance Online Publication. DOI: 10.26650/EurJBiol.2025.1612786

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INTRODUCTION

Lung cancer is a leading cause of cancer-related mortality worldwide. Incidence rates are increasing worldwide.[1] The primary challenge in treating lung cancer with frequently employed chemotherapeutic regimens is cellular resistance. Such resistance can arise from the elevated levels of antioxidant enzymes in drug-resistant cancer cells, potentially leading to acquired multidrug resistance.[2] Furthermore, the diverse heterogeneity among cells in non-small-cell lung cancer (NSCLC) poses a challenge for therapy and contributes to chemoresistance.[3-5] Therefore, when formulating treatment strategies for cancer, it is essential to consider the presence of drug-resistant cells in the tumour. NSCLC, which is known for its low early diagnosis rates, exhibits drug resistance against existing chemotherapeutic agents, necessitating the exploration of new drugs that enhance multiple signaling pathways leading to cancer cell apoptosis. Additionally, it is crucial to identify the most active apoptotic signaling pathways. The pro-apoptotic and anti-apoptotic mechanisms comprise both extrinsic and intrinsic pathways that are modulated by anticancer drugs used for cancer treatment. Technical abbreviations will be explained upon first use. A caspase cascade involved in these pathways initiates apoptotic signalling and causes cell death.[6]

Caspase-3, which is activated in both intrinsic and extrinsic apoptotic pathways, is targeted by numerous anticancer agents and modulates cellular death.[7,8] Regulating the process of cell death by modulating the family of Bcl-2 proteins is a potential approach. The Bax/Bcl-2 ratio in cancer cells is a crucial indicator of the effectiveness of anticancer drugs in cancer treatment.[9] Additionally, apoptosis can be initiated through the DNA damage signaling pathway, which is regulated by p53, with the assistance of the ATM and ATR pathways.[10] The activation of p53, a tumour suppressor protein, stimulates ATM and ATR pathways.[11] The *P53* gene regulates apoptosis through activating the DNA damage response mechanisms in response to external stimuli or stress. Alternatively, DNA repair may be initiated if the level of damage can be repaired.[12] Cell cycle checkpoint proteins, including cyclins and cyclin-dependent kinases (Cdks), constitute a signaling pathway that can regulate apoptosis by controlling cell cycle progression.[13]

The vital role played by plant-derived natural molecules in treating different forms of cancer. Many of these molecules trigger apoptosis and/or regulate mechanisms of the cell cycle in cancer cells.[14-17] Catechol, a compound found naturally in plants such as *Semecarpus anacardium* and *Bonellia macrocarpa* and also in onions, apples, and olive oil, has demonstrated cytotoxic properties in glioblastoma and breast cancer cells.[18-21] The chemotherapeutic effects of catechol on lung cancer require further investigation due to unclear potential. Previous studies have shown that catechol exhibits anticancer properties in various cancer models through mechanisms such as reactive oxygen species (ROS)-mediated apoptosis, cell cycle arrest, and mitochondrial dysfunction. For instance,[15] catechol induces apoptosis in glioblastoma cells via ROS generation and loss of mitochondrial membrane potential, while reported catechol's role in suppressing the proliferation of breast cancer cells by modulating the PI3K/Akt signaling pathway.[16] However, despite these promising findings, catechol pharmacokinetics remains relatively underexplored. Moreover, these studies showed that rapid metabolism and low bioavailability of catechol, which may limit clinical translation without appropriate drug delivery systems. Additionally, its cancer type-specific efficacy remains unclear, and comparative studies on drug-sensitive versus drug-resistant cancer models are lacking. Therefore, this study addresses an important gap by evaluating catechol effects specifically on parental and epirubicin-resistant NSCLC cells. The molecular mechanism of action of the proposed drug is also explored using both experimental and *in silico* approaches.

Thus, the present study aimed to examine the effectiveness of catechol as a chemotherapeutic agent against lung cancer cells by evaluating its impact on DNA damage, cell cycle, and apoptosis. The investigation aimed to provide insights into the therapeutic potential of catechol in lung cancer treatment. We analysed the mRNA expression levels of *PCNA*, *CCND1*, *BAX*, *BCL2*, *ATM*, and *ATR* genes in catechol-treated P-H1299 and R-H1299 cells and compared its efficacy in NSCLC cells with that in healthy fibroblast cells (BJ). Furthermore, comparative molecular docking simulations were performed on DNA models with well-known epirubicin, doxorubicin, and catechol to investigate the underlying mechanism of action.

MATERIALS AND METHODS

Chemicals and Drugs

Catechol, also known as pyrocatechol and brenzcatechin, was procured from Sigma-Aldrich (Darmstadt, Germany. Epirubicin HCl was obtained from Calbiochem (San Diego, CA, USA. Epirubicin HCl solution was prepared with deionised water under sterile conditions, and the drugs were diluted in a growth medium immediately before application. For the measurement of caspase-3 activity, the cell viability assay CellTiter-Blue® and the triplex assay ApoTox-Glo™ were purchased from Promega (Madison, WI, USA). The RNeasy Mini Kit, which is used to purify high-quality RNA from cells, was purchased from Qiagen (Chatsworth, USA). For the RT-

PCR method, the Titan Roche Applied Science (Mannheim, Germany) One Tube RT-PCR System Kit was purchased.

Cell Cultures and Treatments

The H1299 and BJ cell lines were obtained from the American Type Culture Collection (Rockville, MD, CRL-5803 and CRL-2522). Technical terms were explained in their first usage. The cells were cultured in Roswell Park Memorial Institute 1640 medium (RPMI 1640) (ThermoFisher, USA) repeatedly. Additionally, 10% foetal bovine serum (ThermoFisher, USA) and 1% antibiotic-antimycotic solution (penicillin, streptomycin, and amphotericin) (ThermoFisher, USA) were used as supplements. The experiment was carried out in a humid environment with a 5% CO_2 concentration at a temperature of 37°C. After treatment with trypsin/ethylenediaminetetraacetic acid (EDTA) at 37 °C, the cells were harvested for subculture. The cells were used when the monolayer reached 75% confluency. Epirubicin-resistant H1299 cancer cells (R-H1299) were developed from the original cell line by gradually exposing them to higher concentrations of Epirubicin until they successfully multiplied in the presence of 35 ng/mL of the drug. The method of selection was consistent with that presented in previous reports.[2] Amino acid supplementation was added to the production of BJ cells, a healthy human fibroblast cell line, using Dulbecco's Modified Eagle Medium (DMEM). All other procedures were similarly performed on BJ cells.

Cytotoxicity Assay

The cytotoxicity assay was conducted according to the OECD (Document: 46754707) guidelines. The CellTiter-Blue® Cell Viability Assay (Lot No: 0000107812) is a homogeneous, fluorometric technique used to count viable cells in multiwell plates. This assay uses a resazurin indicator dye to detect the metabolic activity of cells, indicating their viability. Viable cells can convert resazurin to the highly fluorescent compound resorufin, whereas non-viable cells, which quickly lose metabolic capacity, cannot reduce resazurin and produce a detectable fluorescent response. In this study, 96-well plates were set up consisting of cells at the desired density, medium, and the substances to be examined, all suitably diluted in the medium. Parenteral H1299 (P-H1299), R-H1299, and BJ cells were added to each well with a volume of medium, resulting in a final volume of 200 µL/well. Catechol was administered to the cells for 24 h. After the treatment period, the appropriate amount of reagent (20 µL/well) was added, and the plate was incubated for 1 h at 37°C in the dark. The fluorescence signal (560 Ex/590 Em) was measured using a spectrofluorometer (PerkinElmer LS 55, Waltham, MA, United Kingdom).[22] The fluorescence signal was directly proportional to the viable cell count. The data were averaged from five wells per concentration, and the percentage of viable was determined by dividing the number of live cells by that of control cells containing only the culture medium. The IC_{50} (50% inhibition constant) values of catechol on cell culture were determined over 24 h. Each experiment was replicated 3 times. IC_{50} values were calculated using two parameters Hill (Equation 1) models, respectively.

$$\text{Cell Viability } (\%) = \frac{100}{1+\left(\frac{\text{Dose}}{\text{IC}_{50}}\right)^{\text{Hill Coefficient}}} \quad (1)$$

Caspase-3 Activity Assay

Caspase-3 activity in P-H1299, R-H1299, and BJ cells was evaluated using the ApoTox-Glo Triplex Assay (Promega). The cells were exposed to catechol for 24 h to assess caspase-3 activity. For the experiment, 10,000 cells were cultured in 96-well assay plates. Following the incubation period, Caspase-Glo 3 reagent was applied to each well. Subsequently, plates were vortexed briefly and allowed to incubate for one hour at ambient temperature. The assay was founded on the notion that active caspases cut purinogenic peptide substrates, releasing a luminescent signal through a reaction between luciferase and luciferin. The luminescence was measured using a microplate reader to determine the activation of caspase. This procedure facilitates the assessment of apoptosis.[23] Caspase-3 activity was measured in triplicate and quantified in relative light units.

RNA Extraction and Study of Gene Expression

The study of gene expression and RNA extraction was conducted using the RT-PCR (Reverse Transcription Polymerase Chain Reaction) method. This was done to determine the rate of gene expression, specifically proliferative cell nuclear antigen *(PCNA), BCL2, BAX, CCND1, ATM* and *ATR* at the transcriptional level in P-H1299, R-H1299, and BJ cells. The cells were exposed to catechol, and gene expression studies were conducted in three distinct trials. After 24 h of incubation, the RNeasy mini kit (Qiagen) was used to extract total RNA from the cells, following the manufacturer's guidelines. RPMI and DMEM were used as controls. The cDNA was synthesised following the manufacturer's instructions using the Titan One Tube RT-PCR System kit (Roche Applied Science) starting with 1 mg of total RNA. The PCR reaction followed a specific experimental protocol: 30 cycles were used, with a denaturation period of 1 min at 94 °C, an annealing period of 1 min at different temperatures depending on the gene (55 °C for *GAPDH* coding glyceraldehyde-3-phosphate dehydrogenase and *BAX*, 58 °C for *CCND1* coding Cylin D1, 56 °C for *ATM* and *ATR*, and 59 °C for *PCNA*), and an extension period

Table 1. Sequence of utilized primers

Gene	Gene ID	F-primer sequence (5′–3′)	R-primer sequence (5′–3′)	References
GAPDH	2597	TTCATTGACCTCAACTACAT	GAGGGGCCATCCACAGTCTT	1
PCNA	5111	CGCGCAGAGGGTTGGTAGTT	AAGCCTTCGGAGCGCAGAGT	1
BAX	581	ACCAAGAAGCTGAGCGAGTGTC	ACAAAGATGGTCACGGTCTGCC	1
BCL2	596	TGCACCTGACGCCCTTCAC	AGACAGCCAGGAGAAATCAAACAG	1
CCND1	595	CCGTCCATGCGGAAGATC	ATG GCCAGCGGGAAGAC	2
ATR	545	GCCGCTCCGATCGTGTAC	TTTGTATGCTCTGTGATAACCTTGTTT	3
ATM	472	ATCTGCTGCCGTCAACTAGAA	GATCTCGAATCAGGCGCTTAAA	4

of 2 min at 72 °C, with a final extension at 72 °C for 90 sec. The *BCL2* gene, a touchdown PCR method was used. This started at 73 °C and decreased by 1 °C per cycle to 63 °C for 10 cycles. PCR analysis was performed with 25 additional cycles. RT-PCR was performed in three replicates with a negative group containing water. The *GAPDH* gene was used as a control. The gene primers used are listed in Table 1. The PCR products were examined by electrophoresis in 2.5% agarose with ethidium bromide (1 µg/mL) in TBE 1× buffer (Tris 40 mM, EDTA 1 mM and boric acid 44 mM) for 30 min at a constant voltage of 120 V, using a 100-bp ladder as a molecular weight marker. Adobe Photoshop CS4 was used to analyse the band density. In order to express the density of the PCR bands, we divided it by the density of the housekeeping gene and then expressed it as a percentage of the density of the control band.[24,25]

Docking Studies

The 3D structures of catechol (ZINC000013512214), Doxorubicin (ZINC000005049216), and Epirubicin (ZINC000003918087) in molecular docking analysis were retrieved from the ZINC Database.[26] Energy minimisation of the 3D structure of the drug molecules was performed using Open Babel v2.3.2 software.[27] 3D structures of B-DNA dodecamer (PDB ID: 195D and PDB ID: 1BNA) were obtained from the protein data bank.[28,29] The existing ligand of the B-DNA (PDB: 195D) was excluded from docking studies. The grid box was determined using AutoDockTools v1.5.7, and docking studies were conducted using Autodock Vina v.1.2.0.[30,31.] The further configuration parameters of the docking study are presented in Supplementary Data. DNA-drug interactions were visualised with Discovery Studio Visualiser 2021. Doxorubicin and epirubicin were selected for comparison and validation studies. The inhibition constants for each result were calculated using Equation 2, where e is Euler's number, R is the gas constant in $J.mol^{-1}.K^{-1}$, and T is the temperature in Kelvin. Because epirubicin-resistant cells were used in the present study, Epirubicin was selected as the reference in the docking study, and doxorubicin was selected as the 2nd reference to control Epirubicin.

$$\text{Inhibition Constant (Ki)} = e^{\frac{\Delta G_{\text{binding affinity}}}{RT}} \quad (2)$$

Statistical Analysis

The mean and standard deviation values are provided for all experimental groups. The data underwent statistical analysis using the SPSS software, and the relationships between groups were examined by employing the ANOVA-GLM (General Linear Model). The significance levels in the statistical analysis were determined using the Tukey Multiple Comparison Test and $p<0.05$ was considered significant.[32]

RESULTS

Effect of Catechol on the Viability of P-H1299, R-H1299, and BJ Cells

The IC_{50} values for P-H1299, R-H1299, and BJ cells treated with catechol for 24 h at 37°C were calculated as 58, 90, and 207 µM, respectively. Based on these IC_{50} values, P-H1299 cells were found to be the most sensitive to catechol, whereas healthy cells were the most resistant. Cell viability rapidly decreased up to 100 µM, but no further changes were observed after 300 µM (Figure 1).

Measuring Effect of Catechol on Caspase-3 Enzyme Activity

The effect of catechol on the activity of caspase-3, an enzyme involved in the apoptotic pathway, was measured after treating the P-H1299, R-H1299, and Bj cell lines with the IC_{50} concentration of catechol for 24 h (Figure 2). The results demonstrated the potential of catechol (IC_{50}) to induce apoptosis in cells. Caspase-3 activity was 2.8-fold higher in P-H1299 cells, 2.3-fold higher in R-H1299 cells, and 1.6-fold higher in BJ cells compared with the control. P-H1299 cells exhibited the highest caspase-3 activity among the three cell lines.

Effect of Catechol on Expression Levels of *PCNA*, *CCND1*, *ATM*, *ATR*, *BAX*, and *BCL2* Genes in Cells

PCNA mRNA expression in P-H1299, R-H1299, and BJ cells treated with catechol was 53%, 24%, and 49% lower than

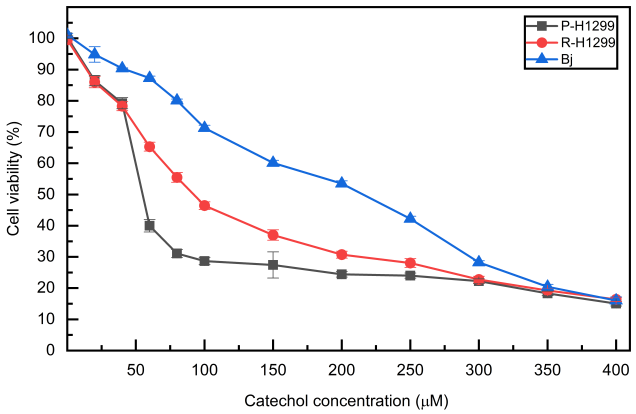


Figure 1. The cytotoxic effects of catechol (20-400 μM) for 24 h on P-H1299, R-H1299, and BJ cells were determined using the CellTiter-Blue Cell Viability Assay. Cell viability results were normalised to the control group of the cell culture. The mean values of the five replicates from three separate experiments are expressed, with error bars indicating the standard deviation ($p<0.05$, ANOVA with Tukey's test).

control cells, respectively (Table 2). The antiproliferative effect of catechol was most pronounced in P-H1299 cells compared with healthy cells, whereas resistant cells showed the least response to catechol (Figure 3). After catechol exposure, Bax mRNA expression increased by 134%, 96%, and 67% in P-H1299, R-H1299, and BJ cells, respectively, compared with the control, whereas Bcl-2 mRNA expression decreased by 88%, 47%, and 84% (Table 2, Figure 3). The Bax/Bcl-2 mRNA ratio was 1.5 in P-H1299 cells, 2 in R-H1299 cells, and 1 in BJ cells. This indicates a tendency towards the proapoptotic pathway in P-H1299 and R-H1299 cells, whereas BJ cells show a tendency towards the antiapoptotic pathway. Cyclin D1 mRNA expression in P-H1299, R-H1299, and BJ cells exposed to catechol decreased by 72%, 47%, and 70%, respectively, compared with the control group (Table 2, Figure 3). The lowest mRNA expression of cyclin D1

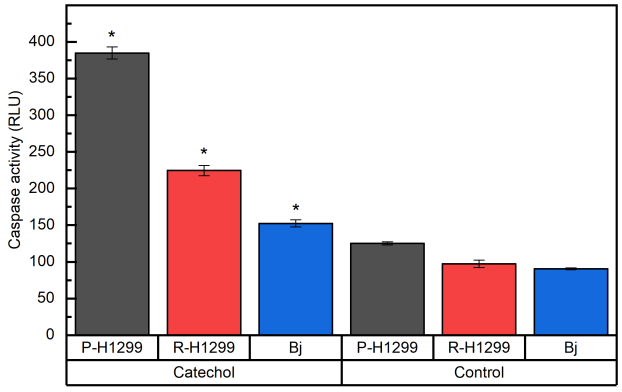


Figure 2. The effect of catechol on caspase-3/7 enzyme activity in P-H1299, R-H1299, and BJ cells was measured using the ApoTox-Glo Triplex Assay kit. The mean values of the three replicates, with error bars indicating the standard deviation and asterisks in the graph indicating significant change compared with the control ($p<0.05$, ANOVA with Tukey's test).

Table 2. Expression of six genes in the P-H1299, R-H1299, and BJ cell lines as a percentage change compared with the control groups

Genes/Cells	P-H1299	R-H1299	BJ
PCNA	-0,53	-0,24	-0,49
CCND1	-0,72	-0,47	-0,7
BAX	1,34	0,96	0,67
BCL2	-0,88	-0,47	-0,84
ATM	1,62	1,12	1,5
ATR	2,17	1,12	1,7

was observed in P-H1299 cells, indicating the inhibitory effect of catechol on cell cycle progression from G1 to S phase. The final step in the apoptotic pathway is the formation of DNA breaks due to DNA damage. ATM and ATR proteins are involved in the cellular response to DNA damage, primarily via double- and single-strand breaks. The expression levels of *ATM/ATR* genes reflect the level of DNA damage. After catechol exposure, ATM mRNA expression was increased by 162%, 112%, and 150% in P-H1299, R-H1299, and BJ cells, respectively, compared with the control (Table 2, Figure 3). Catechol had the most significant effect on increasing ATM and ATR mRNA expression in P-H1299 cells. These findings indicate that catechol induces apoptosis through multiple signaling pathways, with ATM and ATR signaling pathways being the most effective, likely due to the presence of DNA breaks (Figure 3).

As shown in Figure 4, catechol bound to the model DNA chains mostly via guanine nucleotides in accordance with the data of the other experimental studies[38], with a binding affinity of -5.022 and -4.818 kcal/mol for 1BNA and 195D, respectively. In addition to binding affinities, the Ki was calculated via Equation 1 to compare with experimental IC_{50} values and found as 206.42 μM for 1BNA and 291.38 μM for 195D. Moreover, doxorubicin showed ~-9.00 kcal/mol binding affinity and ~0.300 μM for Ki while Epirubicin ~-9.50 kcal/mol and ~0.170 μM. Results from docking studies are illustrated in Figure 4. The binding affinities and Ki values are presented in Table 3.

DISCUSSION

With a mortality rate of approximately 23% for both sexes, lung cancer is the leading cause of cancer-related deaths worldwide.[33] Despite recent advances in the diagnosis and treatment of lung cancer, the prognosis remains poor. One of the biggest reasons for this poor prognosis is the resistance of

Table 3. Binding affinity and inhibition constants in docking studies

Ligand	Receptor	Binding Affinity (kcal/mol)	Inhibition Constant (μM)
Catechol (ZINC000013512214)	PDB: 1BNA	-5022	206.42
	PDB: 195D	-4818	291.38
Doxorubicin (ZINC000005049216)	PDB: 1BNA	-8742	0.3846
	PDB: 195D	-9135	0.1980
Epirubicin (ZINC000003918087)	PDB: 1BNA	-9523	0.1028
	PDB: 195D	-9220	0.1715

cancer cells to the anticancer drugs used for treatment. The need for high-dose anticancer drugs to destroy these resistant cancer cells damages healthy cells. Therefore, there is a need to develop new drugs that will have an anticancer effect on both parental and resistant cancer cells while causing less damage to healthy cells. Investigating the mechanisms of action of herbal products against cancer should be the most crucial strategy in the development of new anticancer drugs.[34] However, no studies have compared the anticancer effects and mechanisms of action of catechol in parental and drug-resistant H1299 cancer cells and BJ healthy fibroblasts.

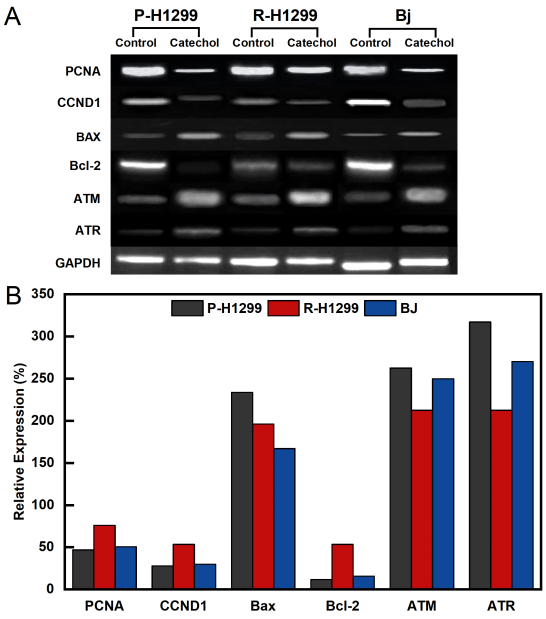


Figure 3. The expression levels of *BAX, BCL2, ATM, ATR, CCND1,* and *PCNA* genes in P-H1299, R-H1299, and BJ cells were measured by RT-PCR. The expression levels of *BAX, BCL2, ATM, ATR, CCND1,* and *PCNA* genes in P-H1299, R-H1299, and BJ cells were measured by RT-PCR (reverse transcriptase polymerase chain reaction). The graph shows the values of mRNA expressions analysed by RT-PCR and measured using the Adobe PS program.

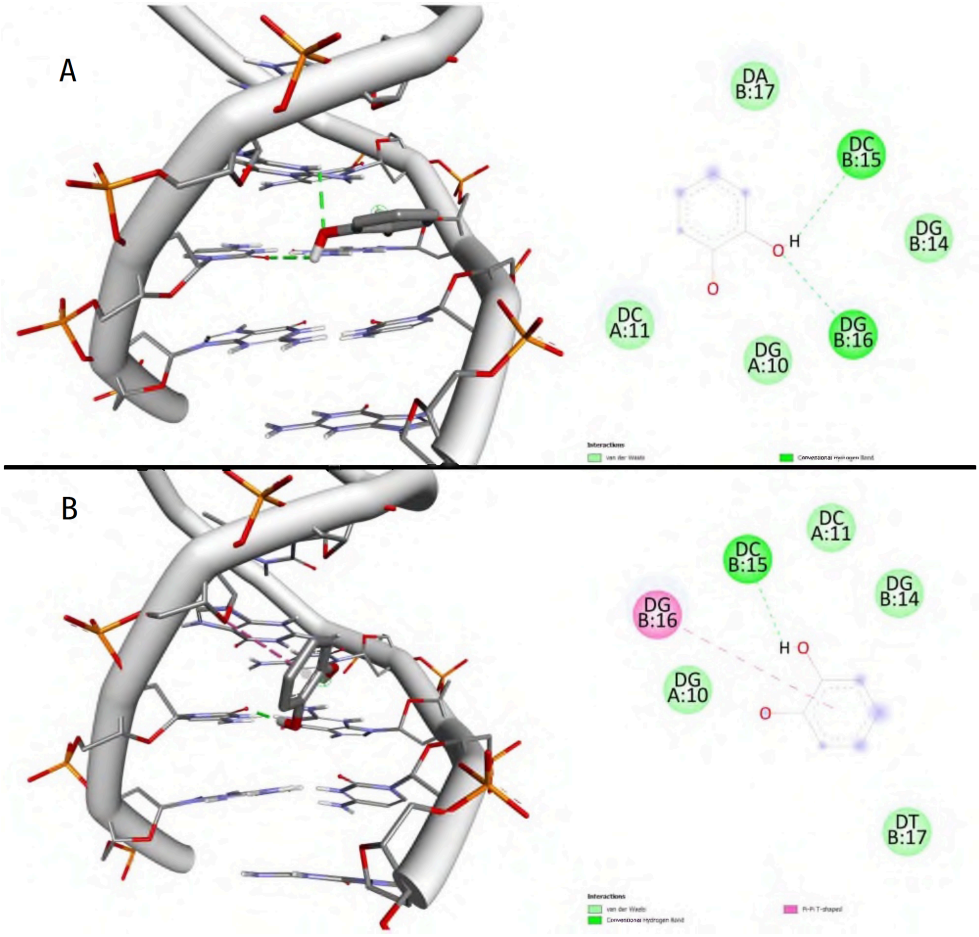


Figure 4. 3D structure of DNA-Catechol complex and 2D visualisation of interactions for PDB:1BNA (A) and PDB:195D (B).

Catechol is a small-ring compound found naturally in plants such as *Semecarpus anacardium*, *Bonellia macrocarpa*, onion, apple, and olive oil. Although literature studies have shown that catechol can be metabolised to compounds such as H_2O_2, semiquinone, and hydroquinone in the presence of metal components, and the formed reactive chemical species cause the destruction of tumour suppressor genes such as p53 and p16, there is no study showing that catechol increases apoptosis in P-H1299, R-H1299, and BJ cells.[35] Therefore, in our study, the cytotoxicity, DNA damage, cell cycle inhibition, and apoptotic effects of catechol in parental and drug-resistant lung cancer and healthy fibroblasts were compared. In the present study, we found that parental and resistant H1299 cells were 3.5 and 2.3 times more sensitive to the cytotoxic effect of catechol (IC_{50}), respectively, compared with BJ cells. In addition, parental cells were approximately 1.5 times more sensitive than resistant cells. In a study in which the growth inhibition of catechol was measured by the MTS test, it was observed that 40 µM catechol concentration did not have a toxic effect on normal lung cancer cells (NL20) but suppressed growth in murine lung cancer (KP2) and human large cell lung (H460) cells.[19] The cytotoxic effect of the catechol compound (SA-3C) isolated from *S. anacardium* on human leukaemia cell lines (CEM), drug-resistant leukaemia cells (CEM/VLB), breast cancer (MCF-7), colon cancer (SW620), and drug-resistant colon cancer (SW620Ad300) cells was higher than that observed in healthy blood mononuclear cells. The cytotoxic effect of SA-3C on leukaemia and colon cancer cells was evaluated in both parental and resistant cells, and the sensitivity was found to be the same.[21]

Catechol has cytotoxic effects on cells and reduces cell proliferation. After catechol exposure, a decrease in the mRNA expression of the DNA polymerase component PCNA, which is a marker of antiproliferative effect in the cell, was observed. In our study, we determined that P-H1299 cells were 8% more sensitive to the effect of catechol on PCNA mRNA expression (IC_{50}) than BJ cells, whereas resistant cells showed resistance at a rate of 51. Parental cells were determined to be 121% more sensitive to catechol (IC_{50}) than resistant cells. After catechol treatment, PCNA expression levels were found to be lower in cells than in the control. The effect of catechol on PCNA was demonstrated for the first time in the present study, that catechol reduces proliferation and slows down the cell cycle. In the present study, we determined that cyclin D1 mRNA expression was 3% lower in P-H1299 cells treated with catechol (IC_{50}) than in BJ cells and 33% higher in resistant cells. Parental cells were determined to be 53% more sensitive than catechol-resistant cells. Lim et al. conducted a study examining the effects of catechol on the CDK-cyclin complex, which are cell cycle regulators in murine and human lung cancer (KP2 and H460), by examining the fluorescence images of propidium iodide staining. They noted a dose-dependent decreases in the expression of CDK2, CDK4, and their regulatory partners, cyclin E, and cyclin D1, in both cell lines.[19] Flow cytometry analysis revealed that the cell cycle was inhibited in a dose-dependent manner in parental and multidrug-resistant leukaemia cancer (CEM and CEM/VLB) cells exposed to the catechol compound (SA-3C) isolated from *S. anacardium*. In CEM cells, the percentage of cells in the G0/G1 stage was 0.4% in the control group and increased to 10.8% after catechol exposure. Similarly, the rate of cell cycle blockade in the G2/M phase increased by 2 times compared to the control. In CEM/VLB cells, cell cycle inhibition in the G0/G1 stage was increased approximately 7 times compared with the control group.[21] Based on the results of this study, we can conclude that parental cells are affected by catechol approximately 1.5 times more than resistant cells.

Catechol causes damage by affecting different molecules in the cell. One of the most important events in the initiation of the death signal in cells is the occurrence of DNA damage. The mechanisms of many known anticancer drugs are based on the principle of DNA damage in cancer cells. Therefore, to examine the effect of catechol on DNA, the mRNA expression levels of ATM and ATR, which are DNA damage response mechanism proteins, were investigated. According to our results, ATM and ATR mRNA expressions in P-H1299 cells treated with catechol (IC_{50}) were 8% and 28% higher, respectively, and 25% and 34% lower in R-H1299 cells compared with BJ cells. Additionally, ATM and ATR mRNA expression was 45% and 94% higher, respectively, in catechol-treated P-H1299 cells than in R-H1299 cells. A previous study showed that catechol can induce p53-dependent but ATM/ATR-independent cell death in MCF-7 cells, whereas catechol can induce p53-independent and ATM/ATR-dependent cell death in MDA-MB-231 cells. Increased expression of Cyclin D1, ATM, and ATR in the cell indicates severe damage to the cell cycle and DNA.

The cell is expected to undergo apoptosis, a programmed death pathway, because of this damage. BID, a pro-apoptotic protein, inactivates Bcl-2, an anti-apoptotic protein, and activates Bax and Bak following apoptotic stimulation by intracellular signals. Bax and Bak enter mitochondria and release cytochrome c. Cytochrome c release induces the formation of the apoptosome-associated caspase-activating complex with other cytoplasmic components. This complex activates procaspase-9, which in turn activates procaspase-3. Apoptosis occurs due to DNA damage.[36] Therefore, in the present study, the Bax/Bcl-2 mRNA expression ratio and

caspase-3 enzyme activation, which are essential markers of apoptosis, were studied. The Bax/Bcl-2 ratio increased 1.5 and 2 times in P-H1299 and R-H1299 cells, respectively. This ratio was calculated as 0.80 in BJ cells, which are healthy cell lines, remaining below the value of 1. This indicates that catechol causes a trend towards the proapoptotic pathway in P-H1299 and R-H1299 cells and towards the antiapoptotic pathway in BJ cells. In one study, the expression level of antiapoptotic Bcl-XL protein was decreased in breast cancer (MCF-7) and resistant breast cancer (MDA-MB-231) cells treated with 80 µM catechol. When treated with different catechol concentrations, a decrease in Bcl-2 expression and an increase in Bax expression were observed in both cell lines. The increased Bax/Bcl-2 ratio in both cell lines indicates that the apoptotic pathway is activated.[37] In the present study, parental and resistant H1299 cells exposed to catechol (IC_{50}) had 1.75- and 1.43-fold higher caspase activity than BJ cells, respectively. The caspase activity of parental cells is approximately 1.2 times higher than that of resistant cells. In a study, when MCF-7 and MDA-MB-231 caspase-3 and caspase-9 expressions were evaluated by western blotting after catechol exposure, it was noted that active caspase-9 expression increased in both cell lines. While caspase-3 expression was increased in MDA-MB-231 cells, caspase-3 expression was not observed in MCF-7 cells, which are cells known for caspase-3 gene deficiency.[37]

In addition to the experimental data, molecular docking studies were conducted to better elucidate the apoptotic mechanism of catechol apoptosis in parental and Epirubicin-resistant cells. In our study, Epirubicin, a well-known DNA intercalating agent, was chosen as the reference anticancer drug in the molecular docking study because epirubicin-resistant cells were involved in our study. Epirubicin results were tested using doxorubicin. In our study, catechol was modelled on appropriate DNA chains.[38] Accordingly, in this study, it bound mostly to DNA chains via guanine nucleotides. Doxorubicin and Epirubicin exhibited binding on minor grooves of the DNA models. Although the inhibition concentration and IC_{50} value are not equivalent, there is a correlation between them.[39] In the literature, the IC_{50} value of doxorubicin in H1299 cells was found to be ~0.10 µM, while the IC_{50} value of catechol was found to be 58 µM in the present study, and these results are consistent with the inhibition coefficients obtained from the docking study.[40] However, catechol showed lower binding affinity than doxorubicin and Epirubicin in docking studies. In this case, catechol causes DNA damage through ROS generated from Fenton reactions in cells instead of acting as an intercalating agent.

CONCLUSION

Based on our experimental data obtained, it was observed that catechol induces apoptotic effects by activating multiple signaling pathways. In addition, catechol-induced ATM and ATR signaling pathways are the most common in P-H1299, R-H1299, and BJ cells, which is explained by the presence of single and double-strand breaks in DNA. When we focus on theoretical data other than experimental data, catechol is thought to cause DNA damage through increased ROS activity, not through the intercalation mechanism like Epirubicin. Although intracellular ROS levels were not directly measured in this study, several prior studies have demonstrated that catechols can autoxidize to form ROS and reactive quinones.[41,42] Combined with our observation of elevated ATM/ATR expression and molecular docking results, these findings support the hypothesis that catechol induces DNA damage via ROS rather than intercalation. The fact that the cytotoxic effect of catechol is lower in Epirubicin-resistant cells than in parental cells but approximately 2-fold higher than in healthy cells can be explained by this theory. Our study is the first to characterise the apoptotic effect of catechol on parental and R-H1299 cells compared with healthy cells and may guide possible future clinical strategies. Catechol's favourable safety profile, cancer cell specificity, and multi-target signaling suggest that it should be developed as a safe and effective chemo-drug against lung cancer.

Acknowledgements: The authors would like to thank TÜBİTAK for the support they provided within the scope of the 2210-A General Domestic Graduate Scholarship Program throughout this study. This work was supported by Akdeniz University (Grant numbers FYL-2022-5902). Author Zeynep DEMİR and Aysun OZKAN have received research support from the Scientific Research Projects Commission.

Ethics Committee Approval: Ethics committee approval is not required for the study.

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- Z.D., A.Ö.; Data Acquisition- Z.D., A.Ö., B.S.; Data Analysis/Interpretation- Z.D., A.Ö., B.S.; Drafting Manuscript- Z.D., A.Ö.; Critical Revision of Manuscript- Z.D., A.Ö., B.S.; Final Approval and Accountability- Z.D., A.Ö., B.S.

Conflict of Interest: Authors declared no conflict of interest.

Financial Disclosure: Authors declared no financial support.

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