

Molecular Effects of Selected Copper Compounds on Novel Oncogenic Signaling Marker Expression in Cancer Cells

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ABSTRACT

This study evaluated the cytotoxic, apoptotic, and gene expression–modulatory effects of CuSO₄, CuCl₂, and CuCO₃ on L929 fibroblasts, DLD-1 colorectal cancer cells, and Capan-1 pancreatic cancer cells. Cells were exposed to concentrations ranging from 7.8 to 500 µM for 24 h. Cell viability was assessed by MTT assay, apoptosis/necrosis by double staining, and gene expression (EZH2, PGK1, HER2, USP28, AURKA, HPRT1, ER-α) by qPCR. At 500 µM, CuSO₄, CuCl₂, and CuCO₃ showed no significant cytotoxicity in L929 cells, with viability values of 99.81±9.4%, 90.69±5.58%, and 104.66±1.48%, respectively. In DLD-1 cells, limited cytotoxicity was observed, with viability remaining above 58% at most concentrations. In contrast, a pronounced, concentration-dependent cytotoxic effect was detected in Capan-1 cells. At 500 µM, viability decreased to 37.28±1.61% (CuSO₄), 36.28±3.29% (CuCl₂), and 31.34±4.93% (CuCO₃). At 250 µM, viability ranged between 42.66±3.44% and 53.50±4.11%, indicating sustained cytotoxic activity. Double staining analysis demonstrated that copper-induced cell death occurred predominantly via apoptosis. In Capan-1 cells, the apoptotic index reached up to 74.2% for CuCl₂ and showed a dose-dependent increase across all compounds, while necrotic rates remained comparatively low. qPCR results revealed significant modulation of cancer-related genes in Capan-1 cells. EZH2 expression was consistently downregulated, particularly in CuSO₄ and CuCO₃ groups. PGK1 and AURKA expression exhibited concentration-dependent changes, with peak upregulation observed at intermediate doses (notably 125–250 µM). HER2 and ER-α expression levels increased across most treatment groups, whereas USP28 expression was generally decreased, with compound-specific variations. HPRT1 expression showed a marked reduction and was nearly undetectable in CuCO₃-treated groups. In conclusion, CuSO₄, CuCl₂, and CuCO₃ exhibit selective and dose-dependent cytotoxic and pro-apoptotic effects in pancreatic cancer cells, while sparing healthy fibroblasts. These compounds also induce significant alterations in gene expression profiles associated with proliferation, metabolism, and tumor progression, highlighting their potential as candidates for targeted anticancer strategies.

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INTRODUCTION

Cancer is recognized as the predominant cause of death in developed nations and the second most common cause of mortality in developing countries (WHO, 2008; Jemal et al., 2011). Pancreatic cancer is a rapidly progressing malignant tumor with a poor prognosis and high mortality rate (Watanapa et al., 1992; Tempero et al., 2011). The 5-

year survival rate for pancreatic cancer is less than 10% (Siegel et al., 2023). Pancreatic cancer, a leading cause of cancer-related deaths due to its aggressive nature, is predicted to become the second leading cause in Western countries within the next decade (Neoptolemos et al., 2018). Colorectal cancer, which arises due to various reasons such as dietary habits, smoking, genetic factors,

and aging, is the most observed cancer among digestive system cancers and is a significant cause of cancer-related deaths (Granados-Romero et al., 2017). In the United States, colorectal cancer constitutes the third major cause of cancer-associated mortality, accounting for nearly 9% of deaths among men and 8% among women. Pancreatic cancer ranks fourth, with an estimated 8% mortality rate in both sexes (Siegel et al., 2023).

Copper is an essential metal involved in various biological processes such as oxidative phosphorylation, iron transport, cross-linking in connective tissue, and pigment formation (Robinson and Winge, 2010). It is known that excess copper can cause various diseases in animals, and Cu(II) complexes have specific effects, such as inducing apoptosis in cancer cells, exhibiting cytotoxic selectivity, and copper compounds are antiproliferative anticancer agents due to the accumulation of copper in high concentrations in cancer cells (Krajčiová et al., 2014).

Cancer is a complex disease characterized by molecular alterations in fundamental biological processes such as epigenetic regulation, cellular metabolism, cell cycle control, and hormone signaling. The enhancer of zeste homolog 2 (EZH2), phosphoglycerate kinase 1 (PGK1), human epidermal growth factor receptor 2 (HER2), ubiquitin-specific protease 28 (USP28), aurora kinase A (AURKA), hypoxanthine phosphoribosyltransferase 1 (HPRT1), and estrogen receptor alpha (ER- α) genes, which play a role in these processes, can be considered potential biomarkers in the molecular characterization of the disease, prognosis, and evaluation of treatment response (Wang et al., 2018; Wolff et al., 2018; Cui et al., 2024; Gao et al., 2024; Lu et al., 2024; Varshney et al., 2024; Xu et al., 2025). In this study, the genes EZH2, PGK1, HER2, USP28, AURKA, HPRT1, and ER- α were selected due to their established roles in these processes.

Enhancer of zeste homolog 2, which serves as a histone methyltransferase in the Polycomb repressor complex, is characterized by recurrent mutations in diverse cancers and by high expression levels in many additional tumor types (Kim and Roberts, 2016). PGK1 is a key enzyme involved in ATP synthesis in glycolysis and has been identified as a molecular regulator associated with tumor progression and patient prognosis in many cancer types, including breast cancer (Yang and Han, 2025). HER2 is a key growth factor receptor encoded by the ERBB2 gene and associated with proliferation, invasion, and treatment resistance in cancer cells (Raggi et al., 2025). USP28 is a critical deubiquitinase that regulates the stability of signaling proteins by removing their ubiquitin tags, thereby influencing key biological processes such as cell proliferation, stress response, and carcinogenesis (Wang et al., 2018). AURKA is a serine/threonine kinase involved in cell division by regulating mitosis and is considered an important therapeutic target due to its overexpression in many cancer types and its involvement of oncogenic signaling pathways (Du et al., 2021). HPRT1 is a key enzyme involved in the purine salvage pathway, regulating nucleotide synthesis and DNA repair, and has recently gained attention as a molecular regulator due to its increased expression in many cancer types and its association with tumor development (Lu et al., 2024). ER- α is a key transcription factor regulating cellular proliferation and hormone signaling, and maintaining its stability through intracellular protein complexes can affect treatment response (Jaiswal et al., 2025).

This study aimed to investigate the effects of various concentrations of three different copper compounds

(CuSO₄, CuCl₂, and CuCO₃) on the healthy cell line L929, the colorectal cancer cell line DLD-1, and the pancreatic cancer cell line Capan-1. We hypothesized that copper compounds would exert selective cytotoxic and gene regulatory effects particularly in pancreatic cancer cells.

MATERIALS AND METHODS

Cell Culture

L929 fibroblast cells, Capan-1 pancreatic cancer cells, and DLD-1 colorectal cancer cells were used. 89% DMEM (Biological Industries), 10% Fetal Bovine Serum (FBS; Biological Industries), 1% L-glutamine (Biological Industries), and 1% Penicillin/Streptomycin (Biological Industries) antibiotics were used as complete culture media for all cells. 0.05% Trypsin-EDTA solution (Trypsin-Ethylenediaminetetraacetic acid) was used to remove cells from the culture flasks, and 1X concentration calcium/magnesium-free phosphate buffer (PBS) was used for washing and cleaning. Trypan blue was used for cell counting. Cell viability was determined using the tetrazolium salt MTT (Serva) in the cytotoxicity test. In double staining, apoptosis/necrosis ratios were determined using ribonuclease-A, propidium iodide (Serva), and hoechst 33342 (Serva). All cell culture studies were performed using serological pipettes, culture dishes, and multi-well plates (Corning, USA).

Frozen cells were thawed briefly at 37°C. Thawed cells were transferred to 15 mL Falcon tubes in a sterile Class II laminar flow cabinet. The Falcon tubes were centrifuged at 250 g for 5 minutes. 3 mL of complete culture medium was added to the Falcon tube and, after homogenization, plated into 25 cm² flasks. The flasks were incubated in a 37°C incubator with 5% CO₂.

Sample Preparation

The prepared materials were sterilized under UV light for 15 minutes and mixed with ready-made culture medium at concentrations of 500 μ M, 250 μ M, 125 μ M, 62.5 μ M, 31.25 μ M, 15.625 μ M, and 7.8 μ M. Cell application was performed without delay.

Cytotoxicity Test (MTT)

For this test method, the MTT method described in reference standard 10993-5 was applied according to the standard. A 96-well plate was used for the toxicity test. After cell counting, calculations were made to ensure there were 1.0×10^4 cells in each well according to the number of live cells. In each well of the 96-well plate, 100 μ L of complete culture medium was placed in cells and incubated for 24 hours. After 24 hours, it was checked whether the cells adhered to the well plate surface. The medium in the wells was drained. The prepared samples were added to the cells at concentrations of 500 μ M, 250 μ M, 125 μ M, 62.5 μ M, 31.25 μ M, 15.625 μ M, and 7.8 μ M. Only the medium was added to the negative control group. The cells were incubated with the applied materials for 24 hours. At the end of the incubation, the medium in the well plates was removed, and 50 μ L of MTT (1 mg/mL) solution was added to the wells. After incubation at 37°C for 2-2.5 hours, the MTT solution in the wells was drained, and 100 μ L of MTT solvent (isopropanol) was added. To determine cell viability, absorbance density values from a 96-well plate were read at 570 nm using an ELISA plate reader. The percentage of cell viability for each group was calculated using the equation "Cell viability (%) = Optical Density, OD570 (sample) / Optical Density, OD570 (control) x 100", assuming 100% viability for the control cells.

Determination of Apoptosis and Necrosis by Double Staining Method

Cells were plated in 48-well plates, with 15×10^3 cells per well. The cells were incubated for 24 hours (5% CO₂, 37°C, >90% humidity). After incubation, the media in the plates were discarded, and 200 µL of samples at concentrations of 500 µM, 250 µM, 125 µM, 62.5 µM, 31.25 µM, 15.625 µM, and 7.8 µM were added. A negative control consisting only of cells and culture medium was also added. Incubation was allowed for 24 hours. After incubation, the media in the plates were discarded, 70 µL of double staining solution was added, the plates were covered to prevent light exposure and incubated for 15 minutes. After incubation, apoptotic cells and necrotic cells were evaluated using an inverted microscope (Leica, DM6000B, Germany) with a DAPI filter (Preparation of the double staining solution: ribonuclease A 10 mg/1 mL PBS, hoechst 33342 200 µg/1 mL PBS, propidium iodide 100 µg/1 mL PBS were prepared from stock solutions, and 500 µL hoechst 33342, 100 µL propidium iodide, and 100 µL ribonuclease A were added to 10 mL PBS).

Quantitative Real-Time PCR (qPCR) Analyses

Total RNA was obtained using cell culture samples as specified in the RNA isolation kit (Norgen, Canada)

protocol. The concentration and purity of total RNA were assessed using a nanodrop spectrophotometer (MultiSkan GO, Thermo Scientific, USA). cDNA synthesis reactions, performed using 500 ng of template total RNA, were carried out in a thermal cycler (Bio-Rad, USA) with the ab185916 cDNA synthesis kit (Abcam, UK). Quantification of the resulting cDNA samples was performed using a nanodrop spectrophotometer (MultiSkan GO, Thermo Scientific, USA). Subsequently, expression analyses were performed on these samples in a qPCR system (Bio-Rad, USA) using the primers given in Table 1. The BrightGreen (ABM, Canada) protocol was applied for this stage, and enzyme activation was performed at 95 °C for 10 minutes. PCR reactions were run for 35 cycles: 15 seconds at 95 °C and 60 seconds at 57-60 °C. Relative quantifications of qPCR amplicons were determined using the $\Delta\Delta C_t$ method. These values were calculated by subtracting the ΔC_t values of the control group from the ΔC_t values of the cancer cells. The relative expression of target genes was determined using the equation $2^{-\Delta\Delta C_t}$, and the levels of EZH2, PGK1, HER2, USP28, AURKA, HPRT1, and ER- α genes were normalized using GAPDH as the primary housekeeping gene (Devrim et al., 2025). In addition, HPRT1 expression variability was also evaluated rather than being used as a stable reference gene.

Table 1. Primer sequences used in qPCR analyses.

Gene	Forward Primer	Reverse Primer
EZH2	GGCTCCTCTAACCATGTTTACAAC	AGCGGTTTGTGACACTCTG AACTAC
PGK1	GGGAAAAGATGCTTCTGGGAA	TTGGAAAGTGAAGCTCGGAAA
HER2	CCAGGACCTGCTGAACCTGGT	TGTACGAGCCGCACATCC
USP28	GCAAATGCTGGACACT	ACTTCCTGCTCAAACC
AURKA	GGAATATGCACCACTTGAACA	TAAGACAGGGCATTGCGCAAT
HPRT1	CCTGGCGTCGTGATTAGTGAT	AGACGTTTCAGTCTGTCCATAA
ER- α	GTGGGAATGATGAAAGGTGG	TCCAGAGACTTCAGGGTGCT
GAPDH	CAATGACCCCTTCATTGACC	GACAAGCTTCCCCTTCTCAG

RESULTS

Cytotoxicity results in the MTT test, the absorbance values of the cells were compared with control cells and the % viability values were calculated. For a material to have an anticancer effect in vitro, it should not be toxic to healthy cells (L929) but should be toxic to cancer cells (DLD-1, Capan-1). Furthermore, it is desirable that the dying cells choose the apoptotic pathway as their death pathway, meaning that necrotic death is not present. According to ISO 10993-5, a material is considered toxic if its viability is less than 70%. After drug design or invention, it is necessary to meet the requirements of the Food and Drug Administration (FDA), and according to the FDA, a viability of 50% lethality is considered acceptable for the drug to be effective in drug development studies. According to the MTT test results; CuSO₄ at a concentration of 500 µM showed 99.81±9.4% viability in L929 cells, while DLD-1 and Capan-1 cancer cells showed 96.02±3.96% and 37.28±1.61% viability, respectively. All concentrations of the prepared material did not show a toxic effect on the healthy cell group, L929 fibroblast cells. It was determined that there was no toxic effect on DLD-1 colorectal cancer cells at the applied concentration; although cell viability changed with decreasing concentration, no dose showed the desired toxic effect. However, the toxic effect on Capan-1 cells is noteworthy, and it was found that the toxic effect decreased and viability increased with decreasing concentration (250 µM and 125 µM, respectively) (50.38±3.89% and 63.18±4.07%, respectively).

At a concentration of 500 µM (CuCl₂), 90.69±5.58% viability was recorded in L929 cells, while viability was observed in DLD-1 and Capan-1 cancer cells at 58.79±1.67% and 36.28±3.29%, respectively. All concentrations of the prepared material showed no toxic effect on the healthy cell group, L929 fibroblast cells, and were almost at the same level as the control group cells. In DLD-1 cells, toxic effects were observed at concentrations of 250 µM and 62.5 µM, with viability rates of 62.61±4.17% and 68.63±5.74%, respectively, while no toxic effects were detected at other concentrations. In Capan-1 cells, toxic effects were detected at concentrations of 250 µM, 125 µM, and 62.5 µM, with viability rates of 42.66±3.44%, 46.97±0.66%, and 68.03±0.62%, respectively. The toxic effect decreased in Capan-1 cells as the material concentration decreased.

At a concentration of 500 µM, CuCO₃ showed viability of 104.66±1.48% in L929 cells, while it showed viability rates of 110.46±6.15% and 31.34±4.93% in DLD-1 and Capan-1 cancer cells, respectively. All concentrations of the prepared material did not show a toxic effect on the healthy cell group, L929 fibroblast cells, and were at almost the same level as the control group cells. Although a decrease in cell viability was detected in DLD-1 colorectal cancer cells as the concentration decreased, it did not reach a level that could be considered toxic. However, it was determined that the concentrations applied at 250 µM and 125 µM showed a toxic effect in Capan-1 cells, with viability rates of 53.50±4.11% and 55.88±1.51%, respectively. The toxic effect decreased and

viability increased in Capan-1 cells as the concentration decreased.

In addition to all these results, during microscopic imaging, CuCO_3 , CuCl_2 , and CuSO_4 induced morphological changes such as cell shrinkage and membrane bulging, especially in the Capan-1 cell line. To determine the relationship between this observed phenomenon and apoptosis, a double staining (apoptosis/necrosis) study was conducted.

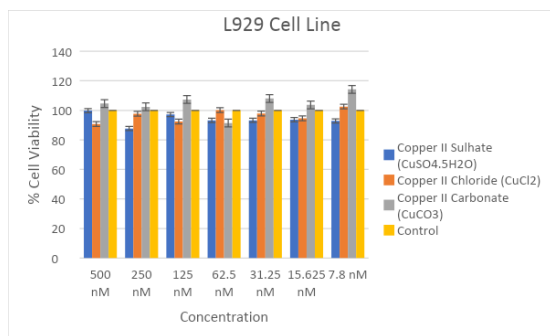


Figure 1. L929 cell line MTT test results.

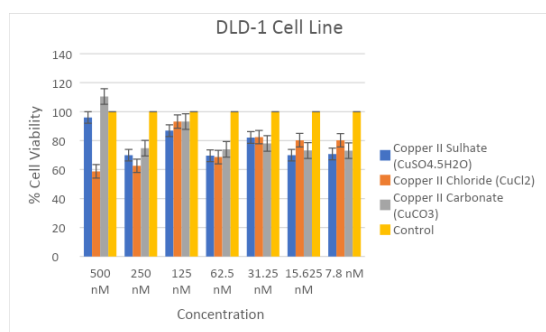


Figure 2. DLD-1 cell line MTT test results.

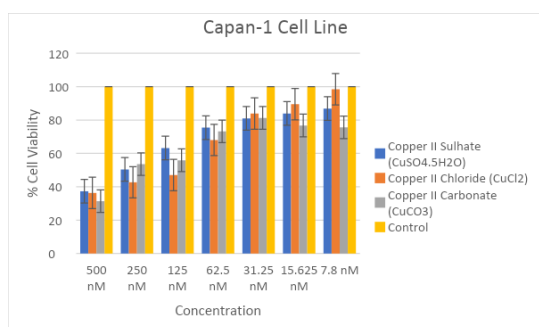


Figure 3. Capan-1 cell line MTT test results.

Double Staining (apoptosis/necrosis) Results

The percentages of apoptosis and necrosis were determined by counting the stained cells using DAPI and FITC filters. The results show that the apoptotic index of CuCO_3 , CuCl_2 , and CuSO_4 applied to DLD-1 and Capan-1 cells was higher than the necrotic index, indicating that the cells predominantly died by apoptosis. Furthermore, in the Capan-1 cell line, the apoptotic index of CuCl_2

increased at concentrations of 500 μM , 250 μM , 125 μM , 62.5 μM , 31.25 μM , 15.625 μM , and 7.8 μM , respectively, with percentages of 74.2%, 67.5%, 65.3%, 35.5%, 24.6%, 22.5%, 3.2%, and 0.5%, respectively, while the apoptotic index of CuSO_4 at the same concentrations was higher. The apoptosis rate was determined to increase with increasing concentrations of CuCO_3 , CuCl_2 , and CuSO_4 , respectively, at the same concentrations of CuCO_3 , CuCl_2 , and CuSO_4 .

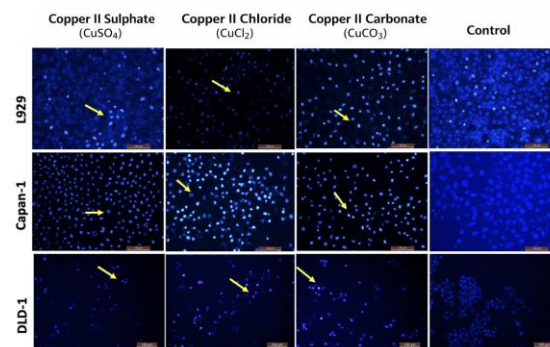


Figure 4. Images of L929, Capan-1, and DLD-1 cells under a DAPI filter after double staining. The material concentration applied in the photographs is 250 μM . Arrows indicate apoptotic cells.

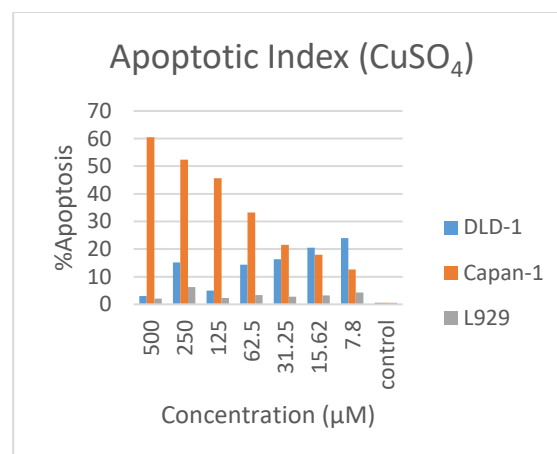


Figure 5. Change in apoptosis rate in DLD-1, Capan-1, and L929 cells depending on CuSO_4 concentration.

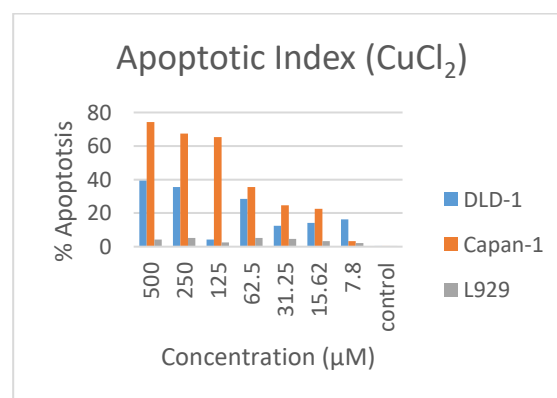


Figure 6. Variation of apoptosis rate in DLD-1, Capan-1, and L929 cells with CuCl_2 concentration.

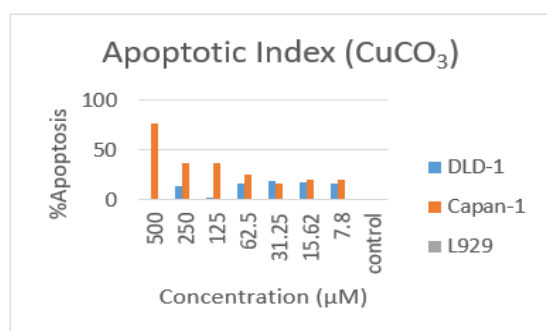


Figure 7. Variation of apoptosis rate in DLD-1, Capan-1, and L929 cells depending on CuCO₃ concentration.

Quantitative Real-Time PCR (qPCR) Analysis Results

When cytotoxicity and apoptosis necrosis findings obtained from different concentrations of CuSO₄, CuCl₂, and CuCO₃ compounds on DLD-1 and Capan-1 cell lines were evaluated, it was decided to use this cell line in the qPCR phase studies due to the clear effect on cell viability in Capan-1 cell lines and the high apoptotic index in Capan-1 cell lines.

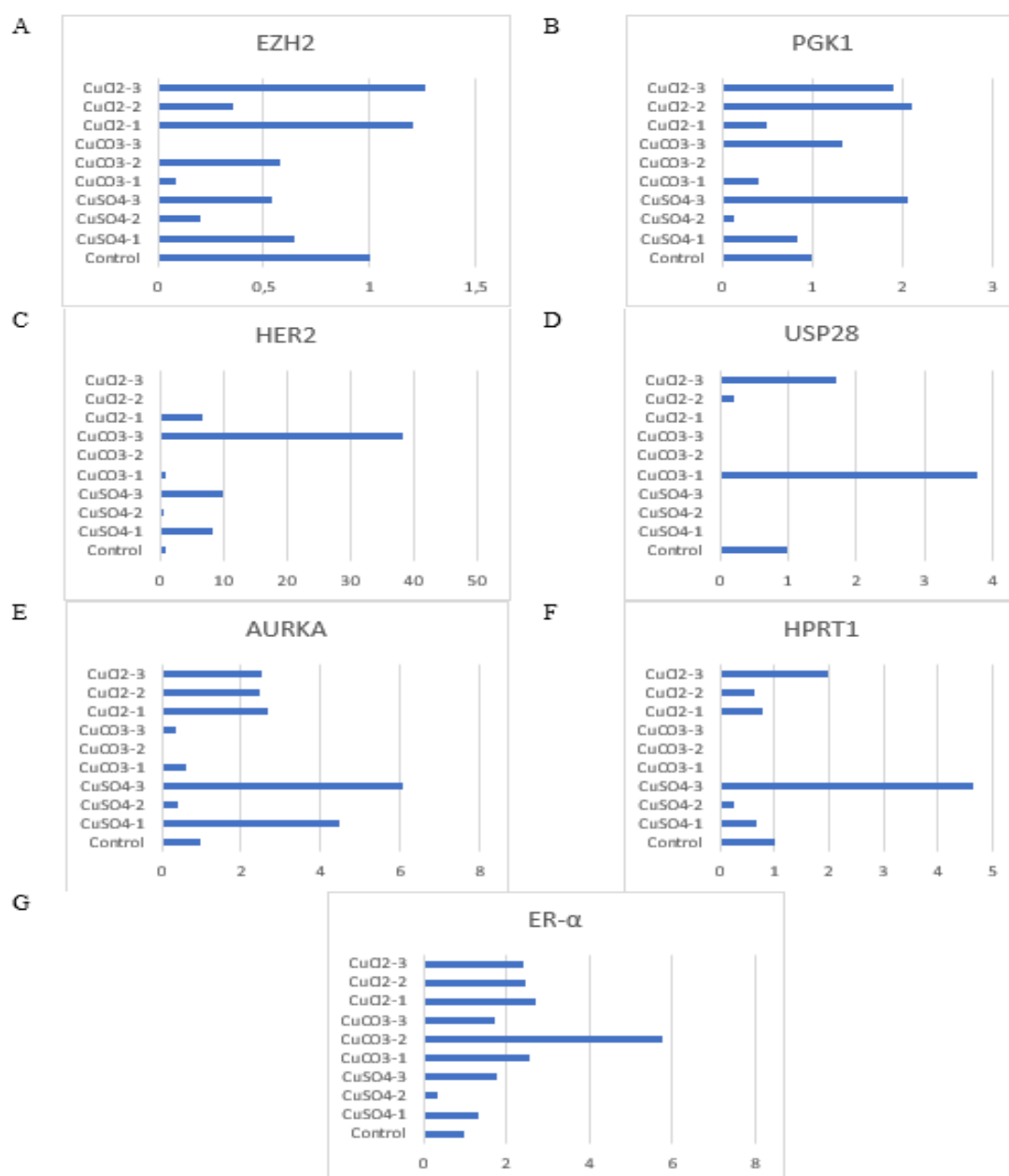


Figure 8. Quantitative real-time PCR (qRT-PCR) analyses of EZH2, PGK1, HER2, USP28, AURKA, HPRT1 and ER-α expression in Capan-1 pancreatic cancer cells treated with CuSO₄, CuCl₂ and CuCO₃. A: EZH2, B: PGK1, C: HER2, D: USP28, E: AURKA, F: HPRT1, G: ER-α. CuSO₄-1: 500 μM, CuSO₄-2: 250 μM, CuSO₄-3: 125 μM, CuCO₃-1: 500 μM, CuCO₃-2: 250 μM, CuCO₃-3: 125 μM, CuCl₂-1: 500 μM, CuCl₂-2: 250 μM, CuCl₂-3: 125 μM. Data are presented as the fold changes.

Compared to the control group, EZH2 gene expression was generally reduced in the groups treated with copper compounds. This reduction was more pronounced with CuSO₄ and CuCO₃, while suppression was more limited in the CuCl₂ group.

When examining the data for the PGK1 gene, the groups treated with copper compounds showed changes in expression levels compared to the control group. Increases in PGK1 gene expression were detected in all three copper compounds, particularly at a concentration of 125 µM. On the other hand, at a concentration of 500 µM, decreases were detected in all three compounds compared to the control. The highest PGK1 RNA expression level occurred at a concentration of 250 µM CuCl₂.

When evaluating HER2 gene RNA expression, it was noted that expression increased significantly in the groups treated with copper compounds at 125 and 500 µM concentrations with CuSO₄. The greatest increase was observed with 125 µM CuCl₂.

The results for the USP28 gene showed a general decrease in expression levels in the groups treated with copper compounds, with this decrease being particularly pronounced in the CuSO₄ group. Increases were observed with 500 µM CuCO₃ and 125 µM CuCl₂ applications.

When evaluating AURKA gene expression, a general upward trend was evident in the groups treated with copper compounds. Although limited decreases were seen in some groups, the dominant response was an increase in expression compared to the control group. This suggests that copper compounds may enhance cell cycle progression and mitotic activity. On the other hand, a gene silencing effect was detected in the AURKA gene at a concentration of 250 µM CuCO₃.

When examining the expression levels of the HPRT1 gene, a general decrease in HPRT1 was detected in the groups treated with copper compounds compared to the control group. Furthermore, it was noted that HPRT1 expression ceased at all three concentrations of copper compounds treated with CuCO₃. In the other two genes, the changes detected at a concentration of 125 µM were noteworthy biological responses.

When RNA expression related to the ER-α gene was evaluated compared to the control group, a consistent increase was observed in the groups treated with copper compounds. This increase was particularly pronounced in the 250 µM CuCO₃ group, while only the CuSO₄ group at the same concentration showed a decrease.

DISCUSSION AND CONCLUSION

Cancer is a complex disease closely related to the mechanism of programmed cell death caused by various factors. Apoptosis is one of the main mechanisms of programmed cell death (Mishra et al., 2018). Copper and copper-based biomaterials currently have various applications in cancer imaging and cancer treatment (Zhong et al., 2022). Because copper activates apoptosis, it can be used in the elimination of cancer cells (da Silva et al., 2022).

CuCl₂ and CuSO₄ are novel compounds exhibiting interesting cytotoxic activity against cancer cell lines and are considered to have potential to be selective for cancer cells compared to non-cancerous cells. CuCO₃, CuCl₂, and CuSO₄ have yielded remarkable results in terms of selectivity against metastatic Capan-1 pancreatic cancer cells. Characterizing the antitumor activity against this cancer type and determining the cellular pathways is considered important for the effective use of the drug.

When we examined the effects of CuSO₄, CuCl₂, and CuCO₃ compounds at various concentrations, we found that they did not show a significant toxic effect in the healthy cell line L929 and the colorectal cancer cell line DLD-1. However, when we examined the effects of these compounds in the pancreatic cancer cell line Capan-1, a toxic effect was found. Subsequently, double staining was performed to determine whether the cell death pathway was apoptosis or necrosis. When the cells were examined under a fluorescence microscope after double staining, it was found that their apoptotic index was higher than their necrotic index.

In this study, the molecular effects of CuSO₄, CuCl₂, and CuCO₃ compounds on the pancreatic cancer cell line Capan-1 were evaluated through the expression levels of genes associated with proliferation, metabolism, and tumor progression. qPCR findings show that copper compounds produce selective and multidimensional gene expression modulation in cancer cells compared to the control group. Since previous studies have reported that EZH2 is overexpressed in pancreatic cancer cells in association with proliferation, tumor progression, and treatment resistance, and that suppression of EZH2 can limit tumor growth, it is thought that the decrease in EZH2 gene expression observed in Capan-1 cells treated with copper compounds in this study may indicate a potential antitumor effect through epigenetic mechanisms (Ougolkov et al., 2008).

Liang et al. (2020) reported that PGK1 can modulate the balance between proliferation and metastasis in pancreatic cancer depending on its cellular localization and metabolic phenotype. In our study, copper compounds were observed to modulate PGK1 expression in cancer cells; this is likely related to cellular energy balance and stress response mechanisms. However, the increases or decreases observed at different concentrations and in different cell lines suggest that PGK1 regulation varies depending on cellular binding and compound properties. These findings indicate that the functional effects of changes in PGK1 expression, such as glycolysis rate, ATP production, and cell proliferation, should be investigated in more detail in future studies.

The literature reports that transition metals, particularly copper, can modulate cell proliferation and growth responses by influencing intracellular signaling pathways (Turski and Thiele, 2009). In our study, copper compounds were observed to increase HER2 expression. This finding suggests that copper may influence oncogenic gene expression by modulating intracellular signaling pathways. This may provide preliminary insights into the potential pro-tumor effects of copper in cancer cells.

The role of the USP28 gene in cancers has been reported in the literature as both tumor suppressor and oncogenic (Prieto-Garcia et al., 2021). In our study, a general decrease in USP28 expression was observed with the application of copper compounds, but increases were also detected in some groups. These results suggest that the role of USP28 may vary depending on the cell type and the dose of the applied compounds.

When AURKA gene expression was evaluated, a general upward trend was observed in the groups treated with copper compounds. Although limited decreases were seen in some groups, the dominant response was an increase in expression compared to the control group (Barr ve Gergely, 2007). This may indicate a potential association with enhanced cell cycle progression and mitotic activity, considering that Aurora kinase A is a key

regulator in the cell cycle, particularly in the G2/M transition, centrosome maturation, and mitotic spindle formation (Barr and Gergely, 2007).

Although HPRT1 is frequently used as a reference “housekeeping gene,” it has been previously reported that its expression can vary under different cellular stress conditions and experimental applications (Suzuki et al., 2000; Vandesompele et al., 2002). This suggests that HPRT1 may not always be a stable reference gene under experimental conditions and should be carefully evaluated, especially in studies involving metal ions or metabolic stress-inducing agents. In this study, it is unclear whether the decrease in HPRT1 expression is transcriptional or a secondary effect to decreased cell viability. Future studies examining cell proliferation, apoptosis markers, and other purine metabolism genes (e.g., IMPDH, GMPS) together will provide a clearer understanding of the mechanism of copper compounds.

It is well-established in the literature that increased ER- α expression can support hormone-dependent tumor growth by activating estrogen-mediated proliferative and anti-apoptotic signaling pathways (Ali and Coombes, 2002; Nilsson and Gustafsson, 2011). Increased ER- α expression in the study groups suggests that hormone-dependent growth may be supported in cancerous cells. This raises the possibility that copper compounds may have the potential to increase proliferation in hormone-sensitive tumors and influence treatment resistance or tumor progression via ER- α . Therefore, the use of copper-based compounds as anticancer agents should be carefully evaluated in terms of their effects on hormone receptor expression.

In conclusion, copper-based compounds can modulate molecular pathways associated with proliferation, metabolism, and tumor progression in pancreatic cancer cells. The more pronounced antitumor potential of CuSO₄ and CuCl₂, in particular, necessitates detailed investigation of their mechanisms of action through further molecular and functional studies. On the other hand, while this study evaluated RNA expression of genes, verification of protein levels, investigation of functional activity, and examination of different dose-time effects are important for future research. These findings are limited to in vitro conditions and require further validation.

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Ethical Declaration

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

Authorship Contributions

Concept: S.E., A.K.D., Design: S.E., A.K.D., E.A.A., Data Collection or Processing: A.Ş., B.M., C.Ç.Ç., Analysis or Interpretation: A.K.D., E.A.A., B.M., C.Ç.Ç., Literature Search: S.E., A.K.D., B.M., Writing: S.E.

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