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COMBINATION THERAPY OF CISPLATIN AND NANOCURCUMIN REDUCES PI3K EXPRESSION AND PROLIFERATION OF HeLa CELLS

Terapi Kombinasi Cisplatin dan Nanokurkumin Menurunkan Ekspresi PI3K dan Proliferasi Sel HeLa

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ABSTRAK

Kanker serviks merupakan salah satu penyebab utama kematian akibat kanker pada wanita, terutama di negara berkembang. Cisplatin adalah obat kemoterapi yang umum digunakan, tetapi penggunaannya dalam dosis tinggi sering menyebabkan efek samping toksik seperti kerusakan ginjal (nefrotoksisitas), yang dapat terjadi pada 25–33% pasien pada dosis 75–100 mg/m². Untuk mengurangi toksisitas dan meningkatkan efektivitas terapi, kombinasi cisplatin dengan senyawa alami seperti nanokurkumin mulai dikembangkan. Penelitian ini bertujuan untuk mengevaluasi potensi kombinasi cisplatin dan nanokurkumin dalam menghambat proliferasi dan menurunkan ekspresi PI3K pada sel kanker serviks HeLa. Penelitian ini menggunakan desain eksperimental dengan post-test only control group. Sel HeLa diberi perlakuan cisplatin (2,5 dan 5 µg/mL), nanokurkumin (100 µg/mL), serta kombinasi cisplatin 2,5 µg/mL dengan nanokurkumin (25, 50, dan 100 µg/mL). Evaluasi mencakup morfologi sel, uji viabilitas menggunakan CCK-8, dan analisis ekspresi PI3K menggunakan flow cytometry. Analisis statistik dilakukan dengan uji Kruskal-Wallis, dilanjutkan uji post hoc Dunn, dengan nilai signifikansi p < 0,05. Hasil menunjukkan bahwa kombinasi cisplatin 2,5 µg/mL dan nanokurkumin 100 µg/mL secara signifikan menurunkan viabilitas sel dibandingkan cisplatin 2,5 µg/mL saja (p = 0,035), serta memiliki efektivitas yang sebanding dengan cisplatin 5 µg/mL (p = 0,553). Penurunan ekspresi PI3K juga signifikan dibandingkan kontrol negatif (p = 0,000), cisplatin 2,5 µg/mL (p = 0,006), dan cisplatin 5 µg/mL (p = 0,010). Hasil ini menunjukkan bahwa kombinasi cisplatin dan nanokurkumin berpotensi sebagai terapi kanker serviks yang efektif.

***Kata kunci**: cisplatin, ekspresi PI3K, nanokurkumin, pengobatan kanker, sel HeLa*

ABSTRACT

Cervical cancer remains a leading cause of cancer-related mortality among women, especially in developing countries. Cisplatin is a standard chemotherapeutic agent, but its high-dose use is associated with severe side effects, including nephrotoxicity, affecting up to 25–33% of patients at doses of 75–100 mg/m². To enhance therapeutic efficacy while reducing toxicity, combining cisplatin with natural compounds such as nanocurcumin has gained attention. This study aimed to elucidate the effects of combining cisplatin and nanocurcumin in inhibiting cell proliferation and suppressing PI3K expression in HeLa cervical cancer cells. An experimental post-test only control group design was used. HeLa cells were treated with cisplatin (2.5 and 5 µg/mL), nanocurcumin (100 µg/mL), and combinations of cisplatin 2.5 µg/mL with nanocurcumin (25, 50, and 100 µg/mL). Assessments included cell morphology, viability using the CCK-8 assay, and PI3K expression via flow cytometry. Statistical analysis used the Kruskal-Wallis test followed by Dunn's post hoc, with significance set at p < 0.05. The combination of cisplatin 2.5 µg/mL and nanocurcumin 100 µg/mL significantly reduced

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cell viability compared to cisplatin 2.5 µg/mL alone (p = 0.035) and showed comparable efficacy to cisplatin 5 µg/mL (p = 0.553). PI3K expression was also significantly reduced compared to the negative control (p = 0.000), cisplatin 2.5 µg/mL (p = 0.006), and cisplatin 5 µg/mL (p = 0.010). These results suggest that combining cisplatin with nanocurcumin could be a more effective strategy for cervical cancer therapy.

Keywords: cisplatin, nanocurcumin, PI3K expression, HeLa cells, cancer treatment

INTRODUCTION

Cervical cancer ranks as the second most prevalent cancer among women in Indonesia, and it is the second leading cancer type in women aged 15 to 44 years.[1] According to recent epidemiological data, approximately 660,000 new cervical cancer cases were reported globally in 2022, with an estimated 350,000 deaths, predominantly occurring in low- and middle-income countries where access to Human Papillomavirus (HPV) vaccination and early screening programs is limited. This disparity highlights the disproportionate burden of cervical cancer in regions with low Human Development Index (HDI), such as parts of Africa and Asia, compounded by inadequate healthcare infrastructure and low public awareness. [2,3]

The impact of cervical cancer is not only mortality, but also imposes significant psychosocial and economic burdens on affected women and their families. It predominantly affects women of reproductive age, leading to loss of productivity and social roles, and adversely affecting dependents, including children who lose their primary caregivers.[4] Consequently, there is an urgent need for innovative, effective, and safer therapeutic strategies to reduce mortality and improve patients' quality of life.

Cisplatin, a platinum-based chemotherapeutic agent, is widely used in advanced cervical cancer for its ability to inhibit DNA replication and induce apoptosis.[5] When combined with radiotherapy, it improves five-year survival rates by 10–20% compared to radiotherapy alone.[6] However, its clinical use is often limited by severe toxicities such as nephrotoxicity, neurotoxicity, and gastrointestinal complications, which reduce patient tolerance and long-term efficacy.[7] A randomized controlled trial by Katke, A. et al, reported grade ≥3 acute toxicities in 35% of patients receiving weekly cisplatin with radiotherapy, while multi-drug regimens showed even higher rates (45% grade 3, 12% grade 4). Late toxicities have been reported in 13–16% of cases.[8] Moreover, cisplatin at doses of 75–100 mg/m² is linked to increased nephrotoxicity, that caused limiting treatment options. Moderate to severe kidney damage occurs in 25–33% of patients, with high doses (100 mg/m²) posing greater risks of irreversible injury.[9]

To address these limitations, combination chemotherapy strategies incorporating natural compounds have gained attention. Curcumin, a bioactive compound derived from turmeric, exhibits antiinflammatory, antioxidant, and antiproliferative properties, making it a promising adjunct in cancer therapy.[10] However, curcumin's clinical application is hindered by its poor bioavailability.

Nanocurcumin, a nanoparticle formulation of curcumin, enhances bioavailability, chemical stability, and therapeutic efficacy compared to conventional curcumin, which suffers from poor absorption and rapid metabolism.[11] Curcumin has poor water solubility and is rapidly degraded in the body, resulting in very low plasma levels even after high oral doses. [12] To overcome these limitations, nanocurcumin has been developed as a more effective delivery system. It has demonstrated the ability to potentiate chemotherapy effects by promoting apoptosis, inhibiting proliferation, and modulating proinflammatory and pro-survival signaling pathways in cancer

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cells.[13] The combination of cisplatin and nanocurcumin is considered to have synergistic potential in optimizing the effectiveness of cervical cancer treatment. [14]

The phosphatidylinositol-3-kinase (PI3K) pathway plays a critical role in cancer cell proliferation and survival, and its dysregulation is implicated in cervical carcinogenesis.[15] Despite this, comprehensive studies investigating the impact of cisplatin and nanocurcumin combinations on PI3K expression in cervical cancer cells remain scarce. Therefore, this study aims to elucidate the effects of combined cisplatin and nanocurcumin therapy on HeLa cell proliferation and PI3K expression, providing foundational data to support the development of novel combination therapies for cervical cancer.

METHODS

This in vitro laboratory experimental study with a quantitative approach aimed to elucidate the effects of combining cisplatin and nanocurcumin on cell proliferation and PI3K expression in HeLa cells. The cultured HeLa cells were divided into seven groups: a negative control roup without any treatment, receiving only complete culture medium; three positive control groups consisting of cells treated with cisplatin at 2.5 µg/mL, cisplatin at 5 µg/mL, and nanocurcumin at 100 µg/mL, respectively; and three treatment groups receiving a combination of cisplatin at 2.5 µg/mL with nanocurcumin at 25 µg/mL, 50 µg/mL, and 100 µg/mL. Cell proliferation was measured using the Cell Counting Kit-8 (CCK-8) assay, while PI3K expression levels were analyzed using flow cytometry.The research was conducted from January to March 2025 at the Biomedical Laboratory, Faculty of Medicine, Universitas Brawijaya.

Ethical Agreements

This research was carried out at the Biomedical Laboratory, Faculty of Medicine, Universitas Brawijaya, and has obtained ethical approval from the Research Ethics Committee of Faculty of Medicine, Universitas Brawijaya University (No: 123/KEP/FK/2024). All procedures adhered to established biosafety and bioethics guidelines, including the safe handling of hazardous.

Cisplatin and Curcumin Preparation

The nanocurcumin stock solution, obtained from the nanoencapsulation process, was diluted into working solutions at concentrations of 25 µg/mL, 50 µg/mL, and 100 µg/mL. These concentrations were selected based on literature and preliminary experiments using the available formulation. The chosen doses were adapted from Aldahoun et al. (2016) and validated through preliminary viability tests [16]. The dilution was carried out using sterile complete culture medium and calculated using the standard dilution formula $M_1 \times V_1 = M_2 \times V_2$, with a final volume of 15 mL for each working solution.

Cisplatin working solutions were prepared from a commercial cisplatin injection (Kalbe, 1 mg/mL). A 5 µg/mL stock solution was further diluted using sterile complete culture medium to obtain a 2.5 µg/mL working concentration, used for both single and combination treatments. Dose selection was based on previous literature and preliminary tests using the available cisplatin preparation. The selected concentrations 2.5 µg/mL and 5 µg/mL (equivalent to 8.33 µM and 16.66 µM, respectively) were within the effective cytotoxic range for HeLa cells after 24-hour incubation, as supported by Becit et al. (2020), while remaining at levels that permit the observation of potential synergistic effects when combined with nanocurcumin.[17] Dilutions were calculated to yield a final volume of 15 mL for each working solution. Cisplatin solutions were stored at 2–8°C, protected from light, and used within 24 hours to ensure stability during the experiment.

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Nanocurcumin Formulation and Characterization

Nanocurcumin used in this study was formulated as nanoliposomes via the thin film hydration method. Curcumin was extracted from *Curcuma longa rhizomes* through ethanol maceration, followed by solvent evaporation to yield a concentrated extract. The liposomal formulation was prepared by dissolving curcumin, phosphatidylcholine (S-100), cholesterol, and Tween 80 in chloroform. After solvent removal using a rotary evaporator at 45–50°C, the resulting lipid film was hydrated with PBS containing Tween 80 and sonicated to produce uniform nanoliposomes. Particle size analysis (Shimadzu SALD-7500nano) showed an average diameter of 32 nm. HPLC analysis confirmed curcumin as the major component, with chromatographic properties matching those of the reference standard (Merck, Cat. No. 8.20354.0010.

Cell Culture

HeLa cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) and 1% Penicillin-Streptomycin (PenStrep) to prevent microbial contamination. The medium components were sterilely mixed and stored at 2–8°C until use. Cells were maintained in 25 cm² culture flasks in a humidified incubator at 37°C with 5% CO_2 to simulate physiological conditions. Cell growth was monitored daily using an inverted microscope, and cell viability was assessed before treatment using the trypan blue exclusion method. When cultures reached approximately 80% confluence, cells were harvested using 0.25% trypsin-EDTA and incubated for 3–5 minutes at 37°C to promote detachment. The cell suspension was then neutralized with complete medium, collected into sterile conical tubes, and centrifuged at 800 rpm for 5 minutes at room temperature. The resulting pellet was resuspended in fresh medium, and total cell count was determined using a hemocytometer. Cell suspensions were then adjusted to appropriate densities for different assays. For the cell viability assay using the CCK-8 kit, cells were seeded into 96-well plates at a density of 5×10^3 cells per well in 100 µL medium. For PI3K expression analysis via flow cytometry, cells were seeded into 6-well plates at a density of 2×10^5 cells per well in 2 mL medium. Plates were incubated overnight to allow cell attachment before treatment was applied.

Cell Viability Assay

HeLa cells were counted and seeded into 96-well plates at a density of 5×10^3 cells per well in 100 µL complete medium. Each treatment group was performed in triplicate (n = 3) to ensure reproducibility. After overnight incubation to allow cell attachment, treatments were applied according to the experimental design. Negative control wells contained untreated cells, while blank wells contained only medium and CCK-8 reagent (no cells) to correct background absorbance.

After treatment, 10 µL of Enhanced Cell Counting Kit-8 *(WST-8/CCK8, Cat. No.: E-CK-A362*) reagent was added to each well. The plates were incubated at 37°C in a humidified 5% CO_2 incubator for 2 hours as the standard incubation period. Absorbance was measured at 450 nm using a microplate reader (Zenix-320). Raw absorbance values were corrected by subtracting the mean absorbance of the blank wells.

Cell viability was calculated using the following formula [18]:

$$Cell\ viability\ (\%) = \left(\frac{Absorbance\ of\ Treatment}{Absorbance\ of\ Control}\right) x\ 100$$

Flow Cytometry Analysis

PI3K protein expression in HeLa cells was evaluated using flow cytometry. Antibody validation was performed in a preliminary test to determine specificity and optimal dilution of the anti-PIK3CA antibody (BS2067R-PerCP, Bioss), conjugated with PE fluorochrome, including titration and isotype control to reduce nonspecific binding. A total of 2×10^5 to 1×10^6 cells per sample were fixed and

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permeabilized using CytoFast™ Fix/Perm Buffer, washed with Perm Wash Buffer, and stained with 50 µL of anti-PIK3CA (1:50) for 20 minutes in the dark at room temperature. After two washes with Cell Staining Buffer, cells were resuspended in 300 µL buffer.

Samples were analyzed using a BD FACSCalibur™ flow cytometer (BD Biosciences, USA). PI3K-PE fluorescence was detected in FL2 (Y-axis), with Forward Scatter Height (FSC-H) on the X-axis. Gating was performed using FSC vs SSC to isolate viable, single-cell populations. Viability gating was based on scatter profiles to exclude debris and dead cells. Data were visualized using CellQuest Pro as quadrant dot plots. PI3K-positive cells appeared in the upper-right quadrant (Q2). Fluorescence thresholds were established using a negative control group. The percentage of PI3K-positive cells was compared across treatment groups to assess treatment effects.

Statistical Analysis

The data were statistically analyzed using IBM SPSS Statistics software version 26. Normality of the data was assessed using the Shapiro-Wilk test, and homogeneity of variances was evaluated with Levene's test. Since the data did not meet the assumptions for parametric tests, the Kruskal-Wallis non-parametric test was used to compare PI3K expression between treatment groups. When a significant difference was detected ($p < 0.05$), a Dunn post hoc test was performed to identify which groups differed significantly. A p-value of less than 0.05 was considered statistically significant throughout the analysis.

RESULT

The results of this study showed differences in the proliferation of HeLa cells treated with cisplatin, nanocurcumin, and a combination of both. After 24 hours of treatment, a clear morphological change was observed in HeLa cells through a inverted microscope (Figure 1). In the control group, the cells showed a distinctive polygonal shape with clear cell boundaries, were tightly attached to the culture base, and showed high confluence growth with no indication of damage. Meanwhile, in the 5 µg/ml cisplatin treatment group, it appeared that the cells changed shape to become rounded, underwent shrinkage, and some began to detach from the culture surface. In the group given nanocurcumin 100 µg/ml single, cell morphology also showed signs of cellular stress such as vacuolization and irregular cell contouring.

The results of the HeLa cell viability test using the CCK-8 method after 48 hours of treatment showed a significant decrease in all treatment groups compared to negative controls ($p<0.05$) (Figure 2). In the control group, cell viability was maintained at 100%, reflecting optimal metabolic activity. Treatment with cisplatin 5 µg/ml resulted in a drastic reduction in viability of up to 16.71%, while nanocurcumin of 100 µg/ml alone lowered viability to 21.82%. This suggests that both agents, both cisplatin and nanocurcumin, have cytotoxic potential against HeLa cells even when used separately. In this study, it was found that the combination of low-dose cisplatin (2.5 µg/ml) and nanocurcumin (100 µg/ml) resulted in the lowest viability, which was only 11.52%. Although the concentration of cisplatin in this combination was lower than that of a single therapy, the results showed a higher ability to suppress cell survival. These findings indicate that the use of combination may be a strategy to increase the efficiency of inhibiting cancer cell viability, while potentially lowering the required dose of cisplatin, which may help reduce the risk of systemic toxicity in future clinical therapies.

Details: The controls showed normal morphology and confluent growth. Treatment of cisplatin 5 µg/ml and nanocurcumin of 100 µg/ml triggered a rounded shape change and partial detachment of cells. The combination of

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cisplatin 2.5 µg/ml + nanocurcumin 100 µg/ml showed the most severe morphological damage with many cells experiencing detachment and irregular shape. Observations were made with an inverted microscope, magnification of 400×.

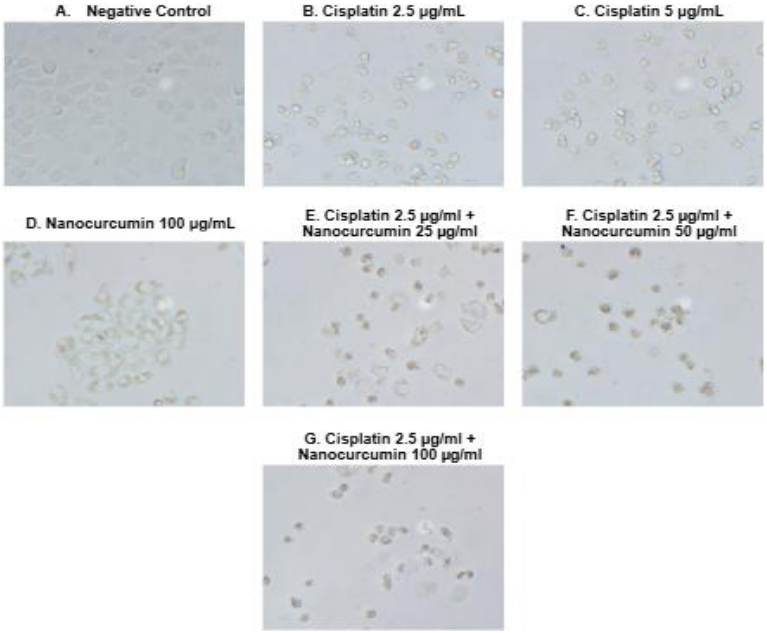

Figure 1. Hela Cell Morphology After 24 Hours of Treatment

Details: The controls showed normal morphology and confluent growth. Treatment of cisplatin 5 µg/ml and nanocurcumin of 100 µg/ml triggered a rounded shape change and partial detachment of cells. The combination of cisplatin 2.5 µg/ml + nanocurcumin 100 µg/ml showed the most severe morphological damage with many cells experiencing detachment and irregular shape. Observations were made with an inverted microscope, magnification of 400×.

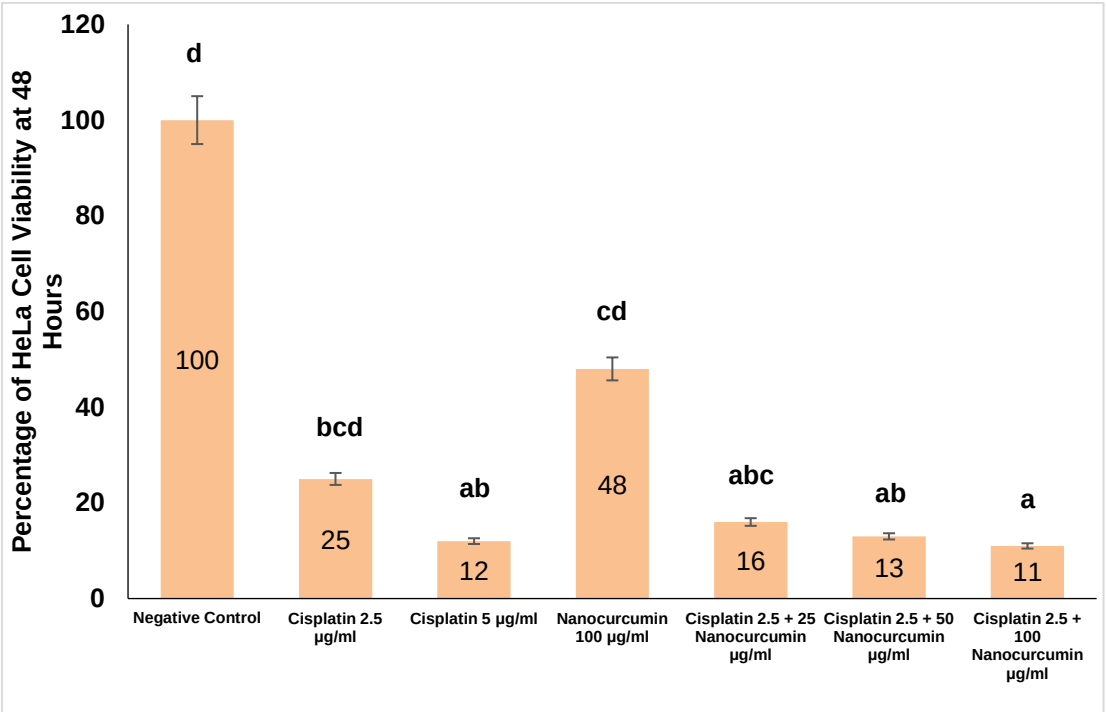

Figure 2. Percentage of Hela Cell Viability After 48 Hours of Treatment

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Details: The viability value is displayed as a percentage (%) against negative controls (K−). The letter notation wasdifferent, showing statistically significant differences between groups ($p < 0.05$, *Dunn's test*).

Statistical analysis using the Kruskal-Wallis test showed a significant difference in viability between treatment groups after 48 hours of incubation ($p = 0.007$). Follow-up tests with Dunn's test revealed that the combination of cisplatin 2.5 µg/mL with nanocurcumin of 100 µg/mL significantly reduced the viability of HeLa cells compared to a single low-dose cisplatin (2.5 µg/mL) ($p = 0.035$). This indicates that the combination has stronger cytotoxic activity than low-dose cisplatin monotherapy.

Furthermore, the combination of cisplatin 2.5 µg/mL with nanocurcumin of 100 µg/mL showed no significant difference in viability when compared to high doses of cisplatin (5 µg/mL) ($p = 0.553$), nor with a combination of lower doses of nanocurcumin, namely 50 µg/mL ($p = 0.948$) and 25 µg/mL ($p = 0.510$). Meanwhile, the combination with 100 µg/mL nanocurcumin showed significant differences compared to 100 µg/mL single nanocurcumin ($p = 0.007$) and negative control ($p = 0.001$), demonstrating the advantage of the combination in suppressing cell viability.

The entire combination of cisplatin 2.5 µg/mL with nanocurcumin (25, 50, and 100 µg/mL) showed cytotoxic efficacy equivalent to high-dose cisplatin (5 µg/mL), with no significant differences between combinations ($p>0.05$). Although the combination with nanocurcumin doses of 25 µg/mL and 50 µg/mL did not show a significant decrease in viability compared to cisplatin 2.5 µg/mL ($p = 0.392$ and $p = 0.147$, respectively), both still showed a consistent downward trend. Overall, these results suggest that the use of a combination of low-dose cisplatin with nanocurcumin, particularly at a dose of 100 µg/mL, is able to significantly degrade cell viability to be equivalent to high-dose cisplatin.

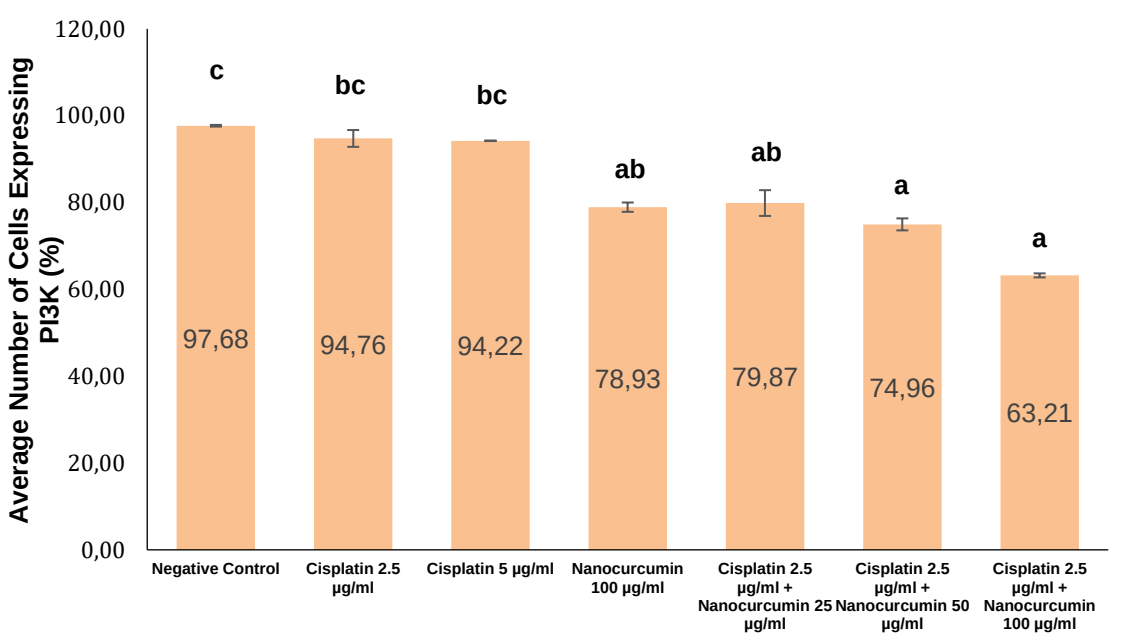

Figure 3. Average cells expressing PI3K

Details: Cisplatin treatment 2.5 µg/ml + Nanocurcumin 100 µg/ml showed significant effects compared to Cisplatin 2.5 µg/ml and Cisplatin 5 µg/ml (p-value < 0.05), while the other treatments showed no significant difference.

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The results of PI3K expression analysis showed that the combination of Cisplatin 2.5 µg/mL with Nanocurcumin 100 µg/mL resulted in the most significant decrease in PI3K expression compared to other groups (Figure 3). This combination showed significant differences against negative controls (p = 0.000), Cisplatin 2.5 µg/mL (p = 0.006), and Cisplatin 5 µg/mL (p = 0.010), indicating that the addition of Nanocurcumin was able to increase the inhibition potential of the PI3K pathway more effectively than the use of Cisplatin alone. In addition, the combination with moderate doses of Nanocurcumin (50 µg/mL) also significantly reduced the expression of PI3K compared to negative controls (p = 0.003), Cisplatin 2.5 µg/mL (p = 0.030), and Cisplatin 5 µg/mL (p = 0.048), although not as potent as the 100 µg/mL dose. In contrast, the combination of Cisplatin with low-dose Nanocurcumin (25 µg/mL) showed only a significant difference against negative control (p = 0.048), with no significant difference compared to low- or high-dose Cisplatin.

The group receiving 100 µg/mL nanocurcumin alone showed only significant differences with negative controls (p = 0.030), but did not differ significantly from Cisplatin 2.5 µg/mL (p = 0.167) or 5 µg/mL (p = 0.236). These findings indicate that nanocurcumin itself has the ability to lower PI3K expression, but the effect becomes more pronounced when used in combination with Cisplatin. Overall, these results suggest that the combination of Cisplatin and Nanocurcumin, particularly at a dose of 100 µg/mL, can significantly decrease PI3K expression than each single agent, supporting the potential of combination therapy in suppressing the proliferative activity of cancer cells through inhibition of the PI3K pathway.

DISCUSSION

Uncontrolled cell proliferation is a hallmark of cancer malignancy, including cervical cancer, indicating the ability of cancer cells to continuously divide and survive without normal regulation.[19] One approach to inhibit this process is the use of chemotherapy agents such as cisplatin, which work by damaging the DNA of cancer cells, inhibiting replication, and promoting the occurrence of apoptosis. wever, the effectiveness of cisplatin is often limited by systemic toxicity.[20]

To overcome these limitations, a combination strategy with nanocurcumin, which is a form of curcumin nanoparticles that has higher bioavailability and strong antioxidant and anticancer activity.[21] Nanocurcumin is able to increase the effectiveness of therapy through inhibition of proliferation, induction of apoptosis, as well as modulation of signaling pathways involved in cancer cell growth.[22] In this study, nanocurcumin was formulated as nanoliposomes with an average particle size of approximately 32 nm. The liposomal delivery system improves curcumin's stability, solubility, and release efficiency, which depend largely on the manufacturing method and carrier structure.[23]

In addition to its bioavailability advantages, recent studies have also shown that nanocurcumin has a selective effect on cancer cells and is able to reduce toxicity in normal cells, thus making it an effective and relatively safe adjuvant agent.[24] [25] However, the selectivity of nanocurcumin for cancer cells was not specifically confirmed in this study and requires further investigation. Thus, the use of nanocurcumin not only increases the cytotoxic effects on cancer cells but also provides protection to healthy tissues.[26]

The results of this study showed that the combination of cisplatin 2.5 µg/mL and nanocurcumin 100 µg/mL significantly reduced the viability of HeLa cells by up to 11% ($p<0.05$), with a greater reduction in viability than that of cisplatin alone. These findings indicate that the addition of nanocurcumin is able to amplify the antiproliferative effects of cisplatin through synergistic mechanisms, in line with the study of Purbadi et al. (2022), which reported a

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significant decrease in viability in ovarian cancer cells, as well as the study of Subandi and Purbadi (2023), which showed an increase in the effectiveness of methotrexate in choriocarcinoma therapy.[27,28] Cheng et al. (2018) demonstrated a strong synergistic effect in hepatocellular carcinoma cells using co-loaded liposomes of cisplatin and curcumin, with a combination index (CI) less than 1 at an optimal drug ratio of 1:8.[14] Although formal CI or isobologram analyses were not conducted in this study, the observed enhanced cytotoxicity in the combination group supports the presence of synergy. These results reinforce the potential of nanocurcumin as a chemotherapy adjuvant that improves cancer cell sensitivity to cytotoxic agents.[24,29]

The 48-hour evaluation period was selected to ensure stability of the observed synergistic effects, as Gross et al. (2020) noted that this timeframe provides valuable information on the durability of therapeutic responses, especially in combination therapies that prolong cytotoxic effects.[30,31]

This study also found that cisplatin alone induced high PI3K expression in cells 94.76% at 2.5 µg/mL and 94.22% at 5 µg/mL, comparable to the negative control (97.68%). This indicating limited inhibition of the PI3K/AKT signaling pathway. These findings indicate that cisplatin has limited inhibitory effects on the PI3K/AKT signaling pathway. The PI3K/AKT pathway plays a crucial role in cervical cancer progression by promoting cell proliferation, survival, and resistance to apoptosis. Although cisplatin effectively inhibits cell proliferation, it does not sufficiently suppress PI3K/AKT activity, which may contribute to chemotherapy resistance. Bhattacharjee et al. (2022) reported that cisplatin activates PI3K/AKT via P21-activated kinases (PAKs), reducing therapeutic efficacy.[32]

In contrast, combining cisplatin (2.5 µg/mL) with nanocurcumin (100 µg/mL) significantly decreased PI3K expression ($p < 0.01$ compared to control and cisplatin alone), indicating enhanced pathway inhibition. This highlights the important role of nanocurcumin in inhibiting PI3K expression specifically in cervical cancer cells, thereby suppressing the PI3K/AKT signaling pathway activity.[22,32] Nanocurcumin acts by inhibiting key molecules such as mTORC1, AKT, and PI3K, as well as modulating upstream effectors like IKKβ and AMPK. This results in proliferation inhibition, autophagy induction, and apoptosis via increased pro-apoptotic proteins and reduced phosphorylation of

The elevated PI3K expression in cisplatin-treated cells likely represents an adaptive response to chemotherapy-induced stress, mediated by activation of genes such as PIK3CA and USP17, enabling cells to evade apoptosis.[32–34] It is important to note that PI3K expression was assessed at the protein level using flow cytometry with anti-PIK3CA antibodies, reflecting post-transcriptional or translational regulation rather than direct gene transcription or kinase activity. Since increased PI3K protein levels may arise from enhanced protein stability or translation as a cellular stress response. [35]

In this context, nanocurcumin has been shown to effectively suppress the activation of the PI3K/AKT pathway when combined with cisplatin, thereby increasing cancer cell sensitivity to chemotherapy. Karami et al. (2022) support these findings, demonstrating that the combination of cisplatin and nanocurcumin synergistically targets key oncogenic signaling pathways, enhances cell death, and inhibits proliferation.[36,37]

Mechanistically, nanocurcumin interferes with multiple levels of the PI3K/AKT/mTOR axis by inhibiting PI3K and AKT activity, reducing mTORC1 phosphorylation, and modulating upstream regulators such as IKKβ and AMPK. It also promotes autophagy by blocking mTORC1 and induces apoptosis by downregulating anti-apoptotic proteins (e.g., Bcl-2) and

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upregulating pro-apoptotic factors (e.g., BAX, BAD). These multifaceted interactions suggest that nanocurcumin may regulate PI3K expression not only through translational suppression but also by altering protein stability and degradation, contributing to its antiproliferative and pro-apoptotic effects. [37]

Limitations and Therapeutic Implications

The combination of cisplatin and nanocurcumin shows enhanced antiproliferative effects and effective suppression of PI3K expression in cervical cancer cells, highlighting nanocurcumin's potential as a bioavailable and selective adjuvant agent. However, the study is limited by the absence of normal cell lines, which restricts assessment of treatment selectivity, and by its focus solely on PI3K without evaluating downstream targets like AKT or mTOR. Despite these limitations, the findings offer valuable insights for developing more effective and targeted chemotherapeutic strategies.

CONCLUSION

In this study, the combination of 2.5 µg/mL cisplatin and 100 µg/mL nanocurcumin significantly reduced the viability of HeLa cervical cancer cells and more effectively suppressed PI3K expression compared to cisplatin alone. The decreased PI3K expression indicates that this combination inhibits the PI3K/AKT signaling pathway, which plays a crucial role in cancer cell proliferation and survival. The combination of cisplatin and nanocurcumin has a similar effect to high-dose cisplatin and may be used as an alternative to reduce toxicity and resistance commonly associated with cisplatin-based chemotherapy. These findings support the need for further investigation into the use of nanocurcumin as an adjuvant agent to enhance the safety and efficacy of cervical cancer therapy.

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