MEDIA PENELITIAN DAN
PENGEMBANGAN KESEHATAN
Vol 35 No 3, September 2025

e-ISSN: 2338-3445
p-ISSN: 0853-9987

SYNERGISTIC EFFECTS OF NANOCURCUMIN AND CISPLATIN COMBINATION THERAPY ON APOPTOSIS AND PROTEIN KINASE B (AKT) IN CERVICAL CANCER

Efek Sinergis Terapi Kombinasi Nanokurkumin dan Cisplatin pada Apoptosis dan Protein Kinase B (AKT) pada Kanker Serviks

Subandi Subandi[1], Nadia Taqiyya[2] , Romadhinniar Febriana[2] , Agustina Tri Endharti[3]

[1]Departement of Midwifery, Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia

[2]Master Program of Midwifery, Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia

[3]Doctoral Program of Medical Science, Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia

*Email: desobg@gmail.com

ABSTRAK

Kanker serviks merupakan salah satu jenis kanker yang sering dijumpai pada wanita dan penyebab utama kematian di negara berkembang, termasuk Indonesia. Kemoterapi berbasis cisplatin merupakan terapi standar, namun penggunaannya dibatasi oleh toksisitas yang bergantung pada dosis, seperti nefrotoksisitas, neurotoksisitas, dan kardiotoksisitas. Penelitian ini bertujuan untuk mengevaluasi efek sinergis kombinasi nanokurkumin dan cisplatin terhadap tingkat apoptosis dan ekspresi Protein Kinase B (AKT) pada sel HeLa. Metode yang digunakan adalah eksperimen dengan desain post-test only control group, dengan kelompok kontrol negative (media standar), kontrol positif monoterapi cisplatin (5 dan 2,5 µg/mL), nanokurkumin (100 µg/mL), serta kombinasi cisplatin 2,5 µg/mL dan nanokurkumin berbagai dosis (25, 50, dan 100 µg/mL). Uji flow cytometry digunakan untuk menganalisis tingkat apoptosis dan ekspresi AKT setelah 48 jam inkubasi. Hasil penelitian menunjukkan bahwa kombinasi cisplatin 2,5 µg/mL dan nanokurkumin 100 µg/mL menyebabkan apoptosis (75,23%) pada sel HeLa yang sebanding ($p<0.05$) dengan efek cisplatin tunggal 5 µg/mL (76,15%) serta secara signifikan ($p<0,05$) menurunkan ekspresi AKT pada sel HeLa (76,53%) dibanding cisplatin 5 µg/mL (97,18%) serta cisplatin 2,5 µg/mL (97,24%). Peningkatan apoptosis dan penurunan ekspresi AKT menunjukkan potensi terapi kombinasi ini dalam mengurangi dosis cisplatin yang diperlukan dengan harapan dapat menurunkan toksisitas.

Kata kunci: *apoptosis, cisplatin, kanker serviks, nanokurkumin, protein kinase B (AKT)*

ABSTRACT

Cervical cancer remains a prevalent malignancy among women globally and is a leading cause of cancer-related mortality in developing countries, including Indonesia. Cisplatin-based chemotherapy is a standard treatment but is limited by dose-dependent toxicities such as nephrotoxicity, neurotoxicity, and cardiotoxicity. This study investigates the synergistic effects of combining nanocurcumin with cisplatin on apoptosis induction and Protein Kinase B (AKT) expression in HeLa cervical cancer cells. The method used was an experimental design with a post-test only control group, including a negative control group (standard medium), positive control groups with cisplatin monotherapy (5 and 2.5 µg/mL), nanocurcumin (100 µg/mL), and combination treatments of cisplatin 2.5 µg/mL with various doses of nanocurcumin (25, 50, and 100 µg/mL). Apoptosis rates and AKT expression levels were quantified using flow cytometry after 48 hours of incubation. The results showed that the combination of cisplatin 2.5 µg/mL and nanocurcumin 100 µg/mL induced apoptosis (75.23%) of HeLa cells, which was comparable ($p<0.05$) to the effect

https://doi.org/10.34011/jmp2k.v35i3.3169

e-ISSN: 2338-3445
p-ISSN: 0853-9987

of single-agent cisplatin 5 µg/mL (76.15%) and significantly ($p<0.05$) reduced AKT expression of HeLa cells (76.53%) compared to cisplatin 5 µg/mL (97.18%), cisplatin 2.5 µg/mL (97.24%). The increase in apoptosis alongside the decrease in AKT expression indicates the potential of this combination therapy to reduce the required dose of cisplatin, with the expectation of reducing toxicities.

Keywords: apoptosis, cervical cancer, cisplatin, nanocurcumin, protein kinase B (AKT)

INTRODUCTION

Cervical cancer is one of the most common types of cancer experienced by women worldwide, ranking fourth with 660,000 new cases in 2022. The burden is disproportionately higher in low- and middle-income countries, where mortality rates remain alarmingly elevated. Indonesia, for instance, reported 36,964 new cervical cancer cases and 20,708 deaths in the same year, underscoring the urgent need for improved therapeutic strategies [1]. A significant challenge in cervical cancer management is the late-stage diagnosis, with 70% of cases detected at advanced stages, contributing to a mortality rate of nearly 50% due to suboptimal treatment outcomes [2].

The etiological agent primarily responsible for cervical carcinogenesis is the Human Papillomavirus (HPV), particularly high-risk types 16 and 18. Integration of the viral genome into host DNA leads to the expression of oncogenic proteins E5, E6, and E7, which disrupt normal cellular regulatory mechanisms. These oncoproteins interfere with tumor suppressor pathways and activate multiple signaling cascades, including the phosphatidylinositol 3-kinase (PI3K)/AKT pathway, which promotes cell survival, proliferation, and malignancy [3],[4].

Cisplatin remains a cornerstone chemotherapeutic agent for cervical cancer due to its DNA-damaging properties that induce apoptosis. However, its clinical application is hampered by dose-limiting toxicities such as nephrotoxicity, neurotoxicity, and myelosuppression [5]. To mitigate these adverse effects and enhance therapeutic efficacy, recent research has focused on combining cisplatin with phytochemicals, notably curcumin, a bioactive compound derived from turmeric (*Curcuma longa*). Curcumin exhibits anticancer properties by increasing Reactive Oxygen Species (ROS) production, inhibiting cell proliferation, and promoting apoptosis in various cancer cell lines, including cervical cancer [6],[7].

Despite its therapeutic potential, curcumin's clinical utility is limited by poor solubility and bioavailability. Nanoparticle formulations of curcumin (nanocurcumin) have been developed to overcome these limitations by enhancing drug stability, bio-distribution, and cellular uptake [8]. Studies have demonstrated that nanocurcumin combined with cisplatin exerts superior apoptotic effects in HeLa cells compared to either agent alone, suggesting a synergistic interaction [9].

Although previous studies have shown the potential of a combination of nanocurcumin and cisplatin in cancer cells, studies focusing on the effect of curcumin in nanoparticle form on AKT expression and apoptosis in cervical cancer are still limited. Therefore, this study was conducted to evaluate the effects of the combination of nanocurcumin and cisplatin on the rate of apoptosis and expression of AKT in cervical cancer, particularly in HeLa cells, in the hope of contributing to the development of nanocurcumin as part of more effective and safe cervical cancer therapies in the future.

METHODS

Study Design, Location, and Duration

This research employed a true experimental post-test only control group design. The experimental procedures, included HeLa cell culture, treatment administration, and flow cytometric analysis of apoptosis and AKT expression. The experiments were conducted from January to March 2025 at the Biomedical Laboratory, Faculty of Medicine, Universitas Brawijaya. Ethical approval was obtained from the Research Ethics

https://doi.org/10.34011/jmp2k.v35i3.3169

e-ISSN: 2338-3445
p-ISSN: 0853-9987

Committee of the Faculty of Medicine, Universitas Brawijaya (Approval number 12/EC/KEP-82/01/2024).

Sample

This study used HeLa cells from ATCC (*American Type Culture Collection*), obtained from the Biomedical Laboratory (catalog number CCL-2.2), Faculty of Medicine, University of Brawijaya. The sample were divided into seven groups that were repeated three times to ensure data validity. The group consisted of negative control (HeLa cells with standard medium), positive control 1 (cisplatin 5 µg/mL), positive control 2 (cisplatin 2.5 µg/mL), positive control 3 (nanocurcumin 100 µg/mL), cisplatin combination group 2.5 µg/mL and nanocurcumin 25 µg/mL, cisplatin 2.5 µg/mL and nanocurcumin 50 µg/mL, lastly cisplatin 2.5 µg/mL and nanocurcumin 100 µg/mL.

Reagent Preparation

Nanocurcumin was synthesized from turmeric powder obtained from Materia Medica Batu in West Java. Curcumin was extracted and purified, then formulated into nanoparticles by combining with phospholipids, cholesterol, and Tween 80, followed by evaporation and hydration with Phosphate-buffered Saline (PBS). The resulting liposomal nanoparticles were sonicated to achieve an average particle size of 32 nm, confirmed by Particle Size Analyzer (PSA). Stock solutions were diluted with sterile standard medium to final concentrations of 25 µg/mL, 50 µg/mL, and 100 µg/mL using the dilution formula $M_1 \times V_1 = M_2 \times V_2$.

Cisplatin (Kalbe brand) was supplied as a 10 mg/10 mL vial and diluted with sterile culture standard medium to working concentrations of 2.5 µg/mL and 5 µg/mL for single and combination treatments. Apoptosis detection was performed using the FITC Annexin V Apoptosis Detection Kit with Propidium Iodide (PI) (Biolegend, catalog number 640914). AKT expression was assessed using FITC-conjugated AKT1+2+3 antibody (Bioss, catalog number bs-5193E-FITC).

Cell Culture Procedure

HeLa cells, sourced from the American Type Culture Collection (ATCC), were cultured in a standard medium composed of RPMI-1640 supplemented with 10% Fetal Bovine Serum (FBS) and 1% penicillin-streptomycin. Cells were thawed from cryopreservation at 37°C, centrifuged, and seeded into T-flasks for incubation at 37°C with 5% CO_2 until reaching approximately 80% confluence.

For experimental treatments, cells were trypsinized, counted using a hemocytometer, and seeded at a density of 500,000 cells per well in triplicate. After 24 hours of incubation or the cells have attached and reached confluence, the cells are treated according to the group and harvested after 48 hours for *flow cytometry* assays.

Flow Cytometry Analysis

Apoptosis Assay and AKT Expression Assay

Harvested HeLa cells were washed twice with Cell Staining Buffer and resuspended in Annexin V Binding Buffer. Cells were incubated with 5 µL of fluorochrome-conjugated Annexin V and PI for 15 minutes at room temperature in the dark. Subsequently, 400 µL of Annexin V Binding Buffer was added, and samples were analyzed using a flow cytometer to quantify apoptotic and necrotic cell populations.

Intracellular staining for AKT involved permeabilization using Cyto-Fast™ Perm Wash Solution diluted with deionized water, followed by incubation with CFixPerm. After washing and centrifugation, cells were incubated with FITC-conjugated AKT antibodies (1:50 dilution) for 20 minutes. Cells were then resuspended in Cell Staining Buffer and analyzed by flow cytometry to determine AKT expression levels.

Statistical Analysis

Data were analyzed using SPSS version 26. Normality was assessed with the Shapiro-Wilk test, and homogeneity of variance was evaluated using Levene's test. For

https://doi.org/10.34011/jmp2k.v35i3.3169

e-ISSN: 2338-3445
p-ISSN: 0853-9987

normally distributed and homogeneous data (p>0.05), one-way ANOVA followed by Tukey's post-hoc test was applied to compare group differences. Non-parametric data (p<0.05) were analyzed using the Kruskal-Wallis test and Pairwise comparisons. Correlations between treatment doses and biological responses were examined using Pearson's for parametric data and Spearman's for non-parametric data. Bootstrap resampling (1000 iterations) was performed to assess the stability of correlation estimates.

RESULT

Flow cytometric analysis revealed distinct apoptotic profiles across treatment groups after 48 hours of incubation (Figure 1).

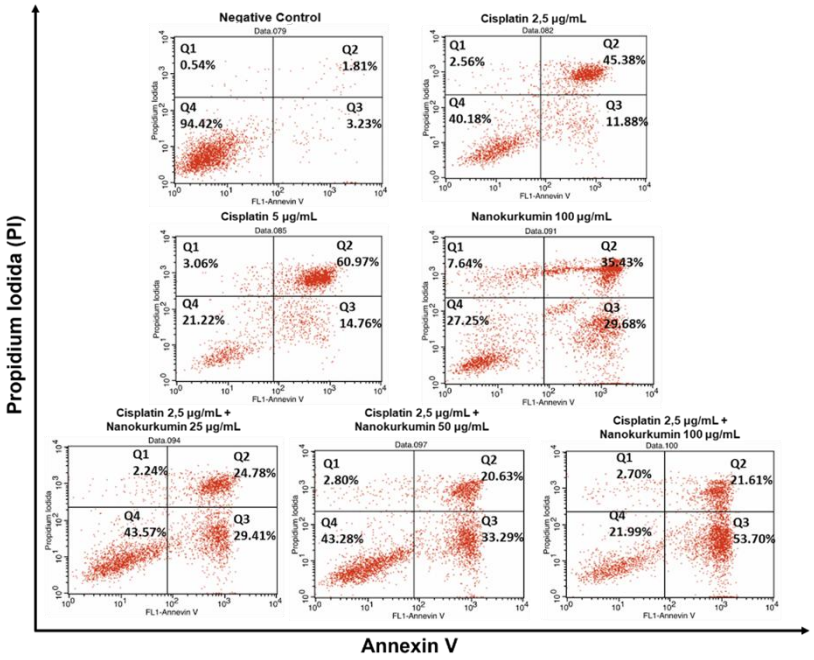

Figure 1. Flow Cytometry Test Results of Apoptosis Rate of HeLa Cells Incubation 48 Hours

Image caption : Q1 (Annexin V^-/PI^+) indicates necrotic cells, Q2 (Annexin V^+/PI^+) indicates advanced apoptosis cells, Q3 (Annexin V^+/PI^-) indicates early apoptosis cells, and Q4 (Annexin V^-/PI^-) indicates live cells

In the negative control group, most of the cells (94.42%) remained viable, showing a very low rate of apoptosis. Treatment with cisplatin at 2.5 µg/mL induced total apoptosis in 57.26% of cells, predominantly in the late apoptotic stage (45.38%). Increasing cisplatin concentration to 5 µg/mL enhanced apoptosis (75.73%), with (60.97%) in late apoptosis. Nanocurcumin at 100 µg/mL alone induced apoptosis (65.11%) of cells, exceeding the apoptotic effect of cisplatin at 2.5 µg/mL but remaining lower than that of cisplatin at 5 µg/mL.

Combination treatments demonstrated dose-dependent effects. Cisplatin 2.5 µg/mL combined with nanocurcumin at 25 µg/mL and 50 µg/mL induced apoptosis rates of 54.19% and 53.92%, respectively, with early apoptosis predominance and a slight increase in necrosis at the 50 µg/mL dose. Notably, the combination of cisplatin 2.5 µg/mL with nanocurcumin 100 µg/mL achieved the highest apoptosis rate (75.31%), comparable to cisplatin 5 µg/mL alone. The average apoptosis percentages across all groups measured in triplicate are illustrated in Figure 2.

https://doi.org/10.34011/jmp2k.v35i3.3169

e-ISSN: 2338-3445
p-ISSN: 0853-9987

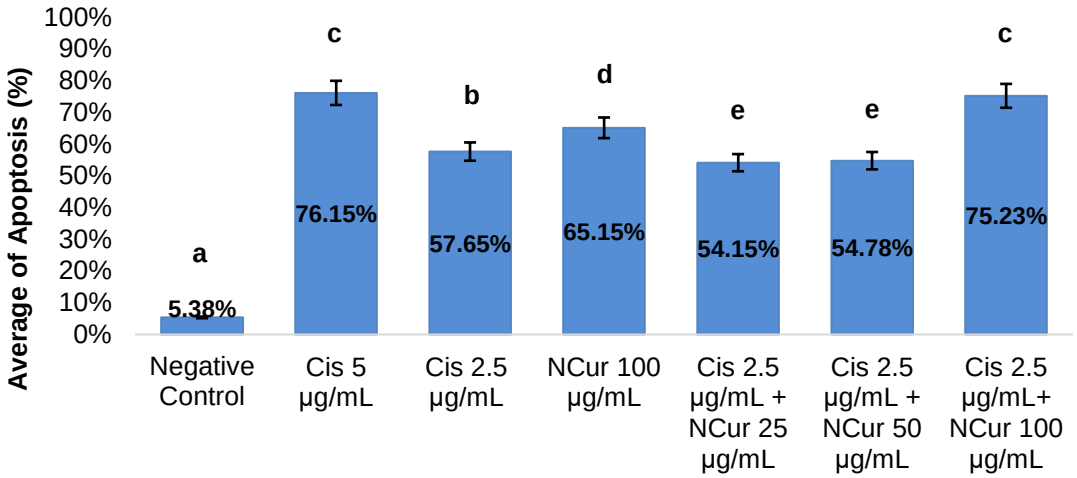

Figure 2. Histogram of Average Percentage of Apoptosis of 48-Hour Incubated HeLa Cells

Image caption : Cis (Cisplatin) and NCur (Nanocurcumin). Q1 (Annexin V^-/PI^+) indicates necrotic cells, Q2 (Annexin V^+/PI^+) indicates advanced apoptosis cells, Q3 (Annexin V^+/PI^-) indicates early apoptosis cells, and Q4 (Annexin V^-/PI^-) indicates live cells. Numbers with different superscript letters indicate statistically significant difference between groups ($p< 0.05$), whereas identical superscript letters indicate insignificant difference ($p>0.05$).

Based on the histogram data above, the single administration of Nanocurcumin 100 µg/mL induced apoptosis (65.15%), higher than Cisplatin 2.5 µg/mL although it was not as high as the effect given by Cisplatin 5 µg/mL (76.15%). In the combination group, Cisplatin 2.5 µg/mL with Nanocurcumin of 25 µg/mL and 50 µg/mL produced apoptosis (54.15%) and (54.78%), respectively, which is lower than Cisplatin 2.5 µg/mL alone. Meanwhile, the combination of Cisplatin 2.5 µg/mL with Nanocurcumin of 100 µg/mL resulted (75.23%) apoptosis, which is close to the apoptosis effectiveness obtained from Cisplatin 5 µg/mL.

The average percentage of apoptosis of HeLa cells was analyzed through normality tests and homogeneity tests. The normality test using *Shapiro-Wilk* yielded $p>0.05$, indicating that the data was normally distributed. The results of the homogeneity test with *the Levene Test* also showed $p=0.099$ ($p>0.05$), which indicates that the data is homogeneous. With the fulfillment of the parametric test prerequisites, *the One Way ANOVA* test was performed, which yielded $p=0.000$ ($p<0.05$), indicating a significant difference in the rate of apoptosis between groups.

Furthermore, *the Post-Hoc Tukey HSD* test was conducted to identify significant differences between groups. The negative control group showed significant differences with all treatment groups ($p<0.05$). The 2.5 µg/mL Cisplatin group also showed significant differences with the rest of the group ($p<0.05$), indicating that this dose was not strong enough to produce apoptosis effects equivalent to higher doses, nanocurcumin, or other combinations of treatments. Meanwhile, Cisplatin 5 µg/mL resulted the highest average rate of apoptosis and did not differ significantly from the combination of Cisplatin 2.5 µg/mL and Nanocurcumin 100 µg/mL.

The 100 µg/mL Nanocurcumin group increased apoptosis significantly higher than Cisplatin 2.5 µg/mL, although it was not equivalent to Cisplatin 5 µg/mL and the combination of Cisplatin 2.5 µg/mL with Nanocurcumin 100 µg/mL. The combination of Cisplatin 2.5 µg/mL with Nanocurcumin 25 µg/mL and 50 µg/mL showed no significant difference with each other, while the combination of Cisplatin 2.5 µg/mL and Nanocurcumin 100 µg/mL differed significantly from the rest of the group, except for Cisplatin 5 µg/mL, showing effectiveness equivalent to Cisplatin 5 µg/mL.

https://doi.org/10.34011/jmp2k.v35i3.3169

e-ISSN: 2338-3445
p-ISSN: 0853-9987

Furthermore, the analysis of the relationship between the combination dose groups of nanocurcumin and cisplatin on the rate of apoptosis in HeLa cells was carried out by Pearson's parametric correlation test. The results of the correlation value (r) 0.878 and *p-value* 0.002 (p<0.01) showed a strong and statistically significant positive relationship between the increased dose of the combination of Cisplatin and Nanocurcumin and the increase in HeLa cell apoptosis. The increase in the dose of Nanocurcumin in this combination is in line with the increasing rate of apoptosis. Bootstrap analysis with 1000 resamples yielded a standard error of 0.082 and a 95% BCa confidence interval between 0.659 to 1.000. Although the lower bound of the confidence interval is negative, the significant p-value and correlation stability indicate the consistency of this relationship.

In the results of *the* flow cytometry test to determine the percentage of HeLa cells expressing AKT after 48 hours of incubation in all groups listed in Figure 3. HeLa cells expressing AKT in the negative control group showed a very high percentage (99.08%). In Cisplatin treatment 2.5 µg/mL, AKT expression was recorded at (95.84%), while in Cisplatin 5 µg/mL (97.13%). A single treatment with Nanocurcumin 100 µg/mL resulted in an AKT expression (87.91%), which is lower compared to the Cisplatin groups of 2.5 µg/mL and 5 µg/mL. In the combination group of Cisplatin 2.5 µg/mL with Nanocurcumin of 25 µg/mL, 50 µg/mL, and 100 µg/mL, the percentage of AKT expression decreased to (84.74%, 82.69%, and 77.08%), respectively.

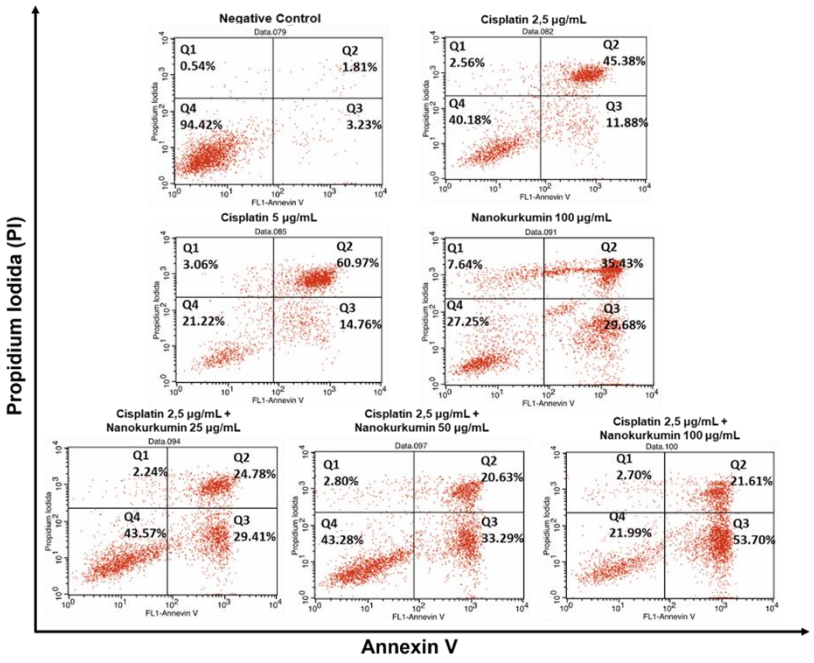

Figure 3. Flow *Cytometry Test* Results of HeLa Cells Expressing AKT

Image caption : The test was performed after 48 hours of incubation, where the horizontal axis (FSC-Height) depicts the cell size, and the vertical axis (FL1-AKT) depicts the fluorescence intensity of the AKT expression.

Statistical tests indicated non-normal distribution (Shapiro-Wilk p<0.05) and heterogeneity (Levene's test p=0.001). The Kruskal-Wallis test confirmed significant differences in AKT expression among groups (p=0.005), showing a significant difference in AKT expression between groups. Furthermore, Pairwise comparation test is used to identify significantly different groups.

Pairwise comparation analysis revealed that the highest AKT expression was in the negative control with insignificant differences with cisplatin 5 µg/mL and cisplatin 2.5 µg/mL groups. The combination of cisplatin 2.5 µg/mL with nanocurcumin 100 µg/mL

https://doi.org/10.34011/jmp2k.v35i3.3169

e-ISSN: 2338-3445
p-ISSN: 0853-9987

exhibited the lowest AKT expression, significantly different ($p<0.05$) compared to the negative control, cisplatin 5 µg/mL, and cisplatin 2.5 µg/mL (Figure 4). Spearman's correlation analysis resulted a strong negative correlation ($r = -0.843, p=0.004$) between combination dose and AKT expression, indicating that higher doses of nanocurcumin combined with cisplatin effectively suppress AKT levels. Bootstrap analysis supported the stability of this correlation. Increased combination doses are closely related to a consistent decrease in AKT expression, suggesting that the combination therapy has the potential to increase apoptosis through a reduction in AKT expression.

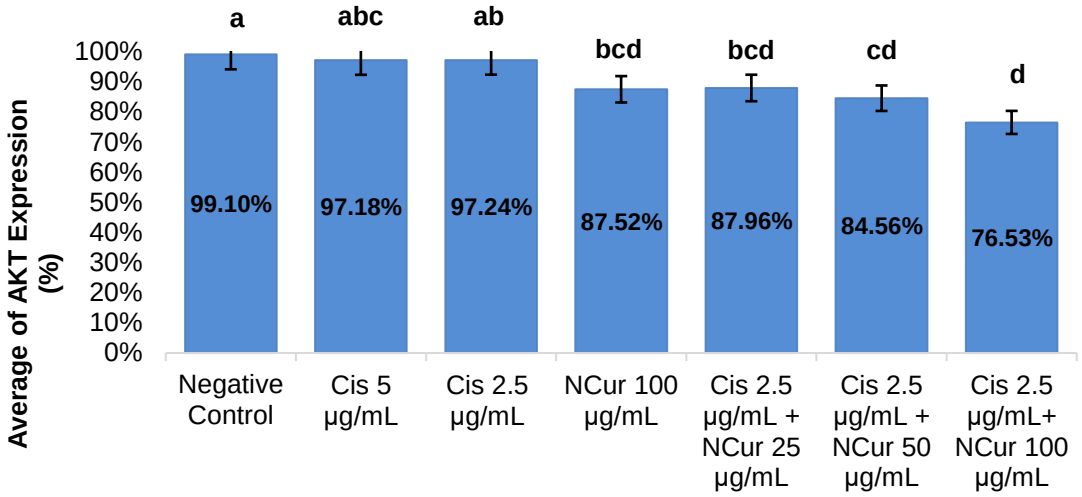

Figure 4. Histogram of Average Percentage of HeLa Cells Expressing AKT

Image caption : Cis (Cisplatin) and NCur (Nanocurcumin). Numbers accompanied by different superscript letters show a significant difference ($p<0.05$), if the superscript letters used are the same, it means that the difference between groups insignificant ($p>0.05$).

DISCUSSION

This study showed that the combination of cisplatin 2.5 µg/mL and nanocurcumin of 100 µg/mL significantly increased HeLa cell apoptosis, achieving effectiveness equivalent to a single 5 µg/mL cisplatin. Significant differences between treatment groups indicated that the addition of nanocurcumin may amplify the effects of cisplatin. These findings are consistent with previous research that stated that the combination of nanocurcumin and cisplatin significantly improves apoptosis in triple-negative breast cancer (TNBC) cells, with greater synergistic effects compared to the use of single agents [10].

Apoptosis, a tightly regulated form of programmed cell death, is essential for maintaining cellular homeostasis and eliminating damaged or malignant cells. In cervical cancer, apoptotic pathways are frequently dysregulated, contributing to tumor progression and resistance to therapy [11]. Cisplatin induces apoptosis primarily through DNA crosslinking and damage, triggering intrinsic apoptotic pathways. However, its clinical use is limited by systemic toxicities, including nephrotoxicity and neurotoxicity, which restrict the maximum tolerable dose [5].

The combination of cisplatin with other agents, such as nanocurcumin, has the potential to enhance therapeutic effects and reduce side effects. Nanocurcumin inhibits the activation of HPV oncoproteins and decreases the expression of key proteins in the PI3K/AKT pathway, which contributes to the induction of apoptosis by activating pro-apoptosis proteins such as BAD and BAX, as well as degrading anti-apoptosis proteins such as Bcl-2 [12]. In addition, nanocurcumin also increases the production of ROS (Reactive Oxygen Species), which causes oxidative stress, damages cancer cells, and

https://doi.org/10.34011/jmp2k.v35i3.3169

e-ISSN: 2338-3445
p-ISSN: 0853-9987

further activates apoptotic pathways. Overall, the combination of cisplatin and nanocurcumin amplifies intrinsic apoptosis signals, leading to a significant increase in apoptosis in HeLa cells [13],[14].

The combination of cisplatin 2.5 µg/mL with nanocurcumin of 25 µg/mL and 50 µg/mL did not provide a significant difference in improved apoptosis. However, the combination of cisplatin 2.5 µg/mL and nanocurcumin of 100 µg/mL showed a significant increase in apoptosis, equivalent to cisplatin 5 µg/mL. Higher doses of nanocurcumin (100 µg/mL) contribute more to the enhancement of apoptosis, potentially reducing the use of high doses of cisplatin in therapy. The results of statistical analysis showed a very strong positive correlation between increased combination dose and increased apoptosis in HeLa cells. These results are also supported by studies showing that increasing doses of nanocurcumin consistently contributes to increased apoptosis [15].

This combination increases the sensitivity of cells to cisplatin, so that the dose of cisplatin required to achieve maximum apoptosis effects can be reduced. Previous studies have also shown that this kind of combination can minimize the side effects of cisplatin toxicity [16]. In addition, increasing the dose of nanocurcumin in the cisplatin and nanocurcumin combination groups may increase pro-apoptosis proteins such as BAD and BAX, which further supports increased apoptosis in cancer cells [17].

Regarding AKT expression, this study showed that the combination of cisplatin 2.5 µg/mL with nanocurcumin of 100 µg/mL significantly decreased AKT expression in HeLa cells, lower than the negative controls, cisplatin 5 µg/mL, and cisplatin 2.5 µg/mL. This decrease in AKT expression suggests that nanocurcumin may amplify the effects of cisplatin in inhibiting the PI3K/AKT pathway which plays a role in cancer cell survival. These results are in line with previous studies that showed that the combination of nanocurcumin and cisplatin was more effective in lowering AKT expression in ovarian cancer tissues [18]. Molecularly, nanocurcumin is known to increase Phosphatase and Tensin Homolog (PTEN) activity, which then inhibits AKT activation, as well as triggering the release of cytochrome c from mitochondria to activate caspase-9 and caspase-3, which contribute to the induction of apoptosis [19].

In the cisplatin monotherapy 5 µg/mL and 2.5 µg/mL groups, AKT expression did not differ significantly compared to negative controls, which supports previous findings that cisplatin affects AKT indirectly by influencing downstream proteins such as PTEN, Mtor, BAD,BAX, Caspase 3 and 9 [20]. However, the combination of cisplatin 2.5 µg/mL with nanocurcumin of 100 µg/mL showed a significant decrease in AKT expression, indicating that nanocurcumin amplifies the effects of cisplatin by inhibiting the PI3K/AKT survival pathway. This decrease in AKT expression contributes to increased activity of pro-apoptosis proteins which in turn increases the induction of apoptosis [19].

The use of nanocurcumin, due to its smaller particle size and enhanced cellular penetration, may improve the efficacy of cisplatin in inducing apoptosis in HeLa cells. The results of this study demonstrate that the combination of these two agents not only significantly promotes apoptosis but also reduces AKT expression. The practical implications of these findings suggest the potential for developing more efficient and safer therapies for cervical cancer by lowering the required cisplatin dose and minimizing its associated toxicity. Furthermore, this study paves the way for the exploration of phytochemical-based therapies as alternatives or adjuncts in cervical cancer treatment. The limitation of this study is that it only evaluates the combined effects of nanocurcumin and cisplatin on apoptosis and AKT expression, without considering other molecular pathways to provide a more comprehensive understanding of the mechanisms involved. This includes the need to examine upstream receptors such as Receptor Tyrosine Kinases (RTKs), PTEN, and p53, as well as downstream apoptosis proteins like caspase 3 and 9.

https://doi.org/10.34011/jmp2k.v35i3.3169

e-ISSN: 2338-3445
p-ISSN: 0853-9987

CONCLUSION

The present study demonstrates that the combination of cisplatin at 2.5 µg/mL with nanocurcumin at 100 µg/mL significantly enhances apoptosis in HeLa cells, the results are similar to cisplatin at 5 µg/mL, and this combination also suppresses AKT expression than either agent alone. This suggests its potential to reduce the required cisplatin dose. This combination therapy amplifies cisplatin's anticancer effects by inhibiting the AKT survival protein, thereby promoting apoptotic induction. These results underscore the promise of nanocurcumin and cisplatin combination therapy as a more effective and safer alternative for cervical cancer treatment, warranting further investigation and development toward clinical application. Further studies are necessary to investigate additional molecular pathways involved in the pathogenesis of cervical cancer, including Receptor Tyrosine Kinases (RTKs), the tumor suppressor p53, and downstream apoptosis effectors such as caspase-3 and caspase-9, to gain a comprehensive understanding of the mechanisms underlying the observed therapeutic effects.

REFERENCES

[1] WHO, "Cervical Cancer," World Health Organization. [Online]. Available: https://www.who.int/news-room/fact-sheets/detail/cervical-cancer?gad_source=1&gclid=Cj0KCQjw3bm3BhDJARIsAKnHoVW3Xwtoe0w_00UOKTQo9prc3PGotxfwc6GS79j9WXGGHsiUGDWSXZ8aApN7EALw_wcB

[2] Kemenkes, "Rencana Aksi Nasional Eliminasi Kanker Leher Rahim," Dec. 2023. [Online]. Available: https://kemkes.go.id/id/rencana-aksi-nasional-ran-eliminasi-kanker-leher-rahim-di-indonesia-tahun-2023-2030

[3] C. A. Burmeister *et al.*, "Cervical cancer therapies: Current challenges and future perspectives," *Tumour Virus Res.*, vol. 13, no. 200238, pp. 1–14, Jun. 2022, doi: 10.1016/j.tvr.2022.200238.

[4] L. de Sanjosé, G. Albero, B. Serrano, M. Mena, J. J. Collado, D. Gómez, J. Muñoz, and F. X. Bosch, "Human Papillomavirus and Related Diseases Report INDONESIA," 2023. [Online]. Available: www.hpvcentre.net

[5] P. B. Tchounwou, S. Dasari, F. K. Noubissi, P. Ray, and S. Kumar, "Advances in our understanding of the molecular mechanisms of action of cisplatin in cancer therapy," *J. Exp. Pharmacol.*, vol. 13, pp. 303–328, 2021, doi: 10.2147/JEP.S267383.

[6] T. Nisar, M. Iqbal, A. Raza, M. Safdar, F. Iftikhar, and M. Waheed, "Turmeric: A Promising Spice for Phytochemical and Antimicrobial Activities," *J. Agric. Environ. Sci*, vol. 15, no. 7, pp. 1278–1288, 2015, doi: 10.5829/idosi.aejaes.2015.15.7.9528.

[7] M. H. Pourhanifeh *et al.*, "Therapeutic role of curcumin and its novel formulations in gynecological cancers," *J. Ovarian Res.*, vol. 13, no. 130, pp. 1–16, Dec. 2020, doi: 10.1186/s13048-020-00731-7.

[8] N. Parmar *et al.*, "Formation, characterization, and analysis of curcumin nanoformulation for evaluating its in vitro cytotoxicity," *Biotechnologia*, vol. 104, no. 3, pp. 275–287, 2023, doi: 10.5114/bta.2023.130730.

[9] R. V. Sadeghi, M. Parsania, M. Sadeghizadeh, and S. Haghighat, "Investigation of Curcumin-Loaded OA400 Nanoparticle's Effect on the Expression of E6 and E7 Human Papilloma-Virus Oncogenes and P53 and Rb Factors in HeLa Cell Line," *Iran. J. Pharm. Res.*, vol. 21, no. 1, Dec. 2022, doi: 10.5812/ijpr-130762.

[10] P. Karami *et al.*, "Nanoformulation of Polyphenol Curcumin Enhances Cisplatin-Induced Apoptosis in Drug-Resistant MDA-MB-231 Breast Cancer Cells," *Molecules*, vol. 27, no. 9, p. 2917, May 2022, doi: 10.3390/molecules27092917.

[11] X. Zhang, L. Zhu, X. Wang, H. Zhang, L. Wang, and L. Xia, "Basic research on curcumin in cervical cancer: Progress and perspectives," *Biomed. Pharmacother.*, vol. 162, no. 114590, pp. 1–10, Jun. 2023, doi: 10.1016/j.biopha.2023.114590.

[12] Y. P. Dang, X. Y. Yuan, R. Tian, D. G. Li, and W. Liu, "Curcumin improves the paclitaxel

https://doi.org/10.34011/jmp2k.v35i3.3169

e-ISSN: 2338-3445
p-ISSN: 0853-9987

induced apoptosis of HPV positive human cervical cancer cells via the NF κB p53 caspase 3 pathway," *Exp. Ther. Med.*, vol. 9, no. 4, pp. 1470–1476, 2015, doi: 10.3892/etm.2015.2240.

[13] K. Mortezaee *et al.*, "Mechanisms of apoptosis modulation by curcumin: Implications for cancer therapy," *J. Cell. Physiol.*, vol. 234, no. 8, pp. 12537–12550, Aug. 2019, doi: 10.1002/jcp.28122.

[14] S. Gökçe Kütük, G. Gökçe, M. Kütük, H. E. Gürses Cila, and M. Nazıroğlu, "Curcumin enhances cisplatin-induced human laryngeal squamous cancer cell death through activation of TRPM2 channel and mitochondrial oxidative stress," *Sci. Rep.*, vol. 9, no. 17784, pp. 1–14, Dec. 2019, doi: 10.1038/s41598-019-54284-x.

[15] S. Harakeh *et al.*, "Novel curcumin nanoformulation induces apoptosis, and reduces migration and angiogenesis in liver cancer cells," *Artif. Cells, Nanomedicine Biotechnol.*, vol. 51, no. 1, pp. 361–370, 2023, doi: 10.1080/21691401.2023.2238756.

[16] B. H. Park *et al.*, "Curcumin potentiates antitumor activity of cisplatin in bladder cancer cell lines via ROS-mediated activation of ERK1/2," *Oncotarget*, vol. 7, pp. 63870–63886, 2016, doi: 10.18632/oncotarget.11563.

[17] W. Dan, X. Cheng, Z. Fei-Fei, and Z. Bao-Jin, "Effect of curcumin on the viability of SKOV3 cells and its probable mechanism of action," *Trop. J. Pharm. Res.*, vol. 20, no. 4, pp. 767–770, Apr. 2021, doi: 10.4314/tjpr.v20i4.15.

[18] N. M. D. Sandhiutami, W. Arozal, M. Louisa, D. Rahmat, and P. E. Wuyung, "Curcumin Nanoparticle Enhances the Anticancer Effect of Cisplatin by Inhibiting PI3K/AKT and JAK/STAT3 Pathway in Rat Ovarian Carcinoma Induced by DMBA.," *Front. Pharmacol.*, vol. 11, p. 603235, 2020, doi: 10.3389/fphar.2020.603235.

[19] M. Boroughani *et al.*, "Nanocurcumin in cancer treatment: a comprehensive systematic review," *Discov. Oncol.*, vol. 15, no. 515, Dec. 2024, doi: 10.1007/s12672-024-01272-x.

[20] L. Chen, J. Qing, Y. Xiao, X. Huang, Y. Chi, and Z. Chen, "TIM-1 promotes proliferation and metastasis, and inhibits apoptosis, in cervical cancer through the PI3K/AKT/p53 pathway," *BMC Cancer*, vol. 22, no. 370, pp. 1–17, Dec. 2022, doi: 10.1186/s12885-022-09386-7.

https://doi.org/10.34011/jmp2k.v35i3.3169