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ORIGINAL ARTICLE

The Effect of Nanoliposomal Curcumin (Cur-NLPs) Proliferation Rate with Cell Counting Kit 8 (CCK-8) and PI3K expression in cervical cancer HeLa cells

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ABSTRACT

INTRODUCTION
Cervical cancer is a major health problem in developing countries. Cisplatin therapy has limitations related to toxicity and drug resistance associated with activation of the PI3K/AKT/mTOR pathway. Curcumin has anticancer activity, but its bioavailability is low; therefore, it has been developed in a nanoliposomal formulation. This study aimed to evaluate the effects of curcumin nanoliposomes (Cur-NLPs) on proliferation and PI3K expression of HeLa cells.

METHODS
An experimental study was conducted using HeLa cells, divided into seven groups: negative control, cisplatin 5 µg/mL, and Cur-NLPs groups at 50, 100, 150, 200, and 250 µg/mL. Cell viability was measured using the cell counting kit-8 (CCK-8). Measurements were performed at 24 and 48 hours at a wavelength of 450 nm. Based on viability results, PI3K expression was analyzed at 48 hours using flow cytometry in five groups: negative control, cisplatin, and Cur-NLPs at 100, 150, and 200 µg/mL. Data were analyzed using one-way ANOVA followed by Games-Howell test.

RESULTS
Cur-NLPs significantly reduced HeLa cell viability at 24 and 48 hours ($p<0.001$). The 200 µg/mL dose showed the most optimal antiproliferative effect, with viability of 38.3% at 24 hours and 18.0% at 48 hours. PI3K expression in the Cur-NLPs group (42.49–45.03%) was lower than that in the cisplatin group (66.78%), but the difference was not statistically significant ($p=0.063$).

CONCLUSION
Cur-NLPs inhibited HeLa cell viability in a dose- and time-dependent manner and showed a trend toward reduced PI3K expression. Further replication and analysis of downstream PI3K markers are needed to confirm these findings.

Keywords: Nanoliposomal curcumin, HeLa cells, cervical cancer, cell proliferation, PI3K, CCK-8, flow cytometry

INTRODUCTION

Cervical cancer is one of the leading causes of death in women worldwide, with its continuously increasing prevalence, especially in developing countries.[1] According to the World Health Organization, 90,000 women worldwide are diagnosed with cervical cancer each year, 80% of whom are in developing countries, including Indonesia.[2] Every minute, one new case appears, and every two minutes, one woman dies from cervical cancer.[3] In Indonesia, it is estimated that 40-45 new cases appear and 20-25 people die every day, so every hour it is estimated that one woman dies from cervical cancer.[4] In Indonesia, cervical cancer ranks second after breast cancer, with around 36,633 new cases and 21,003 deaths in 2020.[5] Standard cervical cancer therapy includes surgery, radiotherapy, and cisplatin-based chemotherapy.[6] Although proven effective, cisplatin has significant limitations, including nephrotoxicity, DNA damage to normal tissues, and chemotherapeutic resistance associated with activation of the PI3K/AKT pathway.[7] The phosphoinositide 3-kinase (PI3K)/AKT/mTOR pathway is a critical signaling pathway involved in regulating cancer cell proliferation, differentiation, and resistance to chemotherapy.[8] Dysregulation of this pathway is common in cervical cancer and plays a role in uncontrolled cell growth and drug resistance.[9]

Curcumin, the main bioactive polyphenol from the rhizome of *Curcuma longa* L., has long been known to possess anticancer activity through various molecular mechanisms, including modulation of the PI3K/AKT pathway, induction of apoptosis, inhibition of angiogenesis, and anti-inflammatory activity.[10] However, the oral bioavailability of curcumin is very low (less than 1%) due to its rapid first-pass metabolism and poor water solubility. These pharmacokinetic barriers have driven the development of nanotechnology-based delivery systems.[11]

Curcumin nanoliposomes (Cur-NLPs) constitute a nanoformulation that encapsulates curcumin within a phospholipid bilayer vesicle. Curcumin-based liposomal and nanoparticle formulations have been reported to improve the stability, solubility, bioavailability, and cellular uptake of curcumin compared to free curcumin, potentially enhancing its anticancer effects in various cancer cell models.[12,13] Several studies have also shown that curcumin nanoformulations are able to suppress cancer cell viability/proliferation and modulate signaling pathways related to cell survival, including PI3K/AKT/mTOR.[14,15]

HeLa cells, derived from cervical adenocarcinoma and containing HPV type 18 integration, are a widely used in vitro model in cervical cancer research because they retain the biological characteristics of cervical cancer and respond to regulatory cell growth pathways. [14] In cervical cancer, activation of the PI3K/AKT/mTOR pathway plays a role in cell growth, proliferation, metabolism, cell survival, angiogenesis, apoptosis resistance, and chemotherapy resistance.[9,14] Biologically, activation of this pathway begins with stimulation of tyrosine kinase receptors, PI3K activation, PIP3 formation, AKT activation, and mTOR activation, which regulate protein synthesis, energy metabolism, growth, and cell cycle progression.[16] Therefore, PI3K was chosen as the initial target because decreasing PI3K expression can reduce the activation of downstream AKT/mTOR signaling, which supports cancer cell proliferation and survival.[16]

Previous experimental studies indicate that curcumin nanoformulations can inhibit cancer-cell viability, induce apoptosis, and modulate pathways involved in cell survival.[17,18] Yu et al.[19] demonstrated that curcumin lipid nanoemulsion inhibited cervical cancer progression by regulating MTA1 and suppressing downstream PI3K, p-AKT, and p-mTOR protein expression. However, their study primarily focused on the role of MTA1 as an upstream regulatory target and evaluated the PI3K/AKT/mTOR axis as part of the MTA1-related molecular mechanism. In contrast, the present study evaluated Cur-NLPs as a single-treatment formulation and focused on its concentration- and time-related antiproliferative effect in HeLa cells using the CCK-8 assay at 24 and 48 hours. In addition, PI3K expression was

assessed directly using flow cytometry after 48 hours in selected Cur-NLP concentration groups based on the viability response. Therefore, the novelty of the present study lies in the evaluation of Cur-NLPs prepared using a liposomal delivery system, the use of two exposure periods for proliferation assessment, and the focused analysis of PI3K expression in relation to the most relevant Cur-NLP concentration range in HeLa cervical cancer cells.

This study aimed to evaluate the effect of Cur-NLPs on the proliferation rate of HeLa cells using the CCK-8 method and PI3K expression using flow cytometry.

METHODS

Research design

An experimental post-test only study was conducted at the Biomedical Laboratory, Faculty of Medicine, Brawijaya University, from January to April 2026.

Study subjects

The research subjects were HeLa cells passage number 4.8 obtained from the American Type Culture Collection (ATCC) that are in the collection of the Biomedical Laboratory, Faculty of Medicine, Brawijaya University. Cell cultures in RPMI 1640 medium supplemented with 10% Fetal Bovine Serum (FBS) and 1% Penicillin-Streptomycin, were incubated at 37°C with 5% CO_2.

Determination of sample size

This study used a two-stage group-selection procedure. In the first stage, the CCK-8 assay included seven experimental groups with three independent experimental replicates per group. Because reliable prior estimates of the expected effect size and within-group variability were unavailable, the sample size was justified using an adapted resource-equation approach for one-way ANOVA. The error degrees of freedom were calculated using the formula (E=N-k), where (N) represents the total number of independent experimental units and (k) represents the number of groups. With 21 experimental units and seven groups, the resulting error degrees of freedom was (E=21-7=14), which falls within the recommended range of 10–20.[20] Therefore, the viability assay comprised 21 independent experimental units (n=21). Independent replication was defined at the experimental level rather than as repeated measurements obtained from the same culture well.

The seven groups consisted of: (1) negative control group, comprising HeLa cells cultured in complete medium without treatment; (2) positive control group, comprising HeLa cells treated with 5 µg/mL cisplatin; (3) group Cur-NLPs 50 µg/mL; (4) group Cur-NLPs 100 µg/mL; (5) group Cur-NLPs 150 µg/mL; (6) group Cur-NLPs 200 µg/mL; and (7) group Cur-NLPs 250 µg/mL. The concentration range of 50–250 µg/mL was selected to characterize the dose–response pattern across a broad concentration interval, because curcumin nanoformulations have been reported to inhibit cervical cancer cell proliferation in a concentration- and time-dependent manner. [19]

Cell intervention

This study used two stages of group selection. The first stage was the CCK-8 test on seven treatment groups with three biological replications in each group, so that the total sample for the viability test was 21 observation units (n=21). The seven groups included: (1) negative control, namely HeLa cells given complete medium without treatment; (2) positive control, namely HeLa cells given 5 µg/mL cisplatin; (3) Cur-NLPs 50 µg/mL; (4) Cur-NLPs 100 µg/mL; (5) Cur-NLPs 150 µg/mL; (6) Cur-NLPs 200 µg/mL; and (7) Cur-NLPs 250 µg/mL. The dose range of 50–250 µg/mL was used to obtain a broader dose-response picture because curcumin nanoformulations can exhibit antiproliferative effects that are influenced by concentration and exposure time.[15,18] In the second stage, PI3K expression was evaluated in five groups: the negative control, cisplatin 5 µg/mL, and Cur-NLPs at concentrations of 100, 150, and 200 µg/mL, with three independent replicates per group (n=15). Flow cytometric analysis was designed as a focused mechanistic assessment rather than a complete dose–response analysis of all Cur-NLP concentrations. The concentrations of 100, 150, and 200 µg/mL were selected because they constituted a consecutive concentration range showing the clearest progressive reduction in HeLa cell viability, culminating in the greatest inhibitory effect at 200 µg/mL

The 50 µg/mL concentration was not included in the PI3K analysis because it did not reduce cell viability relative to the negative control. The 250 µg/mL concentration was also excluded because its cell viability was higher than that observed at 200 µg/mL. Thus, the response at

250 µg/mL did not follow the progressive inhibitory pattern observed from 100 to 200 µg/mL. This interpretation was based on the descriptive CCK-8 results and between-group statistical comparisons; no formal nonlinear regression analysis was performed.

Nanoliposomal curcumin preparation and treatment

Nanoliposomal curcumin was prepared using the thin-film hydration method. Curcumin purified from turmeric powder (*Curcuma longa*) from Materia Medica Batu Malang was combined with soy phosphatidylcholine, cholesterol, and Tween 80 dissolved in chloroform. The solvent was evaporated using a rotary evaporator at 45–50°C for 60 minutes to form a thin lipid film. The lipid film was hydrated with PBS solution containing Tween 80 at 50°C for 30 minutes, then sonicated using a probe sonicator to obtain a uniform particle size. Characterization using a Particle Size Analyzer (Shimadzu SALD-7500nano) revealed an average particle size of 32 nm. Identification of curcumin using HPLC compared to a curcumin standard (Merck) confirmed curcumin as the main component of the preparation.

Cell treatment was performed after HeLa cells were incubated for 24 hours and reached optimal adhesion conditions. The culture medium was then replaced with fresh medium containing Cur-NLPs at concentrations of 50, 100, 150, 200, and 250 µg/mL. The negative control group was given complete medium without treatment agents, while the positive control group was given 5 µg/mL of cisplatin. Cells in 96-well plates were used for proliferation assays using the CCK-8 method, while cells in 6-well plates were used for PI3K expression analysis using flow cytometry. After treatment, the cells were incubated at 37°C with 5% CO_2 for 24 and 48 hours to measure cell viability. PI3K expression examination was performed after 48 hours of incubation because this time period showed a clearer antiproliferative effect of Cur-NLPs based on the results of the viability test.

Cell proliferation assay with CCK-8

Cell viability was measured as an indirect indicator of proliferation using the Cell Counting Kit-8 (CCK-8, Elabscience WST-8). The principle of CCK-8 is based on the reduction of WST-8 by live cell dehydrogenases to water-soluble formazan, so that the absorbance value at 450 nm is directly proportional to the number of live/metabolically active cells. After incubation for 24 and 48 hours, 10 µL of CCK-8 solution was added to each well containing 100 µL of medium. The plates were incubated for 1–4 hours at 37°C, then the absorbance was measured at a wavelength of 450 nm using a microplate reader (Zenix-320). Cell viability was calculated using the formula: Viability (%) = [(sample absorbance – blank absorbance)/(control absorbance – blank absorbance)] × 100%. The proliferation inhibition rate was calculated as 100% minus the percentage of cell viability.[21]

PI3K expression measurement by flow cytometry

PI3K expression analysis using flow cytometry was performed after 48 hours of incubation. The 48-hour time period was chosen because, based on the results of the CCK-8 viability assay, the proliferation-inhibitory effect of Cur-NLPs was more pronounced at 48 hours of incubation compared to 24 hours. Furthermore, changes in protein expression in intracellular signaling pathways, including PI3K, generally require a longer exposure time than changes in initial metabolic viability. Therefore, PI3K assays were focused on 48 hours to illustrate the molecular response of HeLa cells to Cur-NLPs after the antiproliferative effect began to become more pronounced.

After 48 hours of incubation, cells were harvested and processed for flow cytometry analysis. A total of 2×10^5 to 1×10^6 cells were fixed and permeabilized using CytoFast™ Fix/Perm Buffer for 20 minutes in the dark. After washing with Perm Wash Buffer, cells were incubated with anti-PI3KCA antibody conjugated with PE fluorochrome at a dilution of 1:50 for 20 minutes. Cells were then resuspended in 350 µL of Cell Staining Buffer and analyzed using a BD FACSCalibur™ flow cytometer with Cell Quest Pro software. PI3K expression was expressed as the percentage of positive cells in the total cell population analyzed.

Study outcomes

The primary outcome of this study was HeLa cell viability/proliferation, measured using the CCK-8 method at 24 and 48 hours of incubation. The secondary outcome was PI3K expression in HeLa cells, analyzed using flow cytometry after 48 hours of incubation.

Statistical analysis

Data were analyzed using SPSS version 27.0 with a 95% confidence level and a significance value of $p<0.05$. The Shapiro-Wilk test was used to assess data normality, while the Levene test was used to assess homogeneity of variance. One-way ANOVA was used to compare the differences in mean HeLa cell viability between the seven treatment groups at each incubation time, namely 24 and 48 hours. In addition, one-way ANOVA was also used to compare the mean PI3K expression between treatment groups after 48 hours of incubation. If the ANOVA results showed significant differences, the analysis was continued with the Games-Howell post hoc test because the data variance was not homogeneous. The analysis results were declared statistically significant if the p value <0.05.

Ethical clearance

This research received approval from the Health Research Ethics Committee, Faculty of Medicine, Brawijaya University, Malang, under number 06/EC/KEPK/01/2026. All research procedures were conducted in accordance with applicable research ethics principles.

RESULTS

Effect of Cur-NLPs on HeLa cell proliferation

Observations of cell morphology and density were carried out using an inverted microscope (Olympus Imaging Corp IX-71) at a magnification of (400x) which showed variations in response between cells. Furthermore, quantitative proliferation evaluation was carried out using the CCK-8 method at 24 hours and 48 hours of incubation post-treatment. This method is used to assess cell viability based on the metabolic ability of cells to reduce the CCK-8 reagent to formazan which is then measured using a microplate reader (Zenix-320) at a wavelength of 450 nm.

Based on the CCK-8 assay, HeLa cell viability differed significantly among the treatment groups after 24 and 48 hours of incubation ($p<0.001$) (Table 1). At 24 hours, Cur-NLPs at concentrations of 50 and 100 µg/mL did not produce an apparent reduction in cell viability compared with the negative control, with viability values of 108.8% and 101.2%, respectively. Cell viability decreased to 76.5% at 150 µg/mL and reached its lowest level at 200 µg/mL (38.3%), before increasing to 51.9% at 250 µg/mL. At 48 hours, cell viability was 113.6%, 71.3%, 37.9%, 18.0%, and 24.7% at Cur-NLP concentrations of 50, 100, 150, 200, and 250 µg/mL, respectively. Thus, the 200 µg/mL concentration produced the strongest antiproliferative effect at both incubation periods. Moreover, cell viability at concentrations of 100–250 µg/mL was lower after 48 hours than after 24 hours, indicating a time-related increase in the inhibitory effect.

Because the homogeneity of variance assumption was not met, the Games-Howell post hoc test was applied. Pairwise comparisons showed that significant differences were mainly observed between the negative control or lower-concentration groups on the one hand and the 150–200 µg/mL Cur-NLP and cisplatin groups on the other. Overall, Cur-NLPs produced a concentration-related reduction in HeLa cell viability from 50 to 200 µg/mL; however, this pattern was not maintained at 250 µg/mL, where cell viability increased relative to that observed at 200 µg/mL.

Based on the results of the homogeneity of variance test using the Levene Test, the significance value obtained for the 24-hour absorbance data was 0.010 and that obtained for the 48-hour absorbance was 0.014 ($p < 0.05$). This indicates that the data variance between treatment groups at both incubation times was not homogeneous.

Table 1. Distribution of HeLa cell viability (%) at 24 and 48 hours of incubation by treatment groups

Variable	Treatment groups							p value
	NC (n=3)	PC (n=3)	T1 (n=3)	T2 (n=3)	T3 (n=3)	T4 (n=3)	T5 (n=3)	
Cell viability (%)								
24 hrs	100.0	25.9	108,8	101.2	76.5	38.3	51.9	< 0.001
48 hrs	100.0	13.2	113.6	71.3	37.9	18.0	24.7	< 0.001

Note: Cell viability is expressed as a percentage relative to the negative control (medium = 100%). NC: negative control; PC: positive control/cisplatin 5 µg/mL; T1: Cur-NLPs 50 µg/mL; T2: Cur-NLPs 100 µg/mL; T3: Cur-NLPs 150 µg/mL; T4: Cur-NLPs 200 µg/mL; T5: Cur-NLPs 250 µg/mL; the p-value was obtained from One-Way ANOVA test

Table 2. Results of the Games-Howell assay for cell proliferation at 24 and 48 hours

24 hours proliferation	Mean difference	p value	48 hours proliferation	Mean difference	p value
NC vs PC	1.810	0.001	NC vs PC	2.150	0.001
NC vs T1	−0.215	0.480	NC vs T1	−0.346	0.019
NC vs T2	−0.029	0.996	NC vs T2	0.706	0.003
NC vs T3	0.578	0.003	NC vs T3	1.535	<0.001
NC vs T4	1.515	<0.001	NC vs T4	2.030	< 0.001
NC vs T5	1.182	0.003	NC vs T5	1.864	0.001
PC vs T1	−2.025	0.008	PC vs T1	−2.495	0.001
PC vs T2	−1.839	0.002	PC vs T2	−1.443	<0.001
PC vs T3	−1.232	0.003	PC vs T3	−0.614	0.006
PC vs T4	−0.295	0.075	PC vs T4	−0.119	0.009
PC vs T5	−0.627	<0.001	PC vs T5	−0.285	0.001
T1 vs T2	0.186	0.591	T1 vs T2	1.052	0.002
T1 vs T3	0.793	0.029	T1 vs T3	1.881	<0.001
T1 vs T4	1.730	0.003	T1 vs T4	2.376	<0.001
T1 vs T5	1.398	0.016	T1 vs T5	2.210	0.001
T2 vs T3	0.607	0.003	T2 vs T3	0.829	0.001
T2 vs T4	1.544	<0.001	T2 vs T4	1.324	<0.001
T2 vs T5	1.212	0.004	T2 vs T5	1.158	<0.001
T3 vs T4	0.937	0.001	T3 vs T4	0.495	0.007
T3 vs T5	0.604	0.013	T3 vs T5	0.329	0.032
T4 vs T5	−0.333	0.057	T4 vs T5	−0.166	0.012

Note : Comparison after 24 and 48 hours of Games Howell proliferation test; NC: negative control; PC: positive control/cisplatin 5 µg/mL; T1: Cur-NLPs 50 µg/mL; T2: Cur-NLPs 100 µg/mL; T3: Cur-NLPs 150 µg/mL; T4: Cur-NLPs 200 µg/mL; T5: Cur-NLPs 250 µg/mL

Although the homogeneity assumption was not met, the analysis was continued using One-Way ANOVA because the data were normally distributed. The ANOVA results showed significant differences between groups at both incubation times ($p<0.001$).

The Games–Howell post hoc test showed that, after 24 hours of incubation, the negative control did not differ significantly from the Cur-NLPs groups treated with 50 µg/mL ($p=0.480$) and 100 µg/mL ($p=0.996$) (Table 2). However, significant differences were observed between the negative control and the Cur-NLPs groups treated with 150 µg/mL ($p=0.003$), 200 µg/mL ($p<0.001$), and 250 µg/mL ($p=0.003$). Among the Cur-NLPs groups, the 200 µg/mL concentration produced a viability response closest to that of cisplatin, with no statistically significant difference between the two groups ($p=0.075$) (Table 2).

After 48 hours of incubation, all pairwise comparisons among the treatment groups showed statistically significant differences, with p-values ranging from $p<0.001$ to $p=0.032$. The Cur-NLPs group treated with 200 µg/mL showed the lowest cell viability among all Cur-NLPs groups. However, its viability remained significantly different from that of the cisplatin group ($p=0.009$) (Table 2). Overall, the antiproliferative effect of Cur-NLPs was more pronounced after 48 hours, and the 200 µg/mL concentration produced the strongest inhibitory effect among the Cur-NLP concentrations tested.

Effect of nanoliposomal curcumin on PI3K expression in cervical cancer HeLa cells

Evaluation of morphological changes and cell density levels after treatment was carried out by microscopic observation after administration of nanoliposomal curcumin for 48 hours. The results showed variations in cell characteristics in each group (Figure 1).

PI3K expression was analyzed using flow cytometry only after 48 hours of incubation. The 48-hour time was chosen because CCK-8 results showed a more pronounced antiproliferative effect of Cur-NLPs at 48 hours compared to 24 hours, thus this time point was considered more representative for assessing the molecular response of PI3K. Descriptively, the cisplatin positive control group showed the highest PI3K expression at 66.78 ± 13.64%.

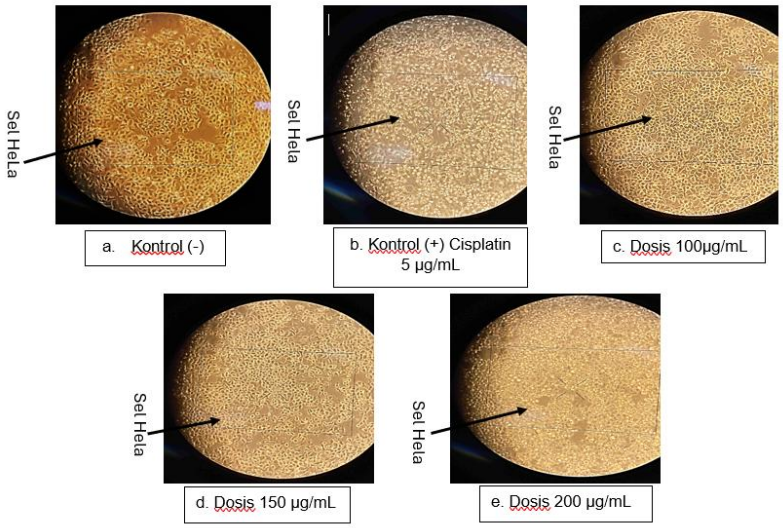

Figure 1. HeLa cells receiving nanoliposomal curcumin and undergoing flowcytometry test for 48 hours. Translation of Indonesian terms in Figure 1: Dosis = dose; Kontrol = Control group; Kontrol (-) = negative control group; Kontrol (+) = positive control group; Sel HeLa = HeLa cells

The 100, 150, and 200 µg/mL Cur-NLPs groups showed PI3K expression of 43.92 ± 11.92%, 42.49±6.68%, and 46.03±9.350%, respectively, while the negative control group showed 48.26±2.44% (Table 3). However, one-way ANOVA showed no statistically significant differences in PI3K expression among the five groups (p=0.063) (Table 3). Therefore, these findings indicate only a descriptive tendency toward lower PI3K expression and should not be interpreted as a statistically confirmed inhibitory effect.

DISCUSISION

This study showed that Cur-NLPs was able to inhibit the proliferation of cervical cancer HeLa cells in a dose- and time-dependent manner, with an optimal inhibitory effect at a dose of 200 µg/mL and 48 hours of incubation resulting in a cell viability of 18.0%. The results of the One-Way ANOVA test showed a significant difference in HeLa cell viability between treatment groups, both at 24 and 48 hours of incubation. The results of the Games-Howell test further confirmed that the difference was especially visible in the 150 µg/mL and 200 µg/mL dose groups compared to the control and low dose groups. These findings indicate that Cur-NLPs have antiproliferative activity against HeLa cells, especially at medium to high concentrations, and the effect becoming stronger with increasing exposure time.

These findings are consistent with previous studies reporting the antiproliferative activity of curcumin against various cancer cell lines, including cervical cancer cells.[14] Nanoliposomal formulations have been shown to enhance the antiproliferative efficacy of curcumin compared to free curcumin by enhancing cellular uptake, protecting it from chemical degradation, and extending its half-life in the biological environment.[12] Encapsulation in nanoliposomes allows curcumin to enter cells more efficiently through endocytosis and passive membrane penetration, thus maintaining its active concentration within the target cells for longer.[15]

Table 3. PI3K expression (%) after 48 hours of incubation across treatment groups

	Treatment groups					p value
	NC (n=3)	PC (n=3)	T100 (n=3)	T150 (n=3)	T200 (n=3)	
PI3K expression (%)	48.26 ±2.44	66.78 ±13.64	43,92 ±11.92	42.49 ±6.68	46,03±9.35	0.063

Note: Data are presented as mean ± standard deviation. NC: negative control; PC: positive control, HeLa cells treated with cisplatin 5 µg/mL; T100: Cur-NLPs 100 µg/mL; T150: Cur-NLPs 150 µg/mL; T200: Cur-NLPs 200 µg/mL. The p-value was obtained using one-way ANOVA.

The increase in cell viability at low doses (50–100 µg/mL) that was found in this study can be explained by the phenomenon of hormesis, a biological response in which a compound stimulates at very low doses but inhibits at higher doses. Curcumin is known as a hormetic agent that at low doses activates cellular adaptive stress response pathways and stimulates proliferation via the MAPK pathway, while at high doses it induces autophagy or cell death.[16] The rebound in viability at the 250 µg/mL dose suggests that the cell response does not follow a simple linear relationship, likely related to the activation of cellular adaptive mechanisms or antiapoptotic pathways, including PI3K/AKT, in response to very high pharmacological stress.

PI3K expression analysis using flow cytometry showed a tendency for decreased expression in the nanoliposomal curcumin group (42.49±6.68% to 46.03±9.35%) compared to the cisplatin group (66.78±13.64%), although this difference did not reach statistical significance. This finding suggests that Cur-NLPs have a tendency to suppress PI3K expression in HeLa cells, but this effect cannot yet be concluded as statistically significant.

The high expression of PI3K in the cisplatin group can be explained as a compensatory response of cancer cells to cytotoxic stress, by the activation of survival pathways. Cisplatin acts through the formation of DNA cross-links that trigger DNA damage and apoptosis, but in some cancer cells, this cytotoxic stress can activate pro-survival pathways such as PI3K/AKT. Activation of the PI3K/AKT pathway plays a role in promoting cell survival, inhibiting apoptosis, and contributing to chemotherapy resistance. Therefore, the increased PI3K expression in the cisplatin group in this study may reflect the activation of adaptive mechanisms in HeLa cells to cytotoxic drug exposure.[18,19]

At the molecular level, curcumin has been reported to inhibit the PI3K/AKT pathway through the regulation of various mediators, including growth factors, protein kinases, and cytokines that support the proliferation and survival of cancer cells.[15] Nanoformulation of curcumin can increase bioavailability, stability, cell penetration, and drug release, thereby strengthening the biological effects of curcumin on cancer cells.[13,17] The PI3K/AKT/mTOR pathway is one of the main pathways in cancer pathogenesis because it regulates proliferation, metabolism, apoptosis, autophagy, and therapy resistance.[19] A study by Sandhiutami et al. [17] showed that curcumin nanoparticles combined with cisplatin were able to reduce the expression of PI3K/AKT and JAK/STAT3 in an ovarian carcinoma model, thus supporting the involvement of the PI3K/AKT pathway in the anticancer effects of nanoparticle-based curcumin. Thus, the decreasing trend of PI3K in the Cur-NLPs group in this study can be explained as part of the modulation of the pro-survival pathway, although this effect is not yet statistically significant and still needs to be confirmed through examination of p-AKT, p-mTOR, Bax, Bcl-2, and caspase-3.

Nanoliposomal curcumin likely act through multiple signaling pathways simultaneously, not just PI3K, including the NF-κB pathway, JAK/STAT3, cell cycle regulation via cyclin D1, and caspase activation in the mitochondrial pathway.[22]

This study has several limitations. First, it was an in vitro study on HeLa cells, therefore the results obtained do not fully reflect the complexity of the biological response in cervical cancer tissue in humans. Second, PI3K expression was only evaluated after a 48-hour incubation period, therefore changes in PI3K expression during the initial phase of exposure, for example up to 24 hours, cannot be explained in this study. Third, PI3K measurements in this study assessed the percentage of PI3K protein expression but did not evaluate the phosphorylation status of AKT and mTOR, key downstream components of the PI3K/AKT/mTOR pathway. Therefore, comprehensive conclusions cannot be drawn about the activity of these pathways based solely on PI3K expression.

The findings of this study imply that nanoliposomal curcumin has potential as a candidate anticancer agent or adjuvant therapy for cervical cancer, particularly due to its ability to decrease HeLa cell viability in a dose- and time-dependent manner. The trend toward decreased PI3K expression in the Cur-NLPs group also suggests that the observed antiproliferative effect is likely related to modulation of the PI3K/AKT/mTOR pathway, although this mechanism still needs to be confirmed through more comprehensive molecular investigations.

Future studies are recommended to evaluate the expression of downstream proteins such as p-AKT, p-mTOR, caspase-3, Bax, and Bcl-2 to

more robustly elucidate the mechanisms of apoptosis and inhibition of cell survival pathways. Furthermore, examination at multiple incubation times, such as 24, 48, and 72 hours, could provide a more complete picture of the dynamics of cell responses to Cur-NLPs. Further studies are also needed in other cervical cancer cell models, normal cells as a toxicity benchmark, and in vivo models to more comprehensively assess the safety, bioavailability, and therapeutic efficacy of Cur-NLPs.

CONCLUSIONS

Nanoliposomal curcumin shown to inhibit the proliferation of HeLa cervical cancer cells in a dose- and time-dependent manner, with the most optimal inhibitory effect at a dose of 200 µg/mL after 48 hours of incubation. resulting in 18.0% cell viability. In addition, there was a tendency for decreased PI3K expression in the Cur-NLPs group compared to the cisplatin group. These findings suggest that Cur-NLPs have potential as an alternative or adjuvant anticancer agent in cervical cancer therapy, primarily through its antiproliferative effect on HeLa cells.

Conflict of Interest

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

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Authors' Contributions

S: responsible for conceptualization, methodological validation, and review and editing of the final manuscript. FS: Implementation of all experimental procedures, data collection, and overall writing of the manuscript. MDA: contributed to conceptualization, methodological development, supervision, data validation, and review of the manuscript and reproductive health perspectives. IWA: provided primary direction in conceptualization, methodology, overall supervision of the study, validation of results, and review and editing of the final manuscript. All authors have read and approved he final manuscipt

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Data Availability Statement

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Declaration of AI Usage in Scientific Writing

None.

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