

Physicochemical Characterization and Anticancer Potential of *Ficus deltoidea*-Silver Nanoparticles (FD-AgNPs) on HeLa Cells: Evidence from Apoptosis and Proliferation Assays

Jihan Salwa Azizah

Biomedical Science Master's Program, Faculty of Medicine, Brawijaya University

Christian Kencana Ngabdi

Biomedical Science Master's Program, Faculty of Medicine, Brawijaya University

Raisha Anis Fatiha

Biomedical Science Master's Program, Faculty of Medicine, Brawijaya University

Soon Wei To

Department of Biosciences, Faculty of Science, Universiti Teknologi Malaysia

Nik Ahmad Nizam Nik Malek

Department of Biosciences, Faculty of Science, Universiti Teknologi Malaysia

Wibi Riawan

Biochemistry and Biomolecular Department, Faculty of Medicine, Brawijaya University

Holipah Holipah

Biochemistry and Biomolecular Department, Faculty of Medicine, Brawijaya University

Hikmawan Wahyu Sulistomo

Pharmacology Department, Faculty of Medicine, Brawijaya University

Bayu Lestari

Pharmacology Department, Faculty of Medicine, Brawijaya University

Happy Kurnia Permatasari

happykp@ub.ac.id

Biochemistry and Biomolecular Department, Faculty of Medicine, Brawijaya University

Article

Keywords: *Ficus deltoidea*, Silver nanoparticles, Physicochemical characterization, Apoptosis, Proliferation

Posted Date: August 22nd, 2025

DOI: <https://doi.org/10.21203/rs.3.rs-7105511/v1>

License:  This work is licensed under a Creative Commons Attribution 4.0 International License.
[Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

Cervical cancer remains a major health problem worldwide, with over 660,000 new cases and 350,000 deaths reported in 2022. *Ficus deltoidea*, a medicinal plant rich in bioactive phytochemicals, was utilized in this study to biosynthesize silver nanoparticles (FD-AgNPs) as an anticancer agent. These nanoparticles were characterized by UV–Vis spectroscopy, showing absorbance at 420–460 nm, while FTIR analysis confirmed the role of plant compounds in reduction and capping. XRD results indicated high crystallinity with a face-centered cubic structure, and TEM images revealed an average crystallite size of 21.01 nm. FD-AgNPs significantly reduced HeLa cell viability in a dose-dependent manner ($p < 0.001$), with greater cytotoxicity observed at 5 and 10 $\mu\text{g/mL}$ compared to cisplatin. Morphological changes highlighting effective induction of apoptosis through intrinsic pathways. The antiproliferative effects of FD-AgNPs evaluated through pAKT and Ki-67 markers, showed significant reduction at 5 and 10 $\mu\text{g/mL}$ ($p < 0.001$) compared to cisplatin. Apoptosis showing significant increases in cleaved caspase-3 levels at 5 $\mu\text{g/mL}$ and 10 $\mu\text{g/mL}$ ($p < 0.0001$) compared to negative control. In conclusion, FD-AgNPs demonstrated significant anticancer activity by inhibiting proliferation and promoting apoptosis in a concentration-dependent manner, suggesting their potential as a promising therapeutic candidate for cervical cancer.

Introduction

Cervical cancer remains one of the leading causes of cancer-related mortality among women worldwide. Globally, cervical cancer remains one of the leading causes of cancer mortality in women, with 660,000 new cases and 350,000 deaths reported in 2022¹. Indonesia has the highest cervical cancer prevalence in Southeast Asia, with an incidence rate of 24.4 per 100,000 women². The high burden of disease highlights the urgent need for effective and accessible therapeutic strategies.

Cervical cancer is a prominent example of malignancy characterized by dysregulated proliferation and apoptotic evasion³. It is primarily caused by persistent infection with high-risk Human Papillomavirus (HPV) types 16 and 18, which promote oncogenesis through the expression of E6 and E7 viral proteins^{4,5}. E6 targets the tumor suppressor protein p53 for degradation, thus inhibiting apoptosis and allowing accumulation of DNA mutations⁶. Meanwhile, E7 inactivates the retinoblastoma (Rb) protein, leading to uncontrolled cell cycle progression and increased proliferation. In normal cells, proliferation is tightly regulated by cell cycle checkpoints and is balanced by apoptosis to maintain tissue homeostasis. However, in cancerous cells, this balance is disrupted, leading to excessive cell growth and resistance to cell death⁷. This dysregulation often arises from mutations and aberrant signaling in key molecular pathways involved in cell cycle control and survival.

At the molecular level, proliferative signaling in cervical cancer is often mediated by the PI3K/AKT/mTOR pathway, which plays a central role in regulating cell survival, growth, and metabolism. Activation of this pathway begins with upstream stimulation of receptor tyrosine kinases (RTKs), leading to PI3K activation, subsequent generation of PIP3, and phosphorylation of AKT which promotes cell

proliferation⁸. Ki-67 is a nuclear protein that is widely used as a marker of cellular proliferation. It is expressed during all active phases of the cell cycle (G1, S, G2, and M) but is absent in quiescent (G0) cells⁹. In parallel, apoptosis is tightly regulated and typically culminates in the activation of caspase-3, a key executioner enzyme that induces DNA fragmentation and cell death. Caspase-3 functions as an endonuclease that facilitates cell death by inducing DNA fragmentation¹⁰. Cancer cells, however, adopt various strategies to evade apoptosis. These include the overexpression of antiapoptotic proteins, downregulation of proapoptotic proteins, or disruption of death receptor signaling through reduced expression of functional receptors or increased expression of decoy receptors lacking death domains¹¹. In many cancer cells, these apoptotic disruptions occur upstream of the proteolytic activation of procaspase-3, resulting in diminished caspase-3 expression and a failure to initiate apoptosis¹². This underscores the importance of identifying bioactive compounds capable of modulating key proliferation and apoptotic pathways to restore controlled cell death in cancerous cells. In this context, attention has been drawn to natural products with multi-targeted properties that can influence various molecular mechanisms associated with cancer progression^{13,14}.

Ficus deltoidea, a traditional medicinal plant widely used in Southeast Asia, has been reported to possess antioxidant, anti-inflammatory, antibacterial, and anticancer activities¹⁵. Its bioactive compounds, including flavonoids, alkaloids, and saponins, have demonstrated the ability to inhibit cancer cell proliferation and induce apoptosis¹⁶. However, the clinical efficacy of plant-derived compounds is often limited by poor solubility, stability, and bioavailability. Besides, nanotechnology offers a promising platform to overcome these limitations. The use of nanoparticles as drug carriers enhances the bioavailability and targeted delivery of therapeutic agents¹⁷. Among these, silver nanoparticles (AgNPs) have gained attention due to their intrinsic cytotoxicity against cancer cells. Green-synthesized AgNPs can induce apoptosis by increasing intracellular reactive oxygen species (ROS), activating caspase-3, and disrupting mitochondrial membrane potential^{18,19}. Morphological changes associated with apoptosis, such as cell shrinkage, chromatin condensation, and membrane blebbing, are commonly observed following AgNPs treatment.

Despite the potential of AgNPs and *Ficus deltoidea* in cancer therapy, there is a lack of research investigating their combined effect, particularly in the form of *Ficus deltoidea*-silver nanoparticles (FD-AgNPs), on cervical cancer cell proliferation and apoptosis. To address this gap, the present study aims to synthesize FD-AgNPs and evaluate their antiproliferative and proapoptotic effects on HeLa cervical cancer cells. This will be assessed by analyzing the expression of pAKT and Ki-67 as markers of proliferation, along with cleaved caspase-3 expression and apoptotic morphology as indicators of cell death. This approach may provide new insights into the development of plant-based nanotherapeutics for cervical cancer.

CTCF = Integrated Density – (Area × Mean Fluorescence of Background) (2)

Based on this formula, Integrated Density refers to the sum of the pixel values within the selected area, Area is the size of the selected region, and Mean Fluorescence of Background is the average pixel intensity of the background. This correction accounts for background fluorescence, providing a more accurate measurement of the true signal within the region of interest.

Statistical Analysis

All experiments were conducted in duplicate. Data were expressed as mean $\pm$ SD. Statistical significance was determined using one-way ANOVA followed by Tukey's post hoc multiple comparison test for normally distributed data, with $p < 0.05$ considered statistically significant versus the control group. For non-normally distributed data, the Kruskal-Wallis test followed by Dunn's post hoc test was applied. All statistical analyses were performed using GraphPad Prism 9 software.

Methods

Plant Material Collection and Authentication

The leaves of *Ficus deltoidea* Jack var. *kunstleri* (King) Corner were collected from the Agricultural Department, Pontian District, Johor, Malaysia. The collection was conducted with verbal authorization from the officer in charge, and an official notification letter was submitted by Universiti Teknologi Malaysia (UTM) to the Agriculture Officer at the Crop Industry Development Division (Ref. No. UTM.J.09.10.01/21.10/1 (15), dated 9 November 2021). The sampling complied with all relevant institutional, national, and international guidelines and legislation for the collection and use of plant materials. The plant species was taxonomically authenticated by Dr. Richard Chung Cheng Kong, Research Officer at the Forest Research Institute Malaysia (FRIM), based on a formal verification letter (Ref. FRIM700-1/1/1Klt. 3(93)). No voucher specimen was deposited in a public herbarium, as the plant material was directly obtained from the Agricultural Department.

Preparation of Plant Extract

Mature, undamaged leaves of *Ficus deltoidea* were collected from Johor Bahru, Malaysia. In the laboratory, leaves were separated, rinsed with tap water, and air-dried to remove surface impurities. They were then dried in a hot air oven at 40°C for 24 hours, reweighed, and ground into powder using a laboratory blender. The powder was sieved (104-mesh) for uniformity. To prepare the aqueous extract, 2 g of the powder was dissolved in 100 mL of distilled water (2% w/v) and stirred at room temperature ($22 \pm 2^\circ\text{C}$) for 30–60 minutes. This preserved thermolabile compounds such as flavonoids and phenolics. The mixture was filtered using Whatman No.1 filter paper to obtain a clear extract, which was stored at 4°C in the dark. This green synthesis approach uses water as a solvent, avoiding organic chemicals to ensure safety and biocompatibility of the final AgNPs formulation. Thus, the resulting mixture was filtered using Whatman No.1 qualitative filter paper (125 mm diameter) to remove insoluble plant debris, producing a clear, light brown extract. The extract was collected in sterile Erlenmeyer flasks and stored at 4°C in the dark to prevent degradation of sensitive phytochemicals prior to its use in the green

synthesis of AgNPs. This process adheres to principles of green chemistry by using water as a solvent and avoiding organic chemicals, ensuring safety, environmental compatibility, and biocompatibility of the final nanoparticle formulation.

Green Synthesis of AgNPs

To initiate the green synthesis process, a 1 mM solution of silver nitrate (AgNO_3) was freshly prepared by dissolving 0.16988 g of analytical-grade AgNO_3 in 1 L of distilled water. Various volumes of *Ficus deltoidea* aqueous extract—specifically 0.1, 0.5, 1.0, 1.5, and 2.0 mL—were separately introduced into 10 mL aliquots of the silver nitrate solution. Each mixture was gently stirred to promote uniform distribution. All reactions were conducted under room temperature conditions, shielded from direct light to prevent photoreduction. The progress of nanoparticle formation was monitored periodically by visual observation, focusing on color changes as an initial indicator of successful synthesis.

Characterization of FD-AgNPs

The synthesized FD-AgNPs were characterized using a combination of spectroscopic and microscopic techniques to confirm their formation and determine their physicochemical properties. As we can see in Fig. 1, the green synthesis of FD-AgNPs was initiated by mixing the aqueous extract of *Ficus deltoidea* with silver nitrate (AgNO_3) solution. The bioreduction of silver ions (Ag^+) into elemental silver (Ag^0) was visually indicated by a gradual color change in the reaction mixture, from pale yellow to dark brown, suggesting the formation of silver nanoparticles. This reaction highlights the dual role of the plant extract as both a reducing and capping agent. The final colloidal suspension of FD-AgNPs was then subjected to various physical characterization techniques, including UV-Vis spectroscopy, FTIR, XRD, and TEM, to analyze its optical properties, functional groups, crystalline structure, and morphology.

UV-Visible (Uv-Vis) Spectrophotometry was conducted to monitor the surface plasmon resonance (SPR) peak of the nanoparticles. The SPR typically appearing between 400–450 nm, as a preliminary indicator of AgNPs synthesis. Fourier-transform infrared (FTIR) spectroscopy was used to identify functional groups involved in the bioreduction and stabilization of the nanoparticles by comparing the spectral profiles of the pure *Ficus deltoidea* extract and the FD-AgNPs. Shifts in characteristic absorption bands provided insight to possible interactions between phytochemicals and silver ions. Transmission electron microscopy (TEM) was employed to observe the morphology and determine the average particle size of the FD-AgNPs at the nanoscale, including size distribution and shape uniformity. Finally, X-ray diffraction (XRD) analysis was performed to evaluate the crystalline structure of the nanoparticles. Distinct diffraction peaks corresponding to the face-centered cubic (FCC) structure of elemental silver confirmed the crystalline nature of the FD-AgNPs. As shown in Eq. (1), the average crystallite size of the synthesized FD-AgNPs was estimated using the Debye-Scherrer²⁰.

$$D = (K \lambda) / (\beta \cos \theta) \quad (1)$$

K is the shape factor (commonly 0.9), λ is the X-ray wavelength, β is the full width at half maximum (FWHM) of the diffraction peak in radians, and θ is the Bragg angle. By applying this equation, the average crystallite size can be determined, providing valuable information about the nanoscale dimensions of the material.

Cell Culture

HeLa cells, obtained from the Laboratory of Biochemistry and Biomolecular Sciences, Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia, were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and antibiotics (100 U/mL penicillin and 100 U/mL streptomycin). The cells were incubated at 37°C in a humidified atmosphere containing 5% CO₂ and maintained at a pH of 7.2–7.4. HeLa cells were routinely passaged and harvested using trypsin-EDTA solution. Once cell confluency reached at least 80%, the cells were seeded into 6 and 96-well plates for subsequent experiments.

Cell Viability Assay

The cytotoxicity of FD-AgNPs against HeLa cells was assessed using the WST-1 assay. HeLa cells were seeded into 96-well plates at a density of 1×10^4 cells per well and incubated for 24 hours. The cells were then treated with FD-AgNPs at concentrations of 2.5, 5, and 10 µg/mL. Untreated cells (0 µg/mL) served as the negative control, while cells treated with cisplatin (10 µg/mL) served as the positive control. After 24 hours of treatment, 10 µL of WST-1 reagent was added to each well and incubated for an additional 2 hours at 37°C. Absorbance was measured at 450 nm using a microplate reader. Cell viability was calculated relative to the negative control.

Morphological Analysis

To assess morphological alterations, approximately 1×10^5 HeLa cells were cultured in 6-well plates and incubated overnight, and then treated with FD-AgNPs at concentrations of 2.5, 5, and 10 µg/mL. The negative control group received only complete culture medium, while the positive control group was treated with 2.5 µg/mL cisplatin. After 24 hours treatment, cellular morphology was examined using an inverted phase contrast microscope, and images were captured at 40x magnification.

Proliferation and Apoptosis Markers Analysis

Indirect immunofluorescence was conducted to evaluate the expression of pAKT, Ki-67, and cleaved caspase-3 in treated HeLa cells. pAKT and Ki-67 are established markers of cellular proliferation, while cleaved caspase-3 serves as an apoptotic marker. Approximately 1×10^4 cells were seeded in 96-well plates in duplicate for each group. After treatment, cells were washed three times with PBS (3, 3, and 2 minutes), fixed with 4% paraformaldehyde for 15 minutes, and permeabilized using 0.1% Triton X-100 in PBS for 15 minutes. Following another PBS wash, cells were blocked with 1% BSA in PBS for 60 minutes at room temperature. Cells were then incubated overnight at 4°C in the dark with rabbit monoclonal primary antibodies against pAKT, Ki-67, or cleaved caspase-3. The next day, after PBS washing, cells

were incubated with fluorescently labeled anti-rabbit secondary antibodies for 2 hours at room temperature, protected from light. Nuclear staining was performed using DAPI for 5 minutes, followed by a final PBS wash. Stained cells were mounted with fluorescence mounting medium and visualized under an inverted fluorescence microscope.

Image analysis was performed using ImageJ software. The intensity and area of red fluorescence indicated the expression levels of proliferation and apoptotic markers. As presented in Eq. (2), the quantification was based on integrated density, mean fluorescence intensity, and area, with Corrected Total Cell Fluorescence (CTCF) calculated to minimize background interference and allow data normalization across samples. The formula used was:

Results

Characterization of the FD-AgNPs

UV-Vis Spectrophotometry

The synthesis and development of FD-AgNPs were characterized through UV-Vis spectrophotometry and visual color observation. As we can see in Fig. 2a, The appearance of a distinct SPR peak between 430–450 nm confirmed the formation of silver nanoparticles, with increasing absorbance over time indicating nanoparticle nucleation, growth, and stabilization by day 4 to day 6. As shown in Fig. 2b, these spectrophotometric results were consistent with visible color changes in the reaction mixture, which gradually shifted from pale yellow to reddish-brown as reaction time and silver nitrate concentration increased. Higher concentrations of AgNO_3 led to more intense coloration and higher absorbance values, reflecting the formation of larger and more stable nanoparticles. Together, these findings confirm the successful, time-dependent green synthesis of FD-AgNPs using *Ficus deltoidea* extract, highlighting the influence of both reaction time and precursor concentration on nanoparticle properties.

Transmission Electron Microscopy

The Transmission Electron Microscopy (TEM) image displays the morphology of AgNPs synthesized using *Ficus deltoidea* extract. As demonstrated in Fig. 3a, the nanoparticles appear predominantly spherical to quasi-spherical and are mostly aggregated in clusters. Based on the scale bar and visible size markers, the particle diameters range approximately between 20 nm to 40 nm, with an average size appearing to cluster around 23–31 nm. This confirms that the particles are within the nanoscale range (1–100 nm). The dense, dark contrast observed in the particles is characteristic of metallic silver due to its high electron density. Although there is noticeable aggregation, the individual particles still maintain distinct borders, indicating partial surface stabilization. The TEM imaging was performed at a magnification of 50,000× and an accelerating voltage of 120 kV, providing high-resolution visualization suitable for accurate nanoparticles characterization.

Fourier Transform Infrared Spectroscopy

FTIR analysis was performed to identify the functional groups involved in the synthesis and stabilization of FD-AgNPs. The FD extract showed peaks corresponding to O-H stretching (3795 cm^{-1} , 3293 cm^{-1}), C-H stretching of alkanes (2918 cm^{-1} , 2851 cm^{-1}), and C = O stretching of carbonyl groups (1730 cm^{-1}), indicating the presence of phenolics, flavonoids, and carboxylic acids. After nanoparticle formation, we can take a look in Fig. 3b that shifts in O-H peaks (3823 cm^{-1} , 3267 cm^{-1}) and the appearance of new peaks at 2112 cm^{-1} and 1968 cm^{-1} suggested the involvement of these functional groups in nanoparticle synthesis. Changes in peaks around 1602 cm^{-1} and $1500\text{--}1000\text{ cm}^{-1}$ further confirmed the capping of AgNPs by bioactive compounds. These findings highlight the role of *Ficus deltoidea* phytochemicals in the green synthesis and stabilization of FD-AgNPs.

X-Ray Diffraction

The crystalline structure and phase identification of FD-AgNPs were analyzed using X-ray diffraction (XRD). According to Fig. 3c, The XRD pattern showed distinct peaks at 2θ values of approximately 38° , 44° , 64° , and 77° , corresponding to the (111), (200), (220), and (311) planes of face-centered cubic (fcc) silver (JCPDS no. 04-0783)²¹. These peaks confirm the successful synthesis of highly crystalline AgNPs, with the most intense peak at 38° indicating a preferred (111) orientation due to its thermodynamic stability. Minor additional peaks at higher angles may be related to bio-organic compounds from *Ficus deltoidea* extract acting as capping agents. The absence of impurity peaks indicates the high purity of the nanoparticles. The average crystallite size, calculated using the Debye-Scherrer equation, was 21.01 nm, confirming the nanoscale nature of the FD-AgNPs. This small particle size is essential for enhancing surface area, biological interactions, and potential biomedical applications.

FD-AgNPs Reduced HeLa Cells Viability

The cytotoxic effects of FD-AgNPs on HeLa cells were evaluated using the WST assay at concentrations of 2.5, 5, and 10 $\mu\text{g/mL}$. As shown in Table 1, FD-AgNPs significantly reduced cell viability in a dose-dependent manner. Treatment with 2.5 $\mu\text{g/mL}$ decreased viability to 73.2% compared to the negative control (104.3%). A sharp decline was observed at 5 $\mu\text{g/mL}$, with viability dropping to 4.4%, and remained low at 10 $\mu\text{g/mL}$ (9.7%). The most substantial reduction occurred at 5 $\mu\text{g/mL}$, suggesting a plateau effect, as no significant difference (ns) was detected between 5 and 10 $\mu\text{g/mL}$. In comparison, the positive control (cisplatin 2.5 $\mu\text{g/mL}$) exhibited 91.6% viability, showing less cytotoxicity than FD-AgNPs at 5 and 10 $\mu\text{g/mL}$ ($p < 0.001$). However, the lowest FD-AgNP concentration (2.5 $\mu\text{g/mL}$) was less effective than cisplatin. No significant difference was found between the control and cisplatin group, which may be due to the low cisplatin dose or exposure time.

Table 1
HeLa Cell Viability (%) after FD-AgNPs Treatment Measured by WST Assay. Cell viability of HeLa cells after treatment with various concentrations of FD-AgNPs (2.5–10 µg/mL), as measured using the WST assay. C–: negative control, C+: positive control (cisplatin 2.5 µg/mL).

Cell Viability (%)					
Replicates	Treatments (µg/mL)				
	C-	FD2.5	FD5	FD10	C+
1	104.39	77.98	6.40	5.08	96.76
2	104.22	68.39	2.38	14.27	86.35
Average	104.3	73.2	4.4	9.7	91.6

The CTCF analysis of cell viability measured by WST assay is presented in Fig. 4. Treatment with FD-AgNPs at concentrations of 5 and 10 µg/mL significantly reduced cell viability compared to both the negative control (C–) and positive control (C+) ($p < 0.001$). No significant difference (ns) was observed between the 5 and 10 µg/mL groups, suggesting a plateau effect at higher doses. Meanwhile, the 2.5 µg/mL group showed no significant reduction compared to controls. These findings confirm that FD-AgNPs induce dose-dependent cytotoxicity in HeLa cells, with the most significant effects reflected by decreased CTCF values at higher concentrations. These findings, supported by CTCF analysis, confirm that FD-AgNPs induce dose-dependent cell death in HeLa cells, with potent cytotoxic effects exceeding those of cisplatin at higher concentrations.

FD-AgNPs Altered HeLa Cells Morphology

Morphology assessment of HeLa cells following FD-AgNPs treatment revealed dose-dependent cytotoxic alterations as seen in Fig. 5. Untreated cells exhibited a typical cobblestone-like epithelial morphology with high confluency and intact cell membranes. Upon exposure to 2.5 µg/mL FD-AgNPs, early morphological signs of cytotoxicity were observed, including partial cell rounding and reduced cell density. At 5 µg/mL, more pronounced changes were evident, such as increased cell shrinkage, membrane irregularities, and detachment from the substrate. These effects were further intensified at 10 µg/mL, where cells displayed severe morphological disruption, including membrane blebbing and loss of intracellular contact, consistent with apoptotic features. Notably, the morphological profile at 10 µg/mL was comparable to that observed in the cisplatin-treated group, suggesting similar levels of cytotoxic impact. These findings indicate that FD-AgNPs induce morphological alterations characteristic of apoptosis in HeLa cells in a dose-dependent manner.

Effects of FD-AgNPs on Proliferation of HeLa Cells

Inhibitory Effect of FD-AgNPs on pAKT Expression

pAKT is one of the key markers used to assess cancer cell survival and progression, reflecting the activation of the PI3K/AKT signaling pathway that promotes proliferation and resistance to apoptosis. Elevated pAKT expression is frequently linked to tumor aggressiveness and poor response to therapy⁸. Indirect immunofluorescence analysis showed that FD-AgNPs reduced pAKT expression in a concentration-dependent manner. Based on the results shown in Fig. 6a, strong red fluorescence indicated high pAKT levels in untreated cells, with merged images displaying intense purple signals. Treatment with 2.5 µg/mL FD-AgNPs visibly reduced red fluorescence, while at 5 and 10 µg/mL, the signal was markedly diminished or nearly absent, resembling the suppression seen in cisplatin-treated cells. This visual pattern was confirmed by Corrected Total Cell Fluorescence (CTCF) analysis referring to Fig. 6b, where pAKT expression significantly decreased across all FD-AgNP-treated groups compared to the control (**p < 0.01 to ***p < 0.001). The strongest reductions were observed at 5 and 10 µg/mL, with no significant difference between them (ns), indicating a plateau effect. These findings demonstrate that FD-AgNPs effectively inhibit pAKT signaling, potentially disrupting cancer cell survival pathways.

FD-AgNPs Suppressed Ki-67 Expression

Ki-67 is one of the established markers of cancer cell proliferation, expressed during active phases of the cell cycle and commonly associated with tumor growth, aggressiveness, and prognosis⁹. As presented in Fig. 6c, immunofluorescence staining revealed that FD-AgNPs reduced Ki-67 expression in a dose-dependent manner. Untreated cells showed strong red fluorescence indicating high Ki-67 expression and active proliferation, with merged images displaying intense purple signals. Treatment with 2.5 µg/mL FD-AgNPs led to weaker, uneven Ki-67 staining, which became markedly reduced at 5 µg/mL. At 10 µg/mL, Ki-67 expression was nearly absent, with merged images dominated by DAPI staining—similar to the cisplatin-treated group. These visual observations were confirmed by CTCF analysis referring to Fig. 6d, which showed significant reductions in Ki-67 expression at 2.5 µg/mL (*p < 0.05) and 5 µg/mL (**p < 0.01) compared to the control. The lowest expression was observed at 10 µg/mL, though no significant differences were found between the 5 µg/mL, 10 µg/mL, and cisplatin groups, suggesting comparable antiproliferative effects. Additionally, Ki-67 expression in the cisplatin group was significantly lower than in the control (***p < 0.001). Collectively, these results highlight the ability of FD-AgNPs to downregulate Ki-67 expression and reduce cancer cell proliferation in a dose-dependent manner.

Effects of FD-AgNPs on Apoptosis of HeLa Cells

FD-AgNPs Increased Cleaved Caspase-3 Expression

Cleaved caspase-3 serves as a definitive indicator of apoptosis, marking the execution phase where cellular components are systematically degraded and DNA fragmentation occurs¹⁰. Immunofluorescence staining using cleaved caspase-3 (red) and DAPI (blue) demonstrated that FD-AgNPs induced apoptosis in HeLa cells in a dose-dependent manner. According to Fig. 7a, the untreated control (C-) showed minimal red fluorescence indicated low basal caspase-3 activation. Treatment with 2.5 µg/mL FD-AgNPs showed a slight increase in red fluorescence, while higher concentrations at 5

µg/mL and 10 µg/mL resulted in progressively stronger caspase-3 expression, as reflected by the intense red signals. The highest expression at 10 µg/mL was comparable to the cisplatin-treated positive control (C+), indicating robust apoptotic induction. As we can see in Fig. 7b, quantitative analysis of Corrected Total Cell Fluorescence (CTCF) further confirmed these observations, showing significant increases in cleaved caspase-3 levels at 5 µg/mL and 10 µg/mL ($p < 0.0001$) compared to the negative control. These findings highlight the ability of FD-AgNPs to activate the apoptotic pathway via caspase-3 activation in a concentration-dependent manner.

Discussion

Biosynthesized AgNPs are gaining attention as eco-friendly anticancer agents due to their targeted cytotoxicity and minimal systemic toxicity²². In this study, the UV–Vis spectra of FD-AgNPs showed strong absorbance between 420–460 nm, confirming nanoparticle formation via phytochemical reduction by *Ficus deltoidea*. The gradual absorbance decline from 0H to 6D suggests nanoparticle maturation and possible agglomeration or sedimentation, while the lack of peaks beyond 500 nm indicates small, stable, likely spherical nanoparticles. Phytochemicals such as flavonoids and phenolic acids from *Ficus deltoidea* may stabilize the nanoparticles and enhance anticancer activity through ROS-mediated pathways. Additionally, based on prior studies, a cellular saturation effect may occur at 5–10 µg/mL, possibly reflecting limited nanoparticle uptake or maximal activation of apoptotic pathways²³. These findings highlight the potential of FD-AgNPs as biocompatible anticancer agents, though further studies on mechanisms and long-term safety are necessary.

AgNPs synthesized using *Ficus deltoidea* and characterized by TEM exhibit favorable physicochemical properties for biomedical applications. The nanoparticles are predominantly spherical, sized around 10–40 nm, which is ideal for cellular uptake, antimicrobial activity, and tumor targeting via the Enhanced Permeability and Retention (EPR) effect²⁴. The mild agglomeration observed may stem from van der Waals forces or incomplete phytochemical stabilization, with natural capping agents like flavonoids and terpenoids from the extract contributing to nanoparticle stability and biological activity. Such spherical nanoparticles are known to enhance internalization and promote apoptosis through oxidative stress and mitochondrial disruption²⁵. The nanometer-scale size (mostly < 100 nm) reflects successful bioreduction and capping, although partial aggregation could be influenced by factors such as pH, extract concentration, or surface coverage²⁶. TEM provides direct visualization of nanoparticle morphology, complementing Dynamic Light Scattering (DLS) measurements of hydrodynamic diameter²⁷. These characteristics support the promising application of FD-AgNPs for anticancer therapy, particularly against cervical cancer cells like HeLa.

FTIR analysis confirmed the successful biosynthesis of AgNPs using *Ficus deltoidea* extract, which serves as both reducing and capping agent. The broad O–H band at 3293 cm⁻¹ in the extract shifted to 3267 cm⁻¹ in FD-AgNPs, indicating involvement of hydroxyl groups in Ag⁺ reduction²⁸. The retained C–H stretching bands (2919 and 2850 cm⁻¹) suggest preservation of aliphatic structures. The diminished

carbonyl peak near 1730 cm^{-1} and new bands at 1515, 1456, 1376, and 1233 cm^{-1} reflect oxidation and the presence of aromatic and carboxylate groups that stabilize the nanoparticles²⁹. Additional fingerprint peaks (1050 cm^{-1} , 718 cm^{-1}) confirm phytochemical binding on the nanoparticle surface. These spectral changes highlight the dual role of *Ficus deltoidea* phytochemicals in reducing silver ions and capping nanoparticles, enhancing their colloidal stability and anticancer potential²³. Overall, the FTIR results support the green synthesis and functionalization of bioactive AgNPs.

XRD analysis revealed sharp diffraction peaks confirming the high crystallinity of FD-AgNPs with a face-centered cubic (FCC) structure. The dominant peak at $2\theta \approx 38.1^\circ$ corresponds to the (111) plane, with additional peaks at 32° , 44° , 64° , and 77° matching the (200), (220), (311), and (222) planes, respectively³⁰. This crystalline nature is crucial for nanoparticle stability, reactivity, and bioactivity³¹. The absence of impurity peaks indicates phase-pure silver, while slight peak variations suggest surface modification by capping phytochemicals like flavonoids and terpenoids²². Using the Debye–Scherrer equation, the average crystallite size of FD-AgNPs was calculated to be approximately 21.01 nm, which falls within the optimal nanometer scale for biomedical applications. These findings align with previous reports of plant-mediated AgNP synthesis, confirming that bioreduction preserves the crystalline nature of silver³². The distinct XRD pattern and nanocrystalline size support the successful formation of pure, crystalline FD-AgNPs with promising biomedical potential, including cancer therapy.

AKT, or protein kinase B, plays a central role in the PI3K/AKT/mTOR pathway, which regulates cancer cell survival, proliferation, and resistance to therapy³³. Phosphorylated AKT (pAKT) promotes cell cycle progression and inhibits apoptosis, with its hyperactivation linked to chemoresistance in cervical cancer³⁴. In this study, FD-AgNPs at 5 and 10 $\mu\text{g/mL}$ significantly reduced pAKT expression, suggesting inhibition of this prosurvival pathway, possibly through blockade of AKT phosphorylation or upstream PI3K³⁵. Phytochemicals in *Ficus deltoidea*, particularly Vitexin, are known to suppress proliferation via PI3K/AKT/mTOR inhibition^{36,37}, and FD-AgNPs may exert similar effects, potentially through ROS-mediated disruption of survival signaling³⁸. Notably, pAKT suppression by 10 $\mu\text{g/mL}$ FD-AgNPs was comparable to cisplatin, which may enhance apoptosis and sensitize cells to treatment³⁹. This downregulation of AKT could also restore GSK3 function, leading to β -catenin degradation and reduced expression of proliferation-related genes⁴⁰. In parallel, FD-AgNPs significantly reduced Ki-67 expression, a key marker of proliferation, in HeLa cells ($p < 0.0001$), with effects comparable to cisplatin ($p < 0.05$)⁴¹. High Ki-67 levels are associated with tumor aggressiveness and poor prognosis⁹. The observed antiproliferative effect is likely driven by ROS-induced DNA damage and p53 pathway activation, resulting in cell cycle arrest at G1/S or G2/M phases⁴². These findings are consistent with prior reports that AgNPs and plant-based nanoparticles downregulate Ki-67 and induce cell cycle arrest in various cancers^{43,44}. Furthermore, the inverse link between Ki-67 and cell cycle regulators like p21 and p27 suggests FD-AgNPs may help restore p53-p21-mediated control, reinforcing their potential to suppress cancer cell proliferation through both direct antiproliferative action and molecular regulation of cell cycle checkpoints⁴⁵.

To further investigate whether growth inhibition was accompanied by apoptotic induction, we evaluated morphological changes and apoptosis-specific markers in FD-AgNPs-treated HeLa cells. Consistent with previous studies, phytochemical-mediated AgNPs, including FD-AgNPs, induce apoptosis through oxidative stress²⁹. FD-AgNPs exhibited strong cytotoxicity at 5 and 10 µg/mL, surpassing cisplatin, with an IC₅₀ ≤ 20 µg/mL. Morphological changes observed—cell rounding, shrinkage, detachment, and membrane blebbing—confirmed dose-dependent apoptosis (2.5–10 µg/mL), consistent with other plant-based nanoparticle studies⁴⁶. The likely mechanism involves ROS overproduction by *Ficus deltoidea* bioactives, leading to oxidative damage and activation of intrinsic apoptotic pathways via Bax and p53⁴⁷. Immunofluorescence analysis further revealed that FD-AgNPs significantly increased cleaved caspase-3 expression in a dose-dependent manner, indicating activation of the intrinsic mitochondrial apoptotic pathway, likely mediated by oxidative stress or mitochondrial dysfunction⁴⁸. This is in line with previous findings where *Ficus deltoidea* extracts activated caspase-3/7 and mitochondrial depolarization in prostate, colorectal, and breast cancer cells^{47–49}. Gene expression studies showed upregulation of pro-apoptotic markers (Fas1, Bax, TNF-α) and downregulation of anti-apoptotic genes (Bcl-2, Cdk-2), suggesting additional activation of extrinsic apoptosis through death receptor signaling and DISC complex formation⁵⁰. Similarly, AgNPs synthesized with *Fagonia indica* induced apoptosis via caspase-3/-9 activation, ROS generation, and DNA fragmentation, highlighting a shared mechanism across green-synthesized nanoparticles^{51,52}.

Conclusions

In summary, this study demonstrates that biogenic FD-AgNPs effectively inhibit the proliferation of HeLa cervical cancer cells and induce apoptosis, as indicated by decreased expression of Ki-67 and pAKT, increased cleaved caspase-3 activation, and apoptosis-related morphological changes. The physicochemical analyses (XRD, TEM, FTIR, UV-Vis) confirmed the successful synthesis of stable and bioactive nanoparticles with characteristics suitable for biomedical applications.

Study Limitations

This study was limited to in vitro assays using a single cancer cell line, without comparison to normal cells or in vivo validation. Therefore, the anticancer potential of FD-AgNPs requires further investigation in animal models and clinical settings to confirm their efficacy and safety. Furthermore, only a limited number of molecular markers were examined, and the underlying signaling pathways remain to be fully elucidated. Additional mechanistic studies, including transcriptomic or proteomic profiling, are necessary to identify key molecular targets and pathways involved in the observed anticancer effects.

Abbreviations

AgNPs

Silver Nanoparticles

FD-AgNPs

Ficus deltoidea-Silver Nanoparticles

FD2.5

Ficus deltoidea 2.5 µg/ml

FD5

Ficus deltoidea 5 µg/ml

FD10

Ficus deltoidea 10 µg/ml

DMEM

Dulbecco's Modified Eagle's Medium

XRD

X-Ray Diffraction

TEM

Transmission Electron Microscopy

FTIR

Fourier-transform infrared

UV-Vis Spectroscopy

Ultraviolet Visible Spectroscopy

Ki-67

pAKT,Phosphorylated Protein Kinase B (AKT)

p53

Tumor Protein p53

PI3K/AKT/mTOR

Phosphoinositide-3 Kinase/Protein Kinase B/Mammalian Target of Rapamycin

PIP3

Phosphatidylinositol (3,4,5)-Trisphosphate

AgNO₃

Silver Nitrate

SPR

Surface Plasmon Resonance

FCC

Face-Centered Cubic

WST-1

Water-Soluble Tetrazolium-1

PBS

Phosphate-Buffered Saline

DAPI

4',6-Diamidino-2-Phenylindole

CTCF

Corrected Total Cell Fluorescence

EPR

Enhanced Permeability and Retention

DLS

Dynamic Light Scattering

TNF- α

Tumor Necrosis Factor Alpha

Fas1

Fas Cell Surface Death Receptor 1

Bax

Bcl-2-Associated X Protein

Cdk-1

Cyclin-Dependent Kinase 1

MCF-7

Michigan Cancer Foundation-7 (a human breast cancer cell line).

Declarations

Consent of publication

Not applicable.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Faculty of Medicine, Brawijaya University (No.62/EC/KEPK/03/2025). Written informed consent was obtained from all participants before enrolment.

Availability and data materials

The datasets and software used for supporting the conclusions of this article are available from the public data repository at the website of <https://doi.org/10.57760/sciencedb.28646>.

Competing interests

The authors declare that they have no competing interests.

Funding

This research was financially supported by the Research Grant of Brawijaya University under the contract number: 7169/UN10.F0701/B/PT.01.05.1/2025.

Author Contribution

J.S.A. was responsible for project administration, investigation, methodology, writing & editing – original draft. C.K.N. contributed to project administration, investigation, methodology, and writing – original draft. R.A.F. was involved in data curation and methodology. S.W.T was involved in data curation and methodology. N.A.N.N.M. contributed to conceptualization, validation, and resources. W.R. contributed to conceptualization, validation & funding acquisition. H.H. contributed to conceptualization, validation & funding acquisition. H.W.S contributed to conceptualization, validation & formal analysis. B.L. contributed to formal analysis, validation, supervision, and writing – review & editing. H.K.P. contributed to formal analysis, supervision, funding acquisition, writing – review & editing, and writing – original draft. All authors reviewed the manuscript.

Acknowledgement

The authors gratefully acknowledge all individuals who contributed to the success of this study, particularly those who provided essential support during the experimental work and manuscript preparation.

Data Availability

The datasets and software used for supporting the conclusions of this article are available from the public data repository at the website of <https://doi.org/10.57760/sciencedb.28646>.

References

1. WHO. Cervical Cancer Fact Sheet. *World Health Organization*; (2022). <https://www.who.int/news-room/fact-sheets/detail/cervical-cancer>
2. Sung, H. et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J. Clin.* **71**, 209–249. <https://doi.org/10.3322/caac.21660> (2021).
3. Yeo-Teh, N. S. L., Ito, Y. & Jha, S. High-Risk Human Papillomaviral Oncogenes E6 and E7 Target Key Cellular Pathways to Achieve Oncogenesis. *Int. J. Mol. Sci.* **19**, 1706. <https://doi.org/10.3390/ijms19061706> (2018).
4. Fowler, J., Maani, E., Dunton, C., Gasalberti, D. & Jack, B. Cervical Cancer. *StatPearls [Internet]* (2023).

5. Estêvão, D., Costa, N. R., Gil da Costa, R. M. & Medeiros, R. Hallmarks of HPV carcinogenesis: The role of E6, E7 and E5 oncoproteins in cellular malignancy. *Biochimica et Biophysica Acta (BBA) - Gene Regulatory Mechanisms*. 1862, 153–162; (2019).
<https://doi.org/10.1016/j.bbagr.2019.01.001>
6. Martinez-Zapien, D. et al. Structure of the E6/E6AP/p53 complex required for HPV-mediated degradation of p53. *Nature* **529**, 541–545. <https://doi.org/10.1038/nature16481> (2016).
7. Rascio, F. et al. The Pathogenic Role of PI3K/AKT Pathway in Cancer Onset and Drug Resistance: An Updated Review. *Cancers (Basel)*. **13**, 3949. <https://doi.org/10.3390/cancers13163949> (2021).
8. Shi, X. et al. Research progress on the PI3K/AKT signaling pathway in gynecological cancer (Review). *Mol. Med. Rep.* **19**, 4529–4535. <https://doi.org/10.3892/mmr.2019.10121> (2019).
9. Sun, X. & Kaufman, P. D. Ki-67: more than a proliferation marker. *Chromosoma* **127**, 175–186. <https://doi.org/10.1007/s00412-018-0659-8> (2018).
10. Eskandari, E. & Eaves, C. J. Paradoxical roles of caspase-3 in regulating cell survival, proliferation, and tumorigenesis. *J. Cell Biol.* **221** <https://doi.org/10.1083/jcb.202201159> (2022).
11. Noor, Z. et al. Caspase 3 and Its Role in the Pathogenesis of Cancer. *Clin. Oncol.* **7**, 1–10 (2022).
12. Boudreau, M. W., Peh, J. & Hergenrother, P. J. Procaspase-3 Overexpression in Cancer: A Paradoxical Observation with Therapeutic Potential. *ACS Chem. Biol.* **14**, 2335–2348. <https://doi.org/10.1021/acscchembio.9b00338> (2019).
13. Aryani, R. et al. Anti-angiogenic activity of *Ficus deltoidea* L. Jack silver nanoparticles using chorioallantoic membrane assay. *F1000Res.* **12**, 544; (2023).
<https://doi.org/10.12688/f1000research.130477.1>
14. Liu, X., Jiang, Q., Liu, H. & Luo, S. Vitexin induces apoptosis through mitochondrial pathway and PI3K/Akt/mTOR signaling in human non-small cell lung cancer A549 cells. *Biol. Res.* **52**, 7. <https://doi.org/10.1186/s40659-019-0214-y> (2019).
15. Abdulrahman, M. D. Crude extract of *Ficus deltoidea* Jack (FD) as a natural biological therapy. *Explor. Target. Antitumor Ther.* **4**, 57–88. <https://doi.org/10.37349/etat.2023.00123> (2023).
16. Putri, R., Kusmardi, K. & Hakim, R. Potensi Ekstrak Daun *Ficus deltoidea* Jack Sebagai Anti Kanker Payudara. *J. Indon Med. Assoc.* **74**, 1–4 (2024).
17. Yetisgin, A. A., Cetinel, S., Zuvun, M., Kosar, A. & Kutlu, O. Therapeutic Nanoparticles and Their Targeted Delivery Applications. *Molecules* **25** <https://doi.org/10.3390/molecules250921932193> (2020).
18. Gurunathan, S., Han, J. W., Eppakayala, V., Jeyaraj, M. & Kim, J. H. Cytotoxicity of Biologically Synthesized Silver Nanoparticles in MDA-MB-231 Human Breast Cancer Cells. *Biomed Res Int.* 1–10; (2013). <https://doi.org/10.1155/2013/535796> (2013).
19. Ahmed, S., Saifullah, Ahmad, M., Swami, B. L. & Ikram, S. Green synthesis of silver nanoparticles using *Azadirachta indica* aqueous leaf extract. *J. Radiat. Res. Appl. Sci.* **9**, 1–7. <https://doi.org/10.1016/j.jrras.2015.06.006> (2016).

20. Cullity, B. & Stock, S. *Elements of X-Ray Diffraction, 3rd Edn* Pearson, Harlow, England,. (2014).
21. Ali, M. H. et al. Analysis of Crystallographic Structures and Properties of Silver Nanoparticles Synthesized Using PKL Extract and Nanoscale Characterization Techniques. *ACS Omega*. **8**, 28133–28142. <https://doi.org/10.1021/acsomega.3c01261> (2023).
22. Azmi, N. A. S. et al. Functional Beverages from Blends of *Ficus deltoidea* Leaves and Brown Rice Powders: Physico-Phytochemical Properties, Antioxidant Activities, Sensory Evaluation and Acute Toxicity Study. *Pharmacognosy J.* **14**, 477–489. <https://doi.org/10.5530/pj.2022.14.125> (2022).
23. Ma, D. D. & Yang, W. X. Engineered nanoparticles induce cell apoptosis: potential for cancer therapy. *Oncotarget* **7**, 40882–40903. <https://doi.org/10.18632/oncotarget.8553> (2016).
24. Subhan, M. A., Yalamarty, S. S. K., Filipczak, N., Parveen, F. & Torchilin, V. P. Recent Advances in Tumor Targeting via EPR Effect for Cancer Treatment. *J. Pers. Med.* **11**, 571. <https://doi.org/10.3390/jpm11060571> (2021).
25. Subramaniam, H., Lim, C. K., Tey, L. H., Wong, L. S. & Djearamane, S. Oxidative stress-induced cytotoxicity of HCC2998 colon carcinoma cells by ZnO nanoparticles synthesized from *Calophyllum teysmannii*. *Sci. Rep.* **14**, 30198. <https://doi.org/10.1038/s41598-024-81384-0> (2024).
26. Jain, A. S., Pawar, P. S., Sarkar, A., Junnuthula, V. & Dyawanapelly, S. Bionanofactories for Green Synthesis of Silver Nanoparticles: Toward Antimicrobial Applications. *Int. J. Mol. Sci.* **22**, 11993. <https://doi.org/10.3390/ijms222111993> (2021).
27. Malatesta, M. Transmission Electron Microscopy as a Powerful Tool to Investigate the Interaction of Nanoparticles with Subcellular Structures. *Int. J. Mol. Sci.* **22**, 12789. <https://doi.org/10.3390/ijms222312789> (2021).
28. Ying, S. et al. Green synthesis of nanoparticles: Current developments and limitations. *Environ. Technol. Innov.* **26**, 102336. <https://doi.org/10.1016/j.eti.2022.102336> (2022).
29. Manzari-Tavakoli, A., Babajani, A., Tavakoli, M. M., Safaeinejad, F. & Jafari, A. Integrating natural compounds and nanoparticle-based drug delivery systems: A novel strategy for enhanced efficacy and selectivity in cancer therapy. *Cancer Med.* **13** <https://doi.org/10.1002/cam4.7010> (2024).
30. Aziz, A. et al. STRUCTURAL,. *Dig. J. Nanomater Biostruct* **13**, 679–683 (2018).
31. Brandelli, A. The interaction of nanostructured antimicrobials with biological systems: Cellular uptake, trafficking and potential toxicity. *Food Sci. Hum. Wellness.* **9**, 8–20. <https://doi.org/10.1016/j.fshw.2019.12.003> (2020).
32. Hudaya, I. R. et al. Biofabrication of silver nanoparticles using *Artocarpus heterophyllus* leaves extract: Characterization and evaluation of its antibacterial, antibiofilm, and antioxidant activities. *J. Cryst. Growth.* **644**, 127827. <https://doi.org/10.1016/j.jcrysgro.2024.127827> (2024).
33. He, Y. et al. Targeting PI3K/Akt signal transduction for cancer therapy. *Signal. Transduct. Target. Ther.* **6**, 425. <https://doi.org/10.1038/s41392-021-00828-5> (2021).
34. Dong, C., Wu, J., Chen, Y., Nie, J. & Chen, C. Activation of PI3K/AKT/mTOR Pathway Causes Drug Resistance in Breast Cancer. *Front. Pharmacol.* **12** <https://doi.org/10.3389/fphar.2021.628690> (2021).

35. Mayer, I. A. & Arteaga, C. L. The PI3K/AKT Pathway as a Target for Cancer Treatment. *Annu. Rev. Med.* **67**, 11–28. <https://doi.org/10.1146/annurev-med-062913-051343> (2016).
36. Najafipour, R., Momeni, A., Mirmazloomi, Y. & Moghbelinejad, S. Vitexin Induces Apoptosis in MCF-7 Breast Cancer Cells through the Regulation of Specific miRNAs Expression. *Int. J. Mol. Cell. Med. (IJMCM)*. **11**, 197–206. <https://doi.org/10.22088/IJMCM.BUMS.11.3.197> (2022).
37. Liang, C., Jiang, Y. & Sun, L. Vitexin suppresses the proliferation, angiogenesis and stemness of endometrial cancer through the PI3K/AKT pathway. *Pharm. Biol.* **61**, 581–589. <https://doi.org/10.1080/13880209.2023.2190774> (2023).
38. Tehrani, M. H., Ziamajidi, N., Abbasalipourkabir, R. & Barartabar, Z. Exploring the potential of AgNPs in modulating the PI3K/ AKT/mTOR pathway via miR-133a regulation in MCF-7 breast cancer cells. *Nanomed. J.* **11**, 52–62. <https://doi.org/10.22038/NMJ.2023.75091.1824> (2024).
39. Wei, Q. Y., Xu, Y. M. & Lau, A. T. Y. Recent Progress of Nanocarrier-Based Therapy for Solid Malignancies. *Cancers (Basel)*. **12**, 2783. <https://doi.org/10.3390/cancers12102783> (2020).
40. Glaviano, A. et al. PI3K/AKT/mTOR signaling transduction pathway and targeted therapies in cancer. *Mol. Cancer*. **22**, 138. <https://doi.org/10.1186/s12943-023-01827-6> (2023).
41. Uxa, S. et al. Ki-67 gene expression. *Cell. Death Differ.* **28**, 3357–3370. <https://doi.org/10.1038/s41418-021-00823-x> (2021).
42. Din, S. M., Malek, N. A. N. N., Shamsuddin, M. & Matmin, J. Effect of plant organs of *Ficus deltoidea* in the synthesis of silver nanoparticles. *Malaysian J. Anal. Sci.* **25**, 716–727 (2021).
43. Swanner, J. et al. Silver nanoparticles selectively treat triple-negative breast cancer cells without affecting non-malignant breast epithelial cells in vitro and in vivo. *FASEB Bioadv.* **1**, 639–660. <https://doi.org/10.1096/fba.2019-00021> (2019).
44. Susanto, H. et al. Moringa oleifera Leaf Powder – Silver Nanoparticles (MOLP-AgNPs) efficiently inhibit metastasis and proliferative signaling in HT-29 human colorectal cancer cells. *J. Agric. Food Res.* **16**, 101149. <https://doi.org/10.1016/j.jafr.2024.101149> (2024).
45. Fischer, M., Quaas, M., Steiner, L. & Engeland, K. The p53-p21-DREAM-CDE/CHR pathway regulates G₂/M cell cycle genes. *Nucleic Acids Res.* **44**, 164–174. <https://doi.org/10.1093/nar/gkv927> (2016).
46. Ooi, T. C. et al. Antimutagenic, Cytoprotective and Antioxidant Properties of *Ficus deltoidea* Aqueous Extract. *Vitro Molecules*. **26**, 3287. <https://doi.org/10.3390/molecules26113287> (2021).
47. Abolmaesoomi, M., Abdul Aziz, A., Mat Junit, S. & Mohd Ali, J. *Ficus deltoidea*: Effects of solvent polarity on antioxidant and anti-proliferative activities in breast and colon cancer cells. *Eur. J. Integr. Med.* **28**, 57–67. <https://doi.org/10.1016/j.eujim.2019.05.002> (2019).
48. Silva, F. et al. Caspase 3 and Cleaved Caspase 3 Expression in Tumorigenesis and Its Correlations with Prognosis in Head and Neck Cancer: A Systematic Review and Meta-Analysis. *Int. J. Mol. Sci.* **23**, 11937. <https://doi.org/10.3390/ijms231911937> (2022).
49. Hanafi, M. M. M. et al. In Vitro Pro-apoptotic and Anti-migratory Effects of *Ficus deltoidea* L. Plant Extracts on the Human Prostate Cancer Cell Lines PC3. *Front Pharmacol.* **8**; (2017). <https://doi.org/10.3389/fphar.2017.00895>

50. Koosha, S., Alshawsh, M. A., Looi, C. Y., Seyedan, A. & Mohamed, Z. An Association Map on the Effect of Flavonoids on the Signaling Pathways in Colorectal Cancer. *Int. J. Med. Sci.* **13**, 374–385. <https://doi.org/10.7150/ijms.14485> (2016).
51. Selvi, B. C. G., Madhavan, J. & Santhanam, A. Cytotoxic effect of silver nanoparticles synthesized from *Padina tetrastromatica* on breast cancer cell line. *Adv. Nat. Sci. NanoSci. NanoTechnol.* **7**, 035015. <https://doi.org/10.1088/2043-6262/7/3/035015> (2016).
52. Ullah, I. et al. Green-Synthesized Silver Nanoparticles Induced Apoptotic Cell Death in MCF-7 Breast Cancer Cells by Generating Reactive Oxygen Species and Activating Caspase 3 and 9 Enzyme Activities. *Oxid Med Cell Longev.* 1–14; (2020). <https://doi.org/10.1155/2020/1215395> (2020).

Figures

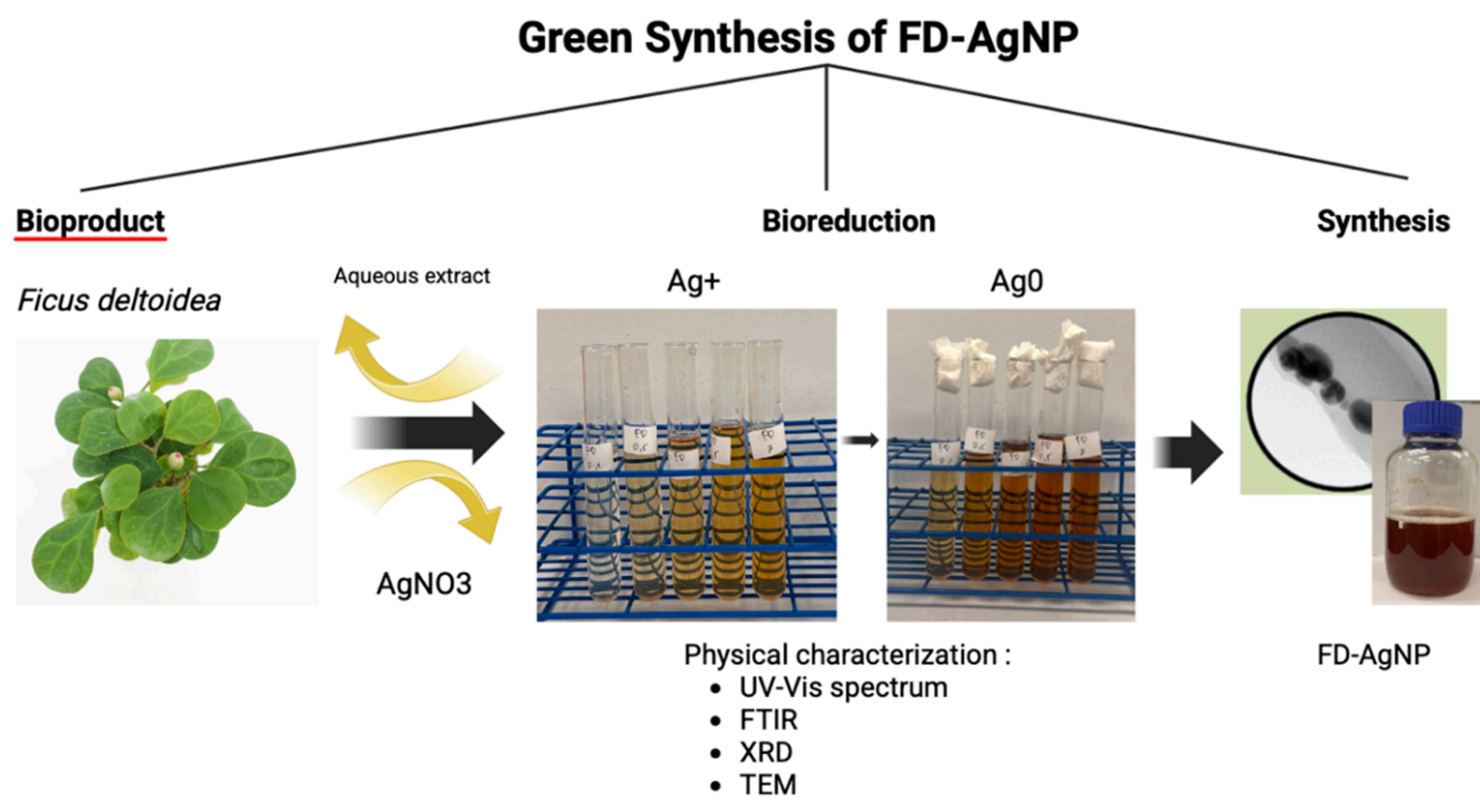


Figure 1

Green Synthesis and Characterization of FD-AgNPs Using *Ficus deltoidea*. The synthesis of silver nanoparticles (FD-AgNPs) was carried out using aqueous extract of *Ficus deltoidea* as a natural reducing and capping agent. The bioreduction of Ag^+ to Ag^0 was indicated by a visible color change, followed by characterization using UV–Vis spectroscopy, FTIR, XRD, and TEM to confirm nanoparticle formation, composition, crystallinity, and morphology. (This figure is created by Biorender.com).

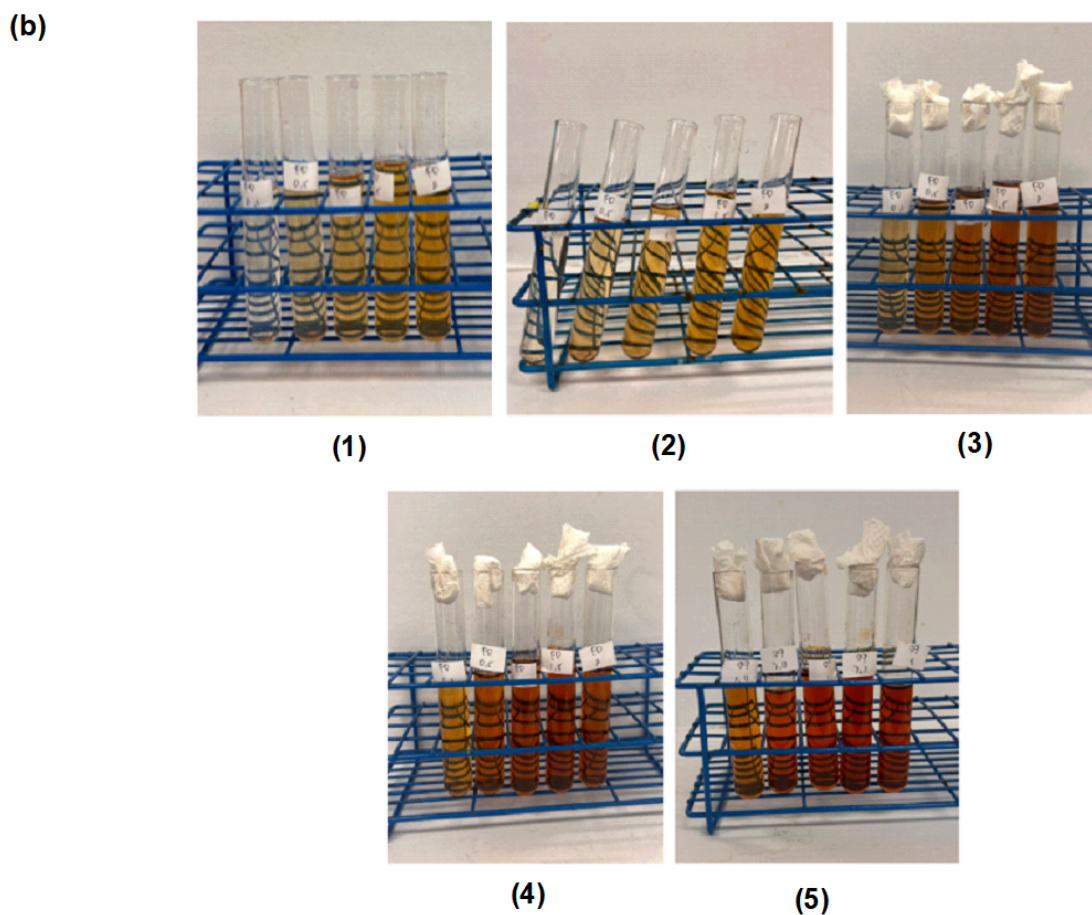
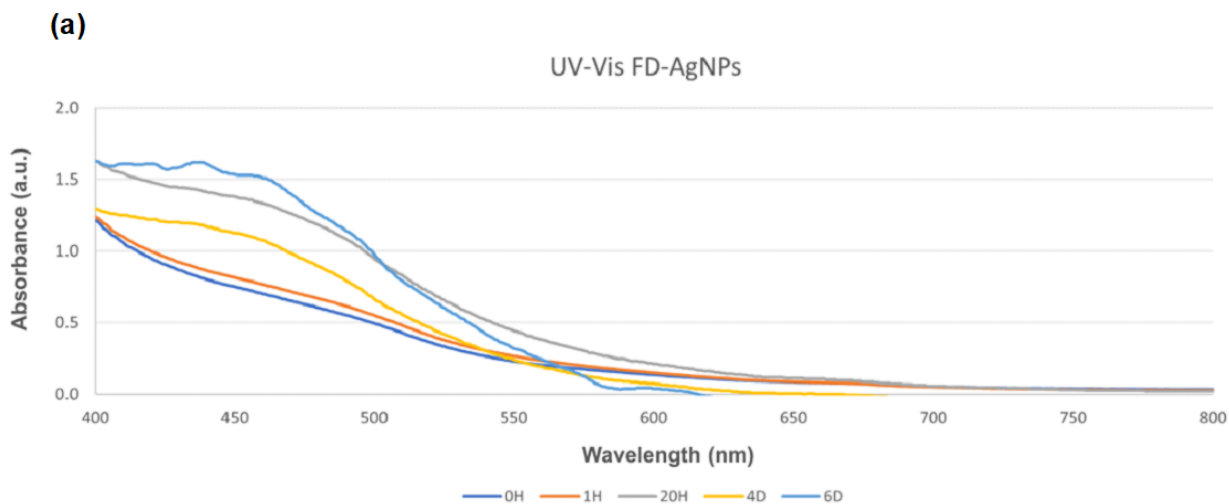


Figure 2

Uv-Vis Characterization and Color Changes of FD-AgNPs Synthesized Using *Ficus deltoidea* Extract. (a) UV-Vis spectrum of FD-AgNPs at different time points (0 h, 1 h, 20 h, 4 d, and 6 d), showing a time-dependent increase in absorbance near 430–450 nm, indicating nanoparticle formation. (b) Color change observations of reaction mixtures with increasing AgNO_3 concentrations (0.1, 0.5, 1.0, 1.5, and 2

mM) from number (1) to (5)) over different synthesis times, showing gradual transition from yellow to reddish-brown, confirming AgNPs formation.

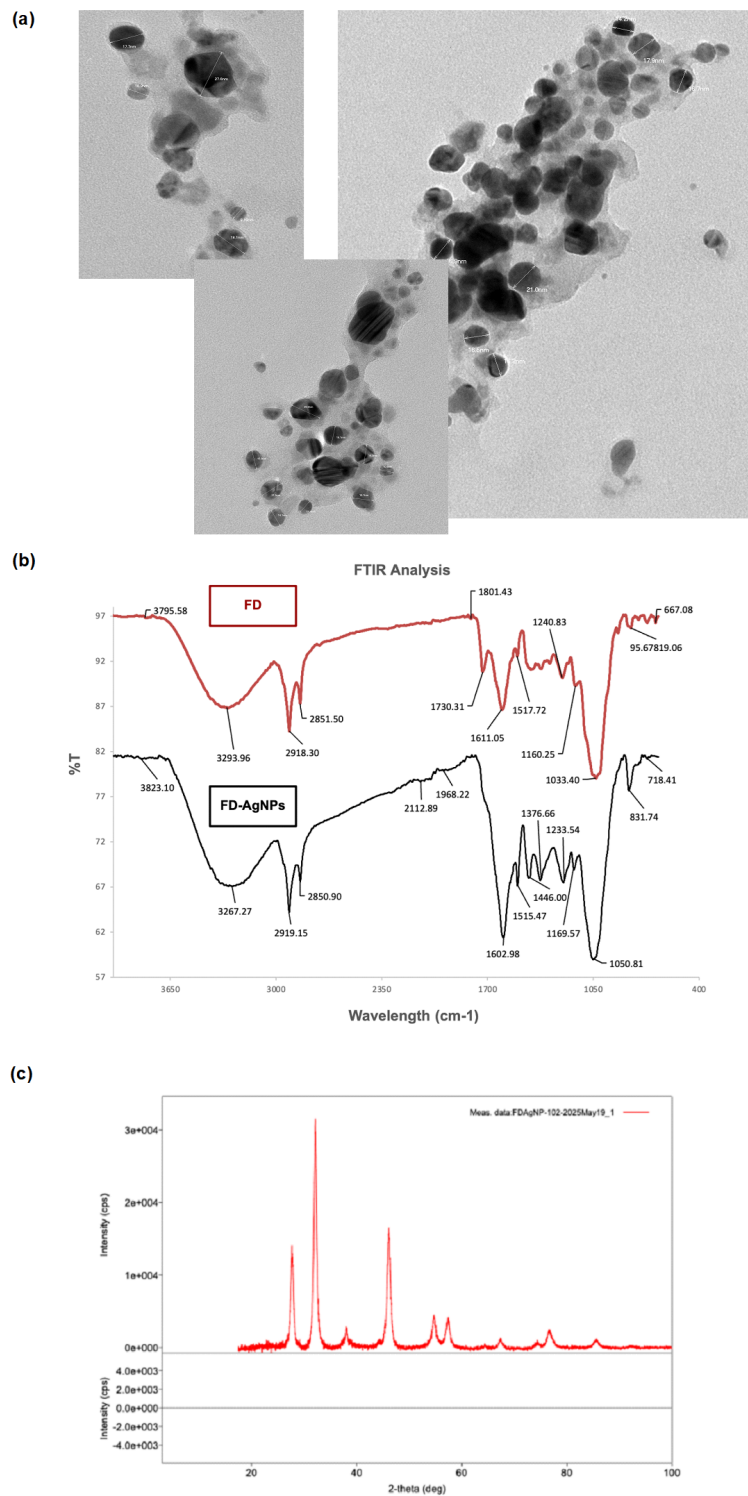


Figure 3

TEM, FTIR, and XRD of FD-AgNPs Synthesized Using *Ficus deltoidea* Extract. (a) TEM image of FD-AgNPs shows mostly spherical particles (20–40 nm), moderately clustered with clear edges, indicating partial stabilization. (b) FTIR spectra of *Ficus deltoidea* extract (FD) and FD-AgNPs show shifts in O-H, C=O, and

C-H peaks, suggesting involvement of phenolics/flavonoids in Ag⁺ reduction and nanoparticle stabilization. **(c)** XRD pattern confirms the crystalline, face-centered cubic structure of FD-AgNPs, with prominent peaks matching standard silver diffraction planes.

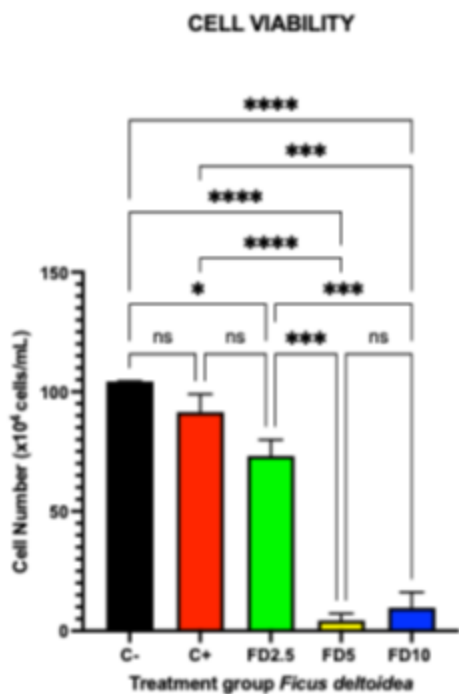


Figure 4

The CTCF of Cell viability measured by WST assay. This result confirmed that FD-AgNPs at 5 and 10 µg/mL significantly reduced viability, exceeding the effect of cisplatin.

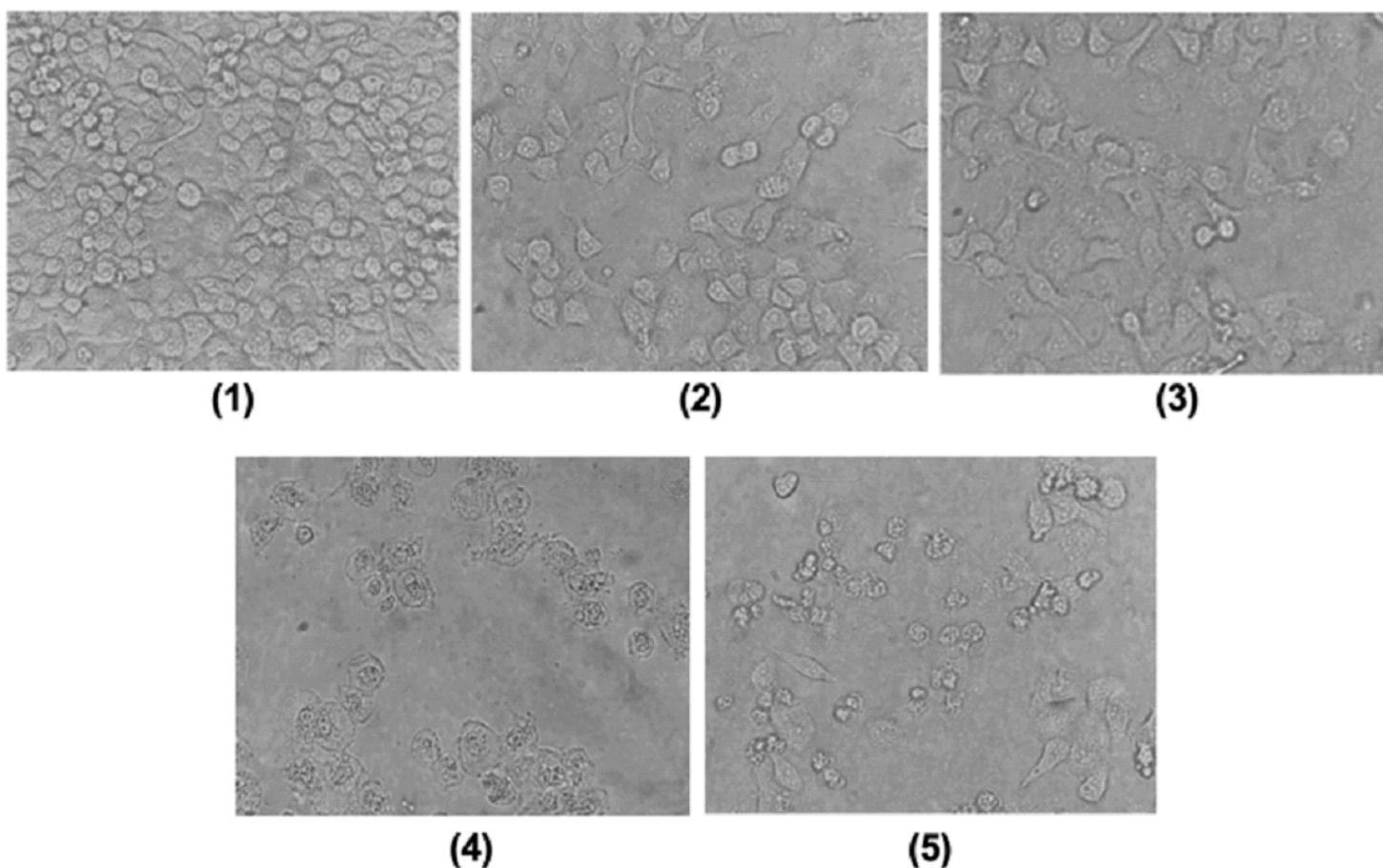


Figure 5

HeLa Cells Morphology after Treated after 24 h of treatment with FD-AgNPs: (1) C-, (2) FD2.5, (3) FD5, (4) FD10, (5) C+. Phase-contrast images show morphological changes in HeLs. Dose-dependent cytotoxicity was observed, including cell shrinkage, membrane blebbing, and detachment.

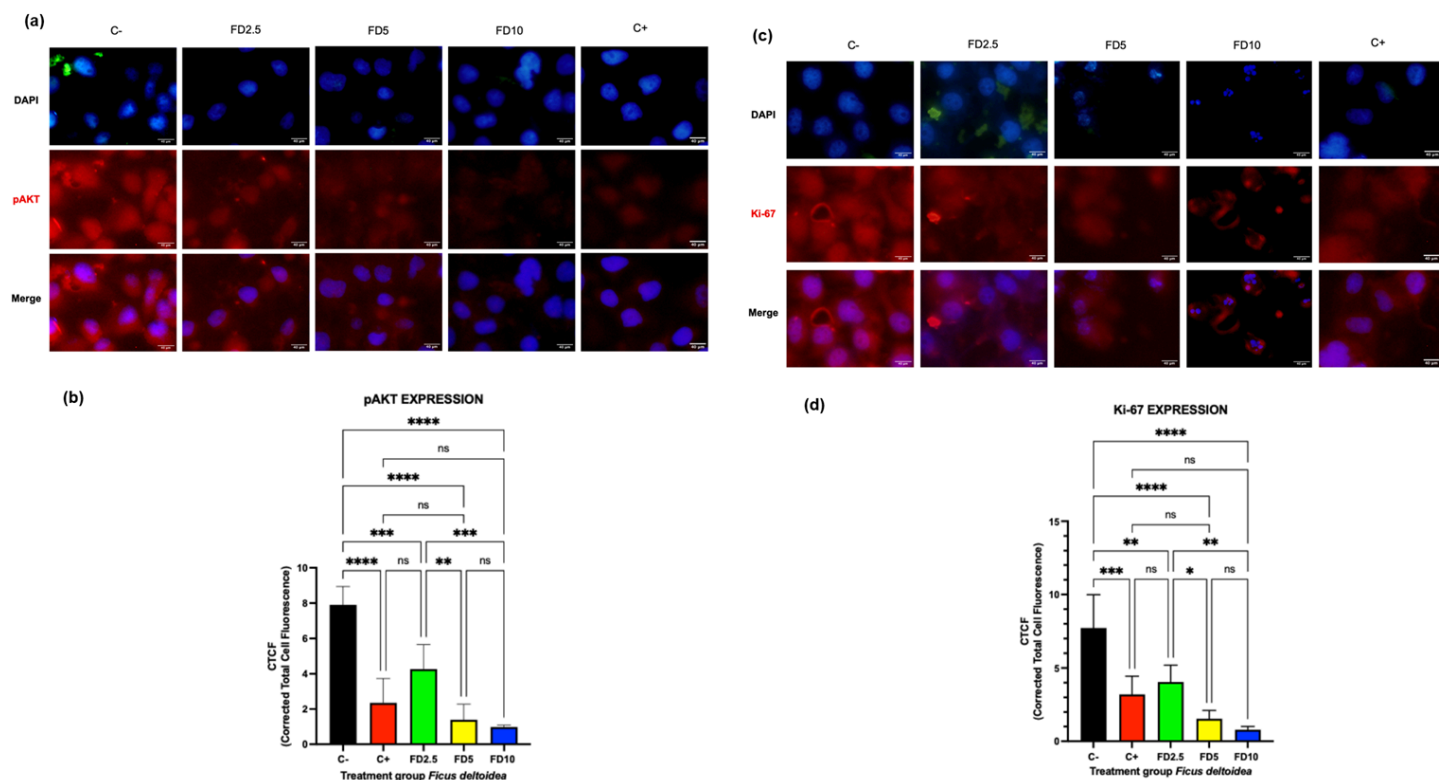


Figure 6

FD-AgNPs effects on Proliferation HeLa cells. **(a)** Immunofluorescence images of pAKT (red) and nuclei (DAPI, blue) showing reduced pAKT with increasing FD-AgNPs doses. **(b)** CTCF quantification of pAKT expression; significant reductions at all FD-AgNPs concentrations (**p < 0.01, ***p < 0.001). **(c)** Immunofluorescence images of Ki-67 (red) showing decreased expression with higher FD-AgNPs doses. **(d)** CTCF quantification of Ki-67 expression showing significant inhibition (*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001).

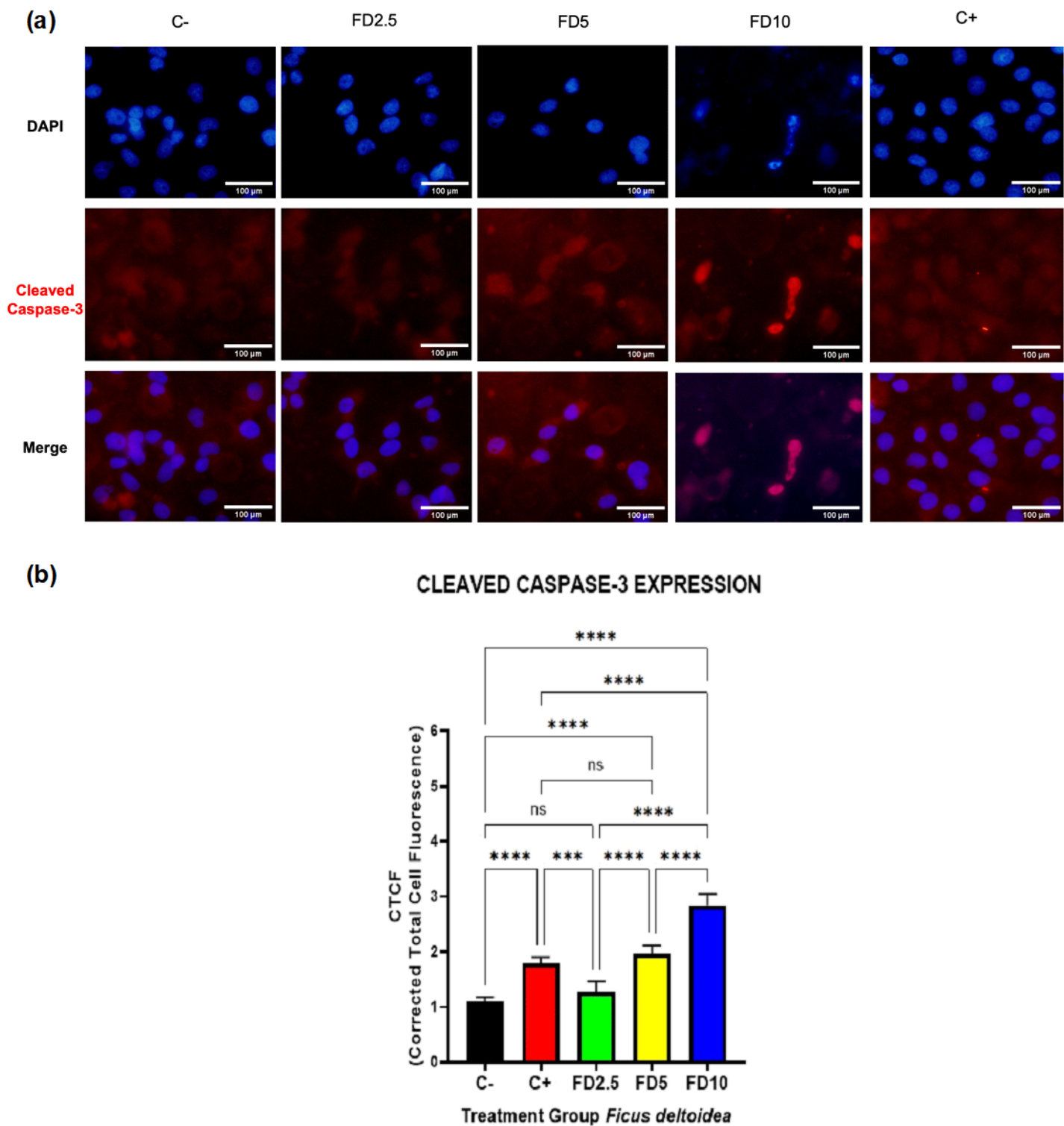


Figure 7

FD-AgNPs Induce Cleaved Caspase-3 Expression in HeLa Cells. **(a)** Representative immunofluorescence images showing cleaved caspase-3 (red) and nuclei (DAPI, blue) in HeLa cells treated with increasing concentrations of FD-AgNPs (2.5, 5, and 10 $\mu\text{g/mL}$) compared to negative control (C-) and cisplatin-treated positive control (C+). Red fluorescence intensity increased with higher FD-AgNPs concentrations, indicating enhanced caspase-3 activation. **(b)** Quantification of cleaved caspase-3 expression using

Corrected Total Cell Fluorescence (CTCF) revealed significant dose-dependent increases, with the highest expression at 10 µg/mL, comparable to cisplatin. Data are presented as mean ± SD; ****p < 0.0001.