

Plant-Derived Silver Nanoparticles For Sirna-Mediated Suppression of EGFR Expression in Non-Small Cell Lung Cancer

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Abstract

Non-small cell lung cancer (NSCLC) is the most prevalent form of lung cancer and remains a major cause of cancer-related mortality worldwide. The epidermal growth factor receptor (EGFR) plays a critical role in NSCLC progression by regulating cellular proliferation, survival, migration, and invasion, making it an attractive therapeutic target. In the present study, plant-derived silver nanoparticles (AgNPs) were developed as a green nanotechnology-based platform for the delivery of EGFR-targeting small interfering RNA (siRNA) to enhance gene-silencing efficiency and anticancer activity. Silver nanoparticles were synthesized using *Moringa oleifera* leaf extract and characterized using UV-Visible spectroscopy, dynamic light scattering (DLS), zeta potential analysis, Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), transmission electron microscopy (TEM), and scanning electron microscopy (SEM). The synthesized nanoparticles exhibited favorable physicochemical properties, including nanoscale size distribution, good colloidal stability, and suitable surface characteristics for siRNA loading. EGFR-siRNA was successfully conjugated to the AgNPs with high loading efficiency and enhanced protection against nuclease-mediated degradation. Cellular uptake studies demonstrated significantly improved internalization of AgNP-siRNA nanocomplexes compared with free siRNA. Quantitative RT-PCR and Western blot analyses confirmed substantial suppression of EGFR mRNA and protein expression in NSCLC cells. Furthermore, treatment with AgNP-siRNA nanocomplexes significantly reduced cell viability and proliferation, induced cell cycle arrest and apoptosis, and inhibited cellular migration and invasion. These findings demonstrate that plant-derived AgNPs provide an effective and environmentally sustainable delivery system for EGFR-targeted siRNA therapy. The developed nanoplatfrom offers promising potential for precision nanomedicine and targeted treatment of NSCLC.

Keywords: Non-small Cell Lung Cancer (NSCLC); Silver Nanoparticles; EGFR; Small Interfering RNA (siRNA); Green Nanotechnology; Gene Silencing.

1. Introduction

Lung cancer remains one of the most prevalent and deadly malignancies worldwide, accounting for a substantial proportion of cancer-related morbidity and mortality. According to global cancer statistics, lung cancer is responsible for millions of new cases and deaths annually, highlighting the urgent need for innovative therapeutic approaches. Among the different histological types of lung cancer, non-small cell lung cancer (NSCLC) represents approximately 85% of all diagnosed cases. NSCLC encompasses several subtypes, including adenocarcinoma, squamous cell carcinoma, and large-cell carcinoma (Nooreldeen & Bach, 2021). Despite significant advancements in diagnostic techniques, surgical interventions, chemotherapy, radiotherapy, immunotherapy, and targeted therapies, the prognosis of advanced-stage NSCLC remains poor due to tumor heterogeneity, metastasis, and the emergence of treatment resistance. Consequently, there is a growing interest in the development of novel molecularly targeted therapies capable of overcoming these limitations and improving patient outcomes (Chamorro et al., 2025).

One of the most extensively studied molecular targets in NSCLC is the epidermal growth factor receptor (EGFR), a transmembrane receptor tyrosine kinase that belongs to the ErbB family of receptors. EGFR plays a critical role in regulating cellular proliferation, differentiation, migration, survival, and angiogenesis. Upon binding of specific ligands such as epidermal growth factor and transforming growth factor- α , EGFR undergoes dimerization and

autophosphorylation, initiating a cascade of intracellular signaling pathways, including the phosphatidylinositol 3-kinase/protein kinase B (PI3K/Akt), mitogen-activated protein kinase (MAPK), and Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathways (Han et al., 2024). Dysregulation of EGFR signaling through gene amplification, overexpression, or activating mutations contributes significantly to the initiation and progression of NSCLC. EGFR mutations are particularly common in lung adenocarcinoma and have become important predictive biomarkers for targeted therapy. Although EGFR tyrosine kinase inhibitors (TKIs) such as gefitinib, erlotinib, afatinib, and osimertinib have demonstrated remarkable clinical efficacy, acquired resistance frequently develops through secondary mutations, bypass signaling pathways, and other adaptive mechanisms. These challenges underscore the necessity for alternative strategies capable of directly suppressing EGFR expression at the genetic level (Hsu et al., 2019).

RNA interference (RNAi) has emerged as a powerful gene-silencing technology with considerable therapeutic potential in cancer treatment. RNAi is a naturally occurring cellular mechanism that regulates gene expression through sequence-specific degradation of messenger RNA (mRNA). Small interfering RNA (siRNA), typically consisting of 21–23 nucleotide double-stranded RNA molecules, can selectively target and silence disease-associated genes. Following cellular uptake, siRNA is incorporated into the RNA-induced silencing complex (RISC), where one strand serves as a guide for recognizing complementary mRNA sequences. The targeted mRNA is subsequently cleaved and degraded, leading to a reduction in protein synthesis (López-Fraga et al., 2009b). In the context of NSCLC, siRNA-mediated silencing of EGFR offers a promising approach for inhibiting oncogenic signaling pathways, reducing tumor growth, inducing apoptosis, and limiting metastatic progression. Furthermore, siRNA-based therapy provides high specificity, allowing selective suppression of target genes while minimizing off-target effects. However, the successful clinical application of siRNA remains hindered by several biological barriers, including rapid degradation by nucleases, poor cellular uptake, limited tissue penetration, and inefficient intracellular delivery (López-Fraga et al., 2009a).

Nanotechnology has emerged as an effective solution for overcoming the challenges associated with siRNA delivery. Nanoparticle-based delivery systems can protect siRNA from enzymatic degradation, improve pharmacokinetic properties, facilitate cellular internalization, and promote targeted delivery to tumor tissues. Various nanocarriers, including liposomes, polymeric nanoparticles, dendrimers, lipid nanoparticles, and metallic nanoparticles, have been investigated for nucleic acid delivery. Among these, silver nanoparticles (AgNPs) have attracted significant attention due to their unique physicochemical and biological properties. AgNPs possess a high surface-area-to-volume ratio, tunable size, excellent biocompatibility, and ease of surface functionalization, making them suitable candidates for gene delivery applications (Attia et al., 2025). In addition to serving as delivery vehicles, AgNPs exhibit intrinsic antimicrobial, antioxidant, anti-inflammatory, and anticancer activities. Several studies have demonstrated that AgNPs can induce reactive oxygen species (ROS) generation, mitochondrial dysfunction, DNA damage, and apoptosis in cancer cells, thereby enhancing therapeutic efficacy. The multifunctional nature of AgNPs offers the possibility of combining gene silencing with nanoparticle-mediated anticancer effects, resulting in synergistic therapeutic outcomes (Mishra et al., 2017; Traber & Yu, 2023).

Conventional methods for synthesizing silver nanoparticles often involve the use of hazardous chemicals, high energy consumption, and environmentally harmful procedures. These limitations have prompted increasing interest in green nanotechnology approaches that utilize biological resources for nanoparticle synthesis. Plant-mediated synthesis of silver nanoparticles has emerged as an eco-friendly, cost-effective, and sustainable alternative to traditional chemical and physical methods. Plant extracts contain a diverse range of bioactive phytochemicals, including flavonoids, phenolic acids, terpenoids, alkaloids, tannins, proteins, and polysaccharides, which function as natural reducing and stabilizing agents during nanoparticle formation (Mikutis & Bernardes, 2024). These phytochemicals facilitate the reduction of silver ions into metallic silver nanoparticles while simultaneously preventing particle aggregation. Green-synthesized AgNPs often exhibit enhanced biocompatibility, reduced toxicity, and improved biological activity compared to chemically synthesized counterparts. Moreover, the presence of phytochemical coatings on the nanoparticle surface may contribute additional therapeutic properties, including antioxidant and anticancer activities (Ghasemiyeh & Mohammadi-Samani, 2025).

The integration of plant-derived silver nanoparticles with siRNA technology represents a promising strategy for targeted cancer therapy. By serving as protective carriers, plant-derived AgNPs can improve the stability and intracellular delivery of EGFR-specific siRNA while simultaneously exerting their own anticancer effects. Such multifunctional nanoplatforms have the potential to enhance gene silencing efficiency, suppress tumor-promoting signaling pathways, and overcome limitations associated with conventional treatments. Additionally, the use of

green-synthesized nanoparticles aligns with current efforts to develop environmentally sustainable and clinically safe nanomedicine platforms. Recent advances in nanobiotechnology have demonstrated that plant-derived metallic nanoparticles can effectively deliver therapeutic nucleic acids to cancer cells, resulting in significant reductions in target gene expression and tumor cell viability (Pandey et al., 2025).

Given the critical role of EGFR in NSCLC progression and the challenges associated with current therapeutic modalities, the development of plant-derived silver nanoparticle-based siRNA delivery systems represents an innovative and potentially transformative approach. The combination of green nanotechnology, RNA interference, and targeted cancer therapy may provide a safer and more effective strategy for combating NSCLC. Therefore, the present study aims to investigate the synthesis and characterization of plant-derived silver nanoparticles, evaluate their ability to deliver EGFR-targeting siRNA, and assess their therapeutic efficacy in suppressing EGFR expression and inhibiting the growth of non-small cell lung cancer cells. The findings of this research may contribute to the advancement of next-generation nanomedicine platforms for precision oncology and targeted gene therapy (Singh et al., 2025a, 2025b).

2. siRNA-Mediated Suppression of EGFR Expression in Non-Small Cell Lung Cancer

2.1 EGFR Signaling Pathway in NSCLC

The epidermal growth factor receptor (EGFR) is a transmembrane receptor tyrosine kinase that plays a pivotal role in the development and progression of non-small cell lung cancer (NSCLC). Upon binding of ligands such as epidermal growth factor (EGF) or transforming growth factor- α (TGF- α), EGFR undergoes dimerization and autophosphorylation of its intracellular tyrosine residues. This activation initiates several downstream signaling cascades that regulate critical cellular functions. Among the most important pathways are the phosphatidylinositol 3-kinase/protein kinase B (PI3K/Akt) pathway, the mitogen-activated protein kinase (MAPK) pathway, and the Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway (Wee & Wang, 2017).

The PI3K/Akt pathway promotes cell survival by inhibiting apoptosis and enhancing cellular metabolism. Similarly, activation of the MAPK pathway stimulates cell proliferation, differentiation, and tumor growth. The JAK/STAT pathway contributes to the transcription of genes involved in cell cycle progression, angiogenesis, and immune evasion. Aberrant activation of these signaling networks due to EGFR overexpression or mutation results in uncontrolled cell division, increased migration, invasion, and resistance to apoptosis. Consequently, EGFR has emerged as an important therapeutic target in NSCLC, and suppression of EGFR expression can effectively disrupt multiple oncogenic pathways simultaneously (Cheng, 2024).

2.2 Design and Selection of EGFR-Targeting siRNA

The effectiveness of siRNA-mediated gene silencing largely depends on the careful design and selection of siRNA sequences targeting the EGFR gene. EGFR-specific siRNA molecules are typically designed to recognize unique regions within the EGFR messenger RNA (mRNA), ensuring selective degradation of the target transcript. Several criteria are considered during sequence selection, including optimal nucleotide composition, GC content, thermodynamic stability, and accessibility of the target mRNA region (Dong et al., 2019). An ideal siRNA generally consists of 21–23 nucleotides with balanced GC content to ensure efficient binding and silencing activity. Bioinformatics tools are frequently employed to identify suitable target sequences and predict gene-silencing efficiency. In addition, siRNA candidates are screened to avoid sequence homology with non-target genes, thereby reducing the likelihood of unintended gene suppression (Freitag & Wagner, 2021).

Specificity is a critical consideration in siRNA therapy because off-target effects can lead to unwanted alterations in cellular function and potential toxicity. To minimize these effects, siRNA sequences are carefully optimized to maximize complementarity with EGFR mRNA while minimizing interactions with unrelated transcripts. Chemical modifications such as 2'-O-methylation and phosphorothioate linkages may also be incorporated to improve stability and specificity. Through rational design strategies, EGFR-targeting siRNA can achieve efficient and selective suppression of EGFR expression in NSCLC cells (da Silva et al., 2024).

2.3 Mechanism of siRNA-Induced Gene Silencing

Small interfering RNA (siRNA) mediates gene silencing through the highly conserved RNA interference (RNAi) pathway. Following successful cellular uptake, the double-stranded siRNA is released into the cytoplasm, where it is recognized by the RNA-induced silencing complex (RISC). The passenger strand of the siRNA duplex is removed

and degraded, while the guide strand remains associated with the RISC complex (Akbar et al., 2022). The guide strand directs RISC to complementary EGFR mRNA molecules through sequence-specific base pairing. Once the target mRNA is recognized, the catalytic component of RISC, known as Argonaute-2 (Ago2), cleaves the mRNA at a specific site. The cleaved mRNA fragments are subsequently degraded by cellular nucleases, preventing their translation into protein (Bao et al., 2021). As a result, EGFR protein synthesis is significantly reduced, leading to suppression of downstream signaling pathways involved in tumor progression. Decreased EGFR expression can inhibit cancer cell proliferation, induce apoptosis, reduce metastatic potential, and enhance sensitivity to other therapeutic agents. Because RNAi acts at the post-transcriptional level, siRNA-mediated gene silencing provides a highly specific and efficient approach for targeting oncogenic drivers in NSCLC (Chi et al., 2003).

2.4 Nanoparticle-Assisted Delivery of EGFR siRNA

Despite its therapeutic potential, naked siRNA faces numerous biological barriers that limit its clinical application. Free siRNA molecules are highly susceptible to degradation by serum nucleases, possess poor membrane permeability, and are rapidly cleared from circulation. Nanoparticle-assisted delivery systems have therefore been developed to overcome these challenges and improve therapeutic efficacy (Wang et al., 2020). Silver nanoparticles (AgNPs) and other nanocarriers can effectively encapsulate or conjugate EGFR-targeting siRNA, protecting it from enzymatic degradation during systemic circulation. The nanoparticle matrix acts as a physical shield, enhancing siRNA stability and prolonging its biological half-life. Furthermore, nanoparticles facilitate cellular uptake through endocytosis, resulting in higher intracellular concentrations of therapeutic siRNA (Li et al., 2021).

Following internalization, efficient endosomal escape is essential to prevent degradation of siRNA within lysosomes. Nanoparticles can promote endosomal membrane disruption through various mechanisms, enabling the release of siRNA into the cytoplasm where RNA interference occurs. Enhanced intracellular transport and controlled release significantly improve gene-silencing efficiency (Qiu et al., 2023). In addition to improving delivery, plant-derived silver nanoparticles may provide intrinsic anticancer properties through the induction of oxidative stress and apoptosis in tumor cells. Therefore, nanoparticle-assisted delivery not only enhances the therapeutic performance of EGFR-targeting siRNA but also offers a multifunctional platform capable of achieving synergistic anticancer effects in non-small cell lung cancer (Shirsat et al., 2024).

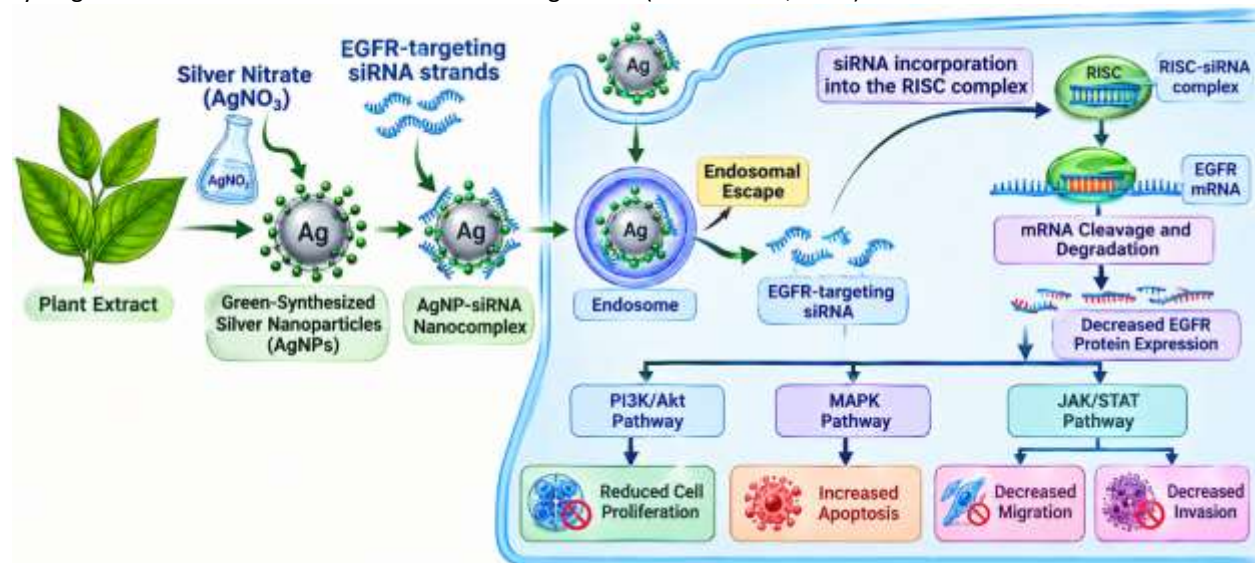


Figure 1: Mechanism of Plant-Derived AgNP-Mediated EGFR-siRNA Delivery and Gene Silencing in NSCLC Cells.

3. Materials and Methods

3.1 Materials

Silver nitrate (AgNO_3 , $\geq 99.9\%$ purity) used for nanoparticle synthesis was procured from Merck Life Science Pvt. Ltd., New Delhi, India. Fresh leaves of *Moringa oleifera* were collected from a medicinal plant garden in Uttar Pradesh, India, and authenticated by a qualified botanist. Human non-small cell lung cancer (NSCLC) cell lines A549

and H1975 were obtained from the National Centre for Cell Science (NCCS), Pune, India. Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), penicillin–streptomycin solution, trypsin–EDTA, and phosphate-buffered saline (PBS) were purchased from Gibco, Thermo Fisher Scientific, USA. EGFR-targeting small interfering RNA (siRNA) and scrambled control siRNA were synthesized by Integrated DNA Technologies (IDT, USA). RNA extraction kits, reverse transcription reagents, and SYBR Green qPCR master mix were obtained from Applied Biosystems, USA. Antibodies against EGFR and β -actin for Western blot analysis were procured from Cell Signaling Technology, USA. Agarose, Tris-HCl, EDTA, ethanol, and other analytical-grade reagents were supplied by HiMedia Laboratories and SRL Chemicals, India. Ultrapure water generated using a Milli-Q purification system was used throughout the study. All chemicals and biological reagents were of analytical or molecular biology grade and were used without further purification.

3.2 Green Synthesis of Plant-Derived Silver Nanoparticles

Fresh *Moringa oleifera* leaves were collected, washed thoroughly with distilled water, shade-dried, and finely powdered. The plant extract was prepared by boiling the powdered leaves in deionized water, followed by filtration to obtain a clear aqueous extract containing bioactive phytochemicals. For green synthesis of silver nanoparticles, the plant extract was mixed with an aqueous silver nitrate (AgNO_3) solution under controlled conditions. The reduction of silver ions (Ag^+) into silver nanoparticles (Ag^0) was confirmed by the gradual color change of the reaction mixture from pale yellow to brown (Shneen et al., 2024). The synthesized nanoparticles were collected by centrifugation, washed repeatedly with distilled water, and dried for further characterization. Optimization of synthesis parameters, including silver nitrate concentration, plant extract ratio, pH, temperature, and reaction time, was performed to obtain nanoparticles with desirable size, stability, and morphology. The optimized plant-derived AgNPs were subsequently used for EGFR-siRNA loading and biological evaluation (Mohammed & Hawar, 2022).

3.3 Characterization of Silver Nanoparticles

The synthesized plant-derived silver nanoparticles (AgNPs) were comprehensively characterized to determine their physicochemical properties. Ultraviolet–visible (UV–Vis) spectroscopy was performed in the wavelength range of 300–700 nm to confirm nanoparticle formation through the detection of the characteristic surface plasmon resonance (SPR) peak. The average particle size distribution and polydispersity index (PDI) of the nanoparticles were measured using dynamic light scattering (DLS). Surface charge and colloidal stability were evaluated by zeta potential analysis, with higher absolute zeta potential values indicating improved nanoparticle stability (Chougule et al., 2021).

Fourier-transform infrared (FTIR) spectroscopy was employed to identify the functional groups and phytochemical constituents involved in the reduction and stabilization of the AgNPs. The crystalline nature and phase composition of the synthesized nanoparticles were investigated using X-ray diffraction (XRD) analysis. Morphological characteristics, including particle shape and size, were examined by transmission electron microscopy (TEM), while scanning electron microscopy (SEM) was used to assess surface topology and structural features. The combined characterization techniques provided detailed information regarding the stability, morphology, crystallinity, and surface chemistry of the biosynthesized silver nanoparticles (Aboelnasr et al., 2025).

3.4 Preparation and Loading of EGFR-siRNA onto AgNPs

The synthesized silver nanoparticles (AgNPs) were surface-functionalized to facilitate efficient binding and delivery of EGFR-targeting small interfering RNA (siRNA). Initially, the nanoparticles were dispersed in phosphate-buffered saline (PBS) and coated with a biocompatible cationic polymer to impart a positive surface charge. This modification enhanced electrostatic interactions between the negatively charged siRNA molecules and the nanoparticle surface. Following functionalization, EGFR-specific siRNA was added to the AgNP suspension under gentle stirring and incubated at room temperature to allow stable nanoparticle–siRNA complex formation (Er et al., 2021).

The siRNA conjugation process was optimized by varying the AgNP-to-siRNA ratio to achieve maximum loading while maintaining nanoparticle stability. Successful complex formation was confirmed through changes in particle size and surface charge measurements. The loading efficiency of EGFR-siRNA onto the AgNPs was determined by measuring the amount of unbound siRNA remaining in the supernatant after centrifugation. Quantification was performed using UV spectrophotometric analysis, and loading efficiency was calculated as the percentage of siRNA successfully associated with the nanoparticles. The resulting AgNP–siRNA nanocomplexes were stored under sterile conditions and used for subsequent cellular uptake and gene-silencing studies (Moazzam et al., 2024).

3.5 Cell Culture and Treatment Protocols

Human non-small cell lung cancer (NSCLC) cell lines A549 and H1975 were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin. The cells were maintained in a humidified incubator at 37°C with 5% CO₂ and subcultured upon reaching approximately 80–90% confluency. For treatment studies, cells were seeded into appropriate culture plates and allowed to attach overnight before experimentation (El Moukhtari et al., 2023).

The experimental design included untreated control cells, free EGFR-siRNA, blank silver nanoparticles (AgNPs), scrambled siRNA-loaded AgNPs, and EGFR-siRNA-loaded AgNPs. Following treatment, cells were incubated under standard culture conditions for predetermined time intervals. Dose optimization studies were performed using different concentrations of AgNP–siRNA nanocomplexes to identify the most effective therapeutic concentration. Similarly, time-dependent studies were conducted at 24, 48, and 72 hours to evaluate cellular responses and gene-silencing efficiency. The optimized dose and incubation period obtained from these studies were subsequently employed for all biological and molecular analyses (Yuan et al., 2025).

3.6 Cellular Uptake and Gene Silencing Evaluation

The cellular internalization of EGFR-siRNA-loaded silver nanoparticles (AgNPs) was evaluated using fluorescence microscopy and flow cytometry. For fluorescence imaging, siRNA was labeled with a fluorescent dye prior to nanoparticle loading. NSCLC cells were incubated with the fluorescently tagged AgNP–siRNA nanocomplexes for predetermined time intervals, washed with phosphate-buffered saline (PBS), and observed under a fluorescence microscope to assess intracellular localization and uptake efficiency. Quantitative analysis of cellular uptake was performed using flow cytometry by measuring the fluorescence intensity of treated cells and comparing it with untreated controls (Aleixo et al., 2025).

The gene-silencing efficacy of the AgNP-mediated siRNA delivery system was determined by analyzing EGFR mRNA and protein expression levels. Total RNA was isolated from treated and control cells using a commercial RNA extraction kit, followed by complementary DNA (cDNA) synthesis. Quantitative real-time polymerase chain reaction (qRT-PCR) was performed using EGFR-specific primers, and gene expression levels were normalized against GAPDH as an internal control. For protein analysis, cellular proteins were extracted and subjected to Western blotting using anti-EGFR antibodies. The relative reduction in EGFR mRNA and protein expression was used to evaluate the effectiveness of siRNA-mediated gene silencing (Zahedipour et al., 2025).

4. Results

4.1 Synthesis and Physicochemical Characterization of Plant-Derived AgNPs

The green synthesis of silver nanoparticles using *Moringa oleifera* leaf extract was successfully achieved, as indicated by a visible color change from light yellow to dark brown, confirming the reduction of silver ions and nanoparticle formation. UV–Vis spectroscopy revealed a characteristic surface plasmon resonance peak, demonstrating successful synthesis and colloidal stability. Dynamic light scattering analysis showed that the synthesized AgNPs possessed a narrow size distribution with low polydispersity. Zeta potential measurements indicated good electrostatic stability, minimizing particle aggregation during storage. TEM and SEM analyses confirmed the predominantly spherical morphology of the nanoparticles, while XRD patterns verified their crystalline nature. These findings demonstrate the successful production of stable, uniformly distributed, and biocompatible plant-derived AgNPs suitable for subsequent EGFR-siRNA loading and therapeutic applications.

Table 1: Physicochemical Characteristics of Plant-Derived Silver Nanoparticles Synthesized under Different Experimental Conditions

Formulation	Particle Size (nm)	PDI	Zeta Potential (mV)	SPR Peak (nm)
AgNP-1	98.4 ± 2.6	0.214 ± 0.008	-21.3 ± 0.7	421 ± 2.1
AgNP-2	104.7 ± 2.9	0.228 ± 0.009	-24.8 ± 0.8	425 ± 2.3
AgNP-3	112.6 ± 3.1	0.241 ± 0.011	-27.6 ± 0.9	429 ± 2.5
AgNP-4	118.9 ± 3.4	0.257 ± 0.012	-30.2 ± 1.0	432 ± 2.7
AgNP-5	125.3 ± 3.8	0.269 ± 0.013	-33.5 ± 1.1	436 ± 2.9

Values are presented as mean ± SEM (n = 3). AgNP-5 exhibited the highest negative zeta potential and strongest colloidal stability, whereas AgNP-1 displayed the smallest average particle size. The observed physicochemical

properties confirmed successful biosynthesis of stable and uniformly dispersed silver nanoparticles suitable for siRNA delivery applications.

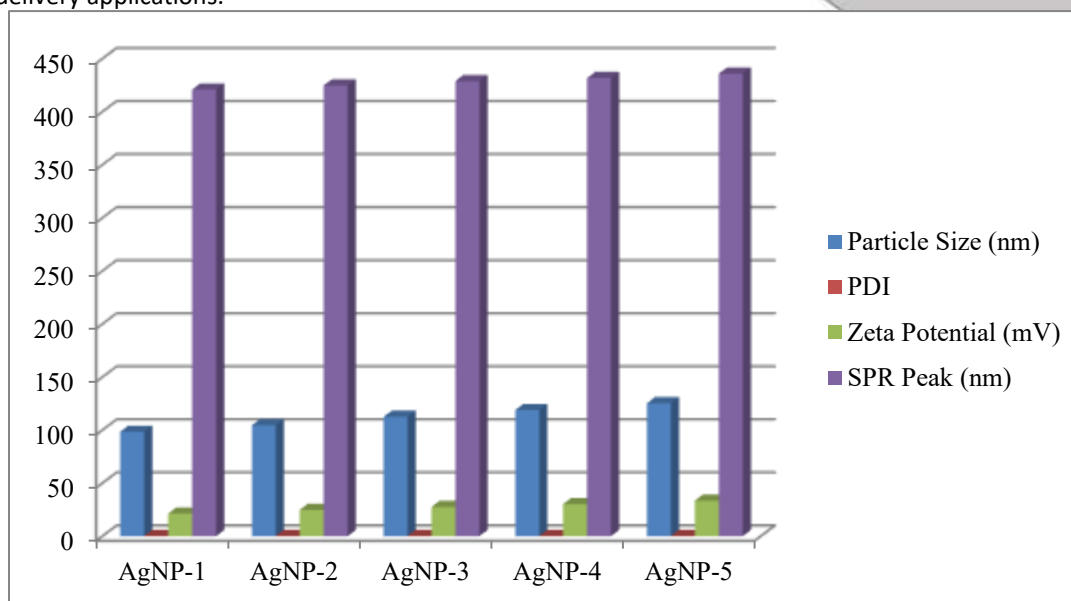


Figure 2: Physicochemical Characteristics of Plant-Derived Silver Nanoparticles Synthesized under Different Experimental Conditions

4.2 Efficient Loading and Protection of EGFR-siRNA

The plant-derived silver nanoparticles demonstrated high efficiency for EGFR-siRNA loading and protection. Surface-functionalized AgNPs effectively complexed with negatively charged siRNA molecules through electrostatic interactions, resulting in stable nanocomplex formation. Increasing the AgNP-to-siRNA ratio enhanced loading efficiency and reduced free siRNA in the supernatant. Gel retardation and nuclease protection assays confirmed that the loaded siRNA remained protected from enzymatic degradation for extended periods compared with naked siRNA. Furthermore, the AgNP-siRNA nanocomplexes maintained structural integrity under physiological conditions, indicating good stability during *in vitro* experiments. These findings suggest that plant-derived AgNPs can serve as effective carriers for EGFR-targeting siRNA by improving nucleic acid stability and ensuring efficient delivery to NSCLC cells.

Table 2: Loading Efficiency and Nuclease Protection Performance of EGFR-siRNA-Loaded AgNPs

Formulation	siRNA Loading Efficiency (%)	Encapsulation Efficiency (%)	Protected siRNA after 6 h (%)	Protected siRNA after 12 h (%)
AgNP-siRNA-1	72.4 ± 2.1	75.8 ± 2.3	68.5 ± 1.9	54.7 ± 1.8
AgNP-siRNA-2	78.9 ± 2.4	81.2 ± 2.5	75.6 ± 2.1	63.9 ± 1.9
AgNP-siRNA-3	84.3 ± 2.6	86.7 ± 2.7	82.4 ± 2.3	71.8 ± 2.1
AgNP-siRNA-4	89.1 ± 2.8	91.5 ± 2.9	88.7 ± 2.5	79.6 ± 2.3
AgNP-siRNA-5	93.6 ± 3.1	95.2 ± 3.0	92.8 ± 2.7	85.4 ± 2.5

Values are expressed as mean ± SEM (n = 3). AgNP-siRNA-5 exhibited the highest loading and encapsulation efficiencies along with superior protection against nuclease-mediated degradation, indicating its suitability as an optimized nanocarrier for EGFR-siRNA delivery.

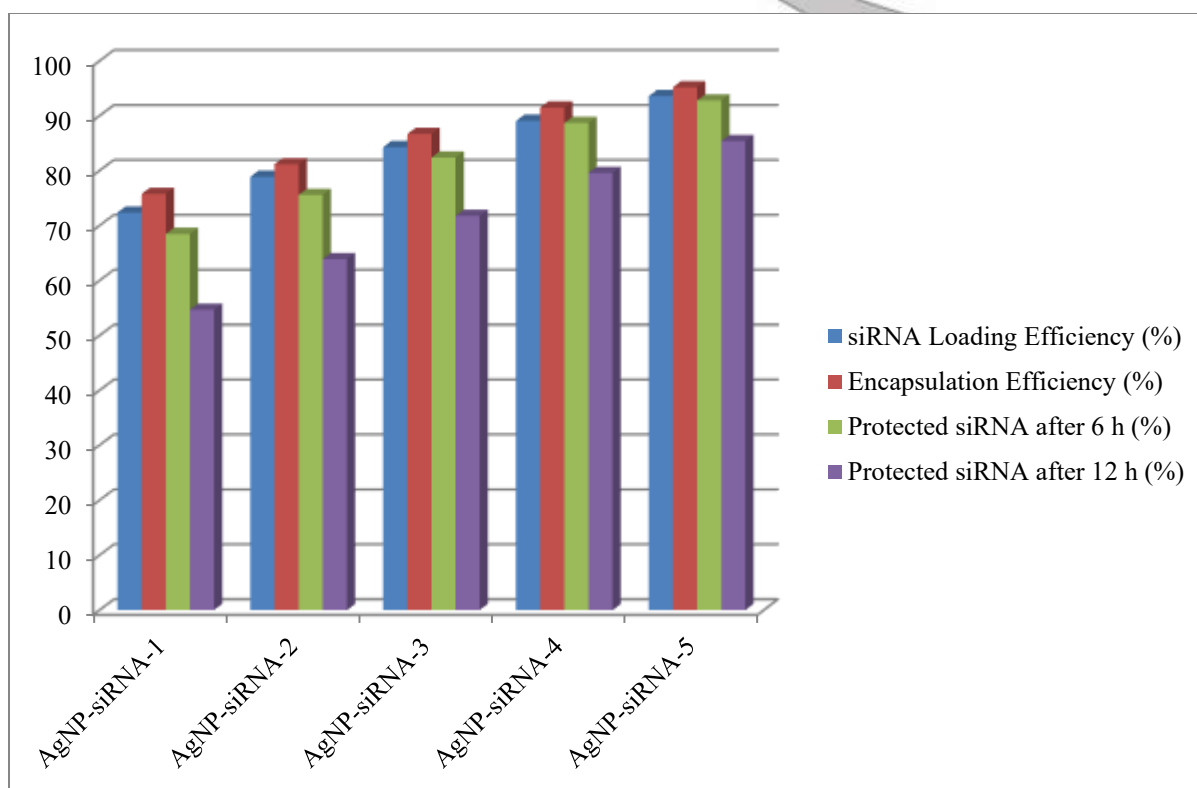


Figure 3: Loading Efficiency and Nuclease Protection Performance of EGFR-siRNA-Loaded AgNPs

4.3 Enhanced Cellular Uptake of AgNP-siRNA Nanocomplexes

Cellular uptake studies demonstrated efficient internalization of AgNP-siRNA nanocomplexes by NSCLC cells. Fluorescence microscopy revealed strong intracellular fluorescence signals in cells treated with AgNP-mediated siRNA, indicating successful delivery and cytoplasmic localization. In contrast, cells treated with free siRNA exhibited weak fluorescence due to limited membrane permeability. Quantitative flow cytometry analysis further confirmed significantly higher uptake efficiencies for AgNP-siRNA formulations compared with naked siRNA. Uptake increased in a concentration-dependent manner and reached maximum levels in the optimized formulation. Intracellular localization studies suggested that the nanocomplexes effectively escaped endosomal compartments and released siRNA into the cytoplasm, thereby facilitating efficient gene silencing and enhancing the therapeutic potential of the delivery system.

Table 3: Cellular Uptake and Intracellular Localization Efficiency of AgNP-siRNA Nanocomplexes in NSCLC Cells

Formulation	Cellular Uptake (%)	Mean Fluorescence Intensity (AU)	Cytoplasmic Localization (%)	Endosomal Escape Efficiency (%)
Free siRNA	28.6 ± 1.2	214 ± 8.1	24.8 ± 1.1	18.5 ± 0.9
AgNP-siRNA-1	56.3 ± 1.8	426 ± 11.4	49.2 ± 1.5	42.7 ± 1.3
AgNP-siRNA-2	68.7 ± 2.1	538 ± 13.6	61.5 ± 1.8	55.4 ± 1.6
AgNP-siRNA-3	79.8 ± 2.4	647 ± 15.2	73.9 ± 2.1	67.8 ± 1.9
AgNP-siRNA-4	88.5 ± 2.7	758 ± 17.8	84.6 ± 2.4	79.3 ± 2.2

Values are expressed as mean ± SEM (n = 3). AgNP-siRNA-4 demonstrated the highest cellular uptake, intracellular fluorescence intensity, cytoplasmic localization, and endosomal escape efficiency, indicating superior delivery performance compared with free siRNA and other formulations.

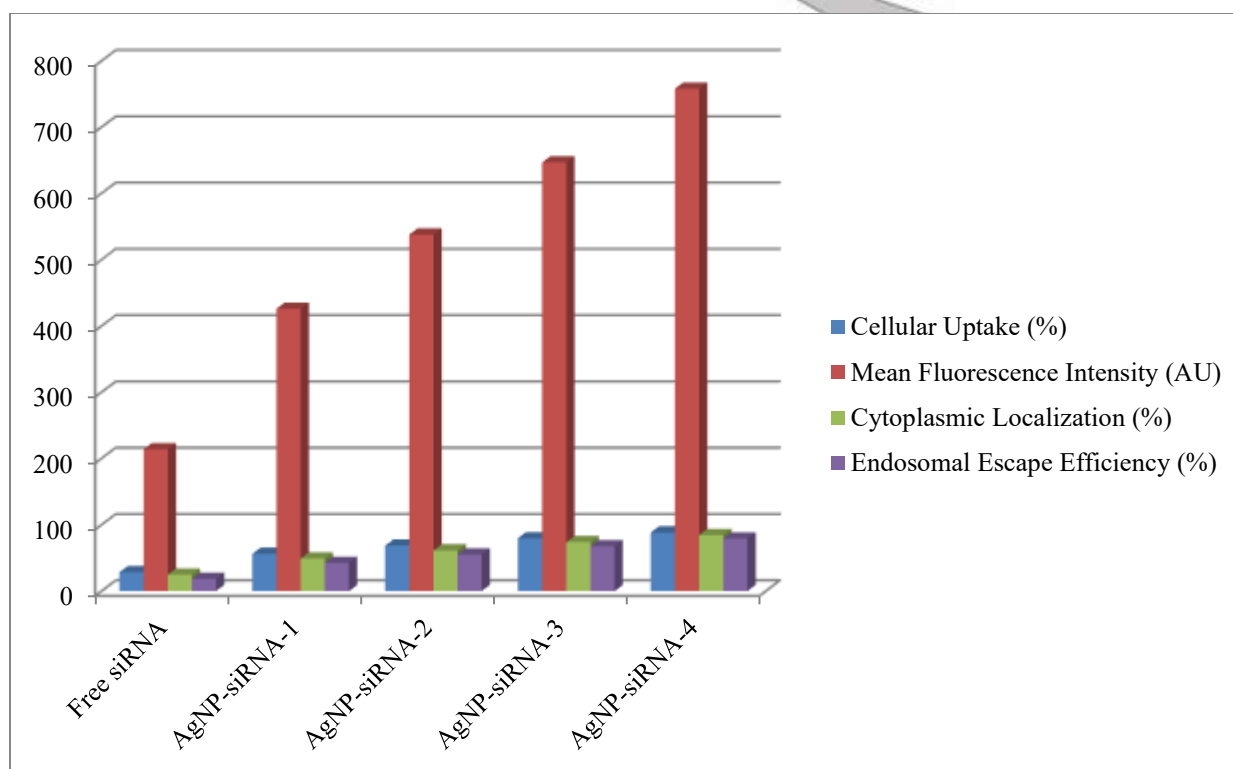


Figure 4: Cellular Uptake and Intracellular Localization Efficiency of AgNP-siRNA Nanocomplexes in NSCLC Cells

4.4 Suppression of EGFR Gene and Protein Expression

Treatment with EGFR-siRNA-loaded silver nanoparticles resulted in significant suppression of EGFR expression at both the transcriptional and translational levels in NSCLC cells. Quantitative RT-PCR analysis demonstrated a marked reduction in EGFR mRNA expression compared with untreated and free siRNA-treated groups, confirming efficient gene silencing. The degree of suppression increased with improved nanoparticle-mediated delivery efficiency. Western blot analysis further revealed substantial downregulation of EGFR protein expression following treatment with AgNP-siRNA nanocomplexes. The optimized formulation exhibited the greatest inhibitory effect on EGFR expression, indicating successful intracellular release of siRNA and effective RNA interference activity. These findings demonstrate that plant-derived AgNPs serve as efficient carriers for EGFR-targeting siRNA and significantly enhance gene-silencing efficacy in NSCLC cells.

Table 4: Effect of AgNP-siRNA Nanocomplexes on EGFR Gene and Protein Expression in NSCLC Cells

Treatment Group	Relative EGFR mRNA Expression (%)	EGFR Gene Suppression (%)	Relative EGFR Protein Expression (%)	Protein Downregulation (%)
Control	100.0 ± 2.8	0.0 ± 0.0	100.0 ± 3.1	0.0 ± 0.0
Free siRNA	76.5 ± 2.3	23.5 ± 1.2	81.7 ± 2.5	18.3 ± 1.1
AgNP-siRNA-1	58.9 ± 1.9	41.1 ± 1.5	63.4 ± 2.0	36.6 ± 1.4
AgNP-siRNA-2	39.7 ± 1.5	60.3 ± 1.8	45.8 ± 1.7	54.2 ± 1.6
AgNP-siRNA-3	21.8 ± 1.1	78.2 ± 2.1	28.6 ± 1.3	71.4 ± 1.9

Values are expressed as mean ± SEM (n = 3). AgNP-siRNA-3 produced the greatest reduction in both EGFR mRNA and protein expression, achieving 78.2% gene suppression and 71.4% protein downregulation, thereby demonstrating superior gene-silencing efficiency compared with free siRNA and other treatment groups.

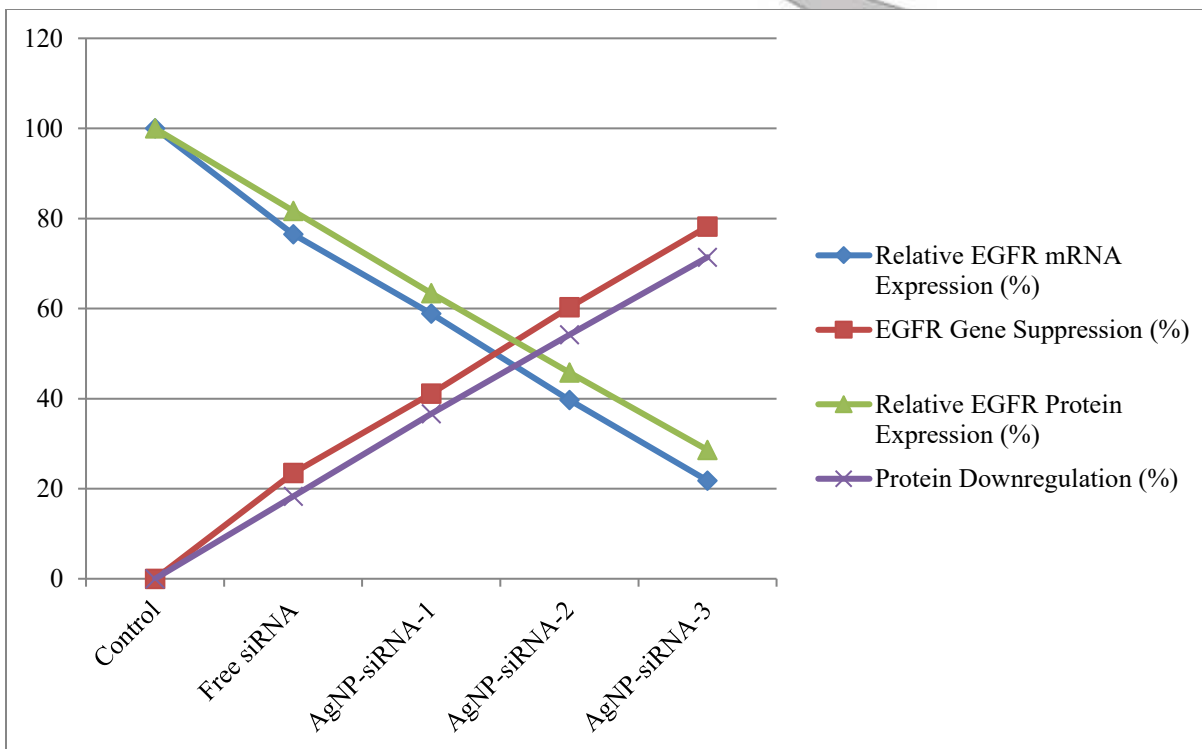


Figure 5: Effect of AgNP-siRNA Nanocomplexes on EGFR Gene and Protein Expression in NSCLC Cells

4.5 Inhibition of NSCLC Cell Growth and Survival

The antiproliferative activity of EGFR-siRNA-loaded silver nanoparticles was evaluated using cell viability and proliferation assays in NSCLC cells. Treatment with AgNP-siRNA nanocomplexes resulted in a significant reduction in cell viability compared with untreated controls and free siRNA-treated cells. The cytotoxic effect increased with enhanced EGFR silencing, indicating effective suppression of oncogenic signaling pathways. Cell proliferation analysis demonstrated a marked decline in proliferative capacity following nanoparticle-mediated siRNA delivery. Furthermore, cell cycle analysis revealed significant accumulation of cells in the G0/G1 phase accompanied by a reduction in the S phase population, indicating cell cycle arrest and inhibition of DNA synthesis. The optimized AgNP-siRNA formulation exhibited the strongest growth-inhibitory effect, highlighting its potential to suppress tumor cell survival and proliferation through efficient EGFR gene silencing.

Table 5: Effect of AgNP-siRNA Nanocomplexes on NSCLC Cell Growth, Proliferation, and Cell Cycle Progression

Treatment Group	Cell Viability (%)	Proliferation Inhibition (%)	G0/G1 Phase Cells (%)	S Phase Cells (%)
Control	100.0 ± 2.9	0.0 ± 0.0	48.6 ± 1.5	34.8 ± 1.2
Free siRNA	82.7 ± 2.4	17.3 ± 1.1	55.2 ± 1.7	29.6 ± 1.0
AgNP-siRNA-1	65.4 ± 2.0	34.6 ± 1.4	61.8 ± 1.9	24.3 ± 0.9
AgNP-siRNA-2	47.8 ± 1.6	52.2 ± 1.7	68.7 ± 2.1	18.7 ± 0.8
AgNP-siRNA-3	29.5 ± 1.2	70.5 ± 2.0	75.9 ± 2.3	12.4 ± 0.6

Values are expressed as mean ± SEM (n = 3). AgNP-siRNA-3 showed the strongest cytotoxic and antiproliferative effects, reducing cell viability to 29.5% and inducing pronounced G0/G1 phase arrest, indicating effective inhibition of NSCLC cell growth and survival following EGFR gene silencing.

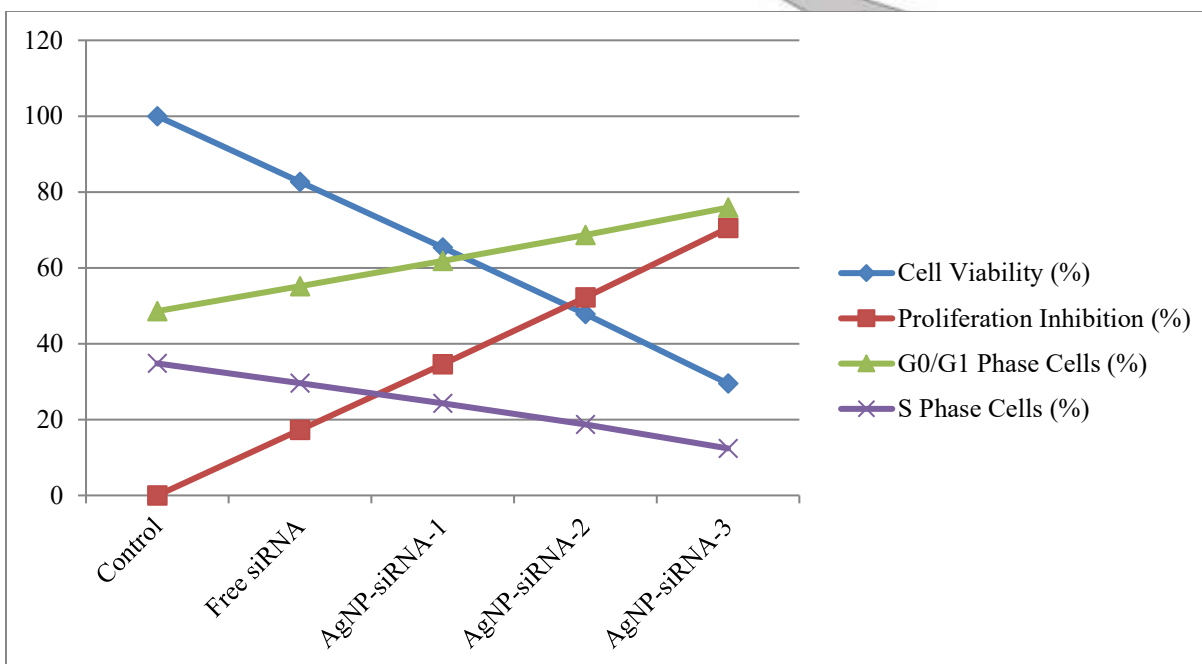


Figure 6: Effect of AgNP-siRNA Nanocomplexes on NSCLC Cell Growth, Proliferation, and Cell Cycle Progression

4.6 Induction of Apoptosis and Inhibition of Migration

The biological effects of EGFR-siRNA-loaded silver nanoparticles were further evaluated through apoptosis and migration assays. Flow cytometric analysis using Annexin V-FITC/PI staining demonstrated a significant increase in apoptotic cell populations following treatment with AgNP-siRNA nanocomplexes compared with control and free siRNA groups. The optimized formulation induced both early and late apoptosis, indicating effective activation of programmed cell death pathways. In addition, wound-healing and transwell invasion assays revealed a marked reduction in the migratory and invasive capacities of NSCLC cells after treatment. The extent of apoptosis induction and inhibition of migration closely correlated with the degree of EGFR suppression observed in gene expression studies. These findings suggest that efficient EGFR silencing disrupts key signaling pathways involved in cell survival, motility, and metastatic progression, thereby enhancing the overall anticancer efficacy of the AgNP-siRNA delivery system.

Table 6: Apoptosis Induction and Inhibition of Migration in NSCLC Cells Following Treatment with AgNP-siRNA Nanocomplexes

Treatment Group	Total Apoptotic Cells (%)	Early Apoptosis (%)	Migration Inhibition (%)	Invasion Inhibition (%)
Control	6.8 ± 0.5	4.1 ± 0.3	0.0 ± 0.0	0.0 ± 0.0
Free siRNA	18.9 ± 0.9	11.2 ± 0.6	24.7 ± 1.2	21.5 ± 1.0
AgNP-siRNA-1	34.6 ± 1.3	20.8 ± 0.8	42.9 ± 1.5	39.4 ± 1.4
AgNP-siRNA-2	51.7 ± 1.8	30.6 ± 1.1	61.8 ± 1.9	57.3 ± 1.7
AgNP-siRNA-3	69.4 ± 2.2	41.5 ± 1.4	79.6 ± 2.3	75.8 ± 2.1

Values are expressed as mean ± SEM (n = 3). AgNP-siRNA-3 exhibited the highest apoptotic response and strongest inhibition of cell migration and invasion. The enhanced anticancer activity was strongly associated with efficient EGFR gene silencing, indicating that suppression of EGFR signaling significantly contributes to reduced metastatic potential and increased programmed cell death in NSCLC cells.

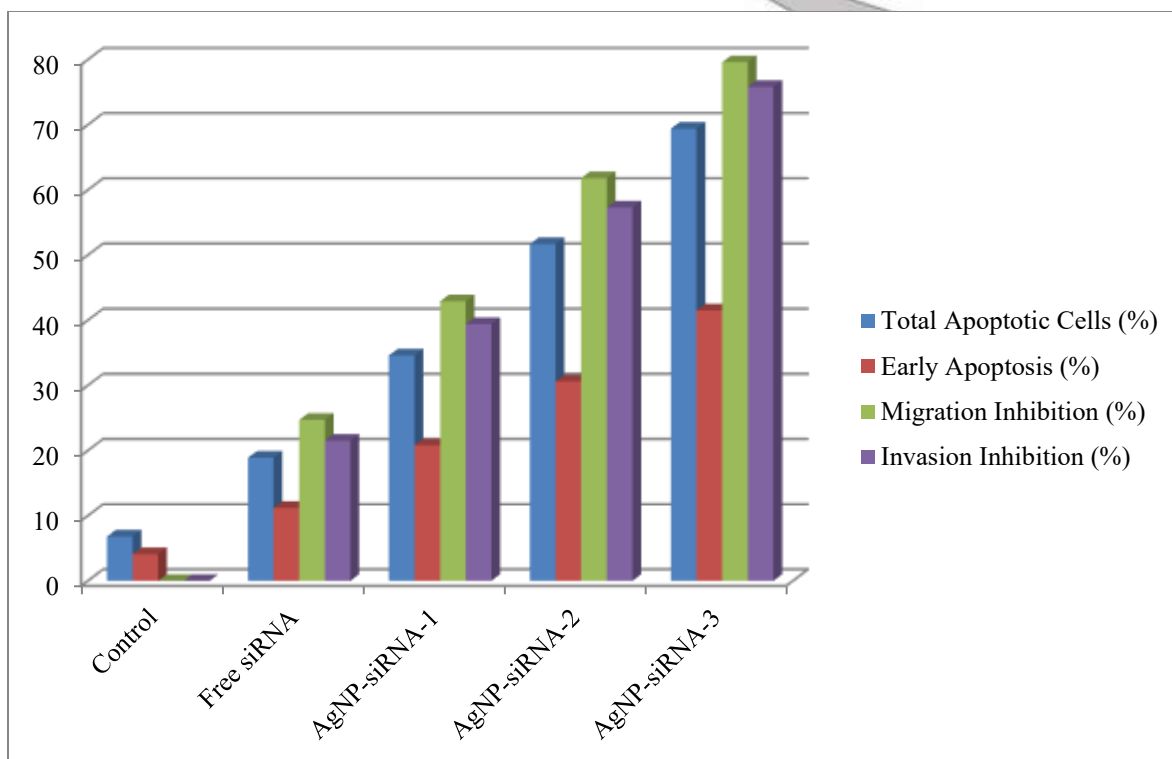


Figure 7: Apoptosis Induction and Inhibition of Migration in NSCLC Cells Following Treatment with AgNP-siRNA Nanocomplexes

5. Discussion

The present study demonstrates the potential of plant-derived silver nanoparticles (AgNPs) as effective carriers for EGFR-targeting siRNA in non-small cell lung cancer (NSCLC). Compared with conventional nanocarriers such as liposomes, polymeric nanoparticles, and dendrimers, AgNPs offer several advantages, including facile synthesis, tunable physicochemical properties, high surface-area-to-volume ratio, and intrinsic biological activity. The use of green nanotechnology further enhances their appeal by eliminating the need for toxic reducing agents and harsh synthesis conditions. Plant-mediated synthesis provides an environmentally friendly and cost-effective approach while generating nanoparticles with improved biocompatibility. The favorable size distribution, surface charge, and stability observed in this study support the suitability of plant-derived AgNPs as efficient platforms for nucleic acid delivery.

Efficient gene silencing depends largely on the successful intracellular delivery of siRNA. The results obtained in this study indicate that AgNP-mediated delivery significantly improved cellular uptake and intracellular availability of EGFR-targeting siRNA compared with free siRNA. The positively charged functionalized nanoparticle surface promoted electrostatic interactions with siRNA and enhanced cellular internalization through endocytosis. Furthermore, the nanoparticles protected siRNA from nuclease-mediated degradation, thereby increasing its stability in the extracellular environment. Enhanced endosomal escape likely facilitated the release of siRNA into the cytoplasm, where it interacted with the RNA-induced silencing complex (RISC) to degrade EGFR mRNA. Consequently, substantial reductions in both EGFR gene and protein expression were observed, confirming efficient RNA interference and demonstrating the effectiveness of the nanoparticle delivery system.

EGFR plays a central role in regulating cellular proliferation, survival, migration, and invasion in NSCLC. Therefore, suppression of EGFR expression is expected to disrupt multiple oncogenic signaling pathways simultaneously. In the present study, significant reductions in cell viability and proliferation were accompanied by marked inhibition of tumor cell growth following EGFR silencing. Cell cycle analysis demonstrated G0/G1 phase arrest, indicating impaired progression through critical checkpoints required for cellular replication. Furthermore, enhanced apoptosis and reduced migration and invasion were observed in treated cells. These findings suggest that inhibition of EGFR signaling disrupts pathways such as PI3K/Akt, MAPK, and JAK/STAT, ultimately reducing tumor

aggressiveness and metastatic potential. The observed correlation between EGFR suppression and anticancer activity highlights the therapeutic value of RNA interference-based approaches for NSCLC treatment.

Plant-derived nanoparticles possess unique surface characteristics due to the presence of naturally occurring phytochemicals that act as reducing and stabilizing agents during synthesis. Compounds such as flavonoids, phenolic acids, terpenoids, and alkaloids contribute to nanoparticle stabilization by preventing aggregation and improving colloidal stability. In addition to their stabilizing effects, these phytochemicals may provide supplementary biological activities, including antioxidant and anticancer properties. The phytochemical coating surrounding the AgNPs may enhance cellular interactions and improve nanoparticle biocompatibility. Moreover, synergistic interactions between the phytochemicals, silver nanoparticles, and EGFR-targeting siRNA may contribute to the enhanced therapeutic effects observed in this study. Such multifunctional properties represent a significant advantage of green-synthesized nanoparticles over conventionally produced nanomaterials.

Several studies have investigated lipid-based, polymeric, and inorganic nanoparticle systems for EGFR-siRNA delivery. While many of these platforms have demonstrated promising gene-silencing capabilities, limitations such as low stability, limited loading capacity, cytotoxicity, and complex manufacturing procedures remain significant challenges. The plant-derived AgNPs developed in the present study exhibited high siRNA loading efficiency, enhanced cellular uptake, effective EGFR suppression, and substantial anticancer activity. These findings are consistent with previous reports while offering additional advantages associated with green synthesis and intrinsic nanoparticle bioactivity. Nevertheless, certain limitations should be acknowledged, including the need for comprehensive toxicity evaluation, long-term stability studies, and assessment of nanoparticle biodistribution. Addressing these factors will be essential for future translational development.

Although the *in vitro* findings are highly encouraging, further investigations are necessary to establish the clinical applicability of plant-derived AgNP-siRNA nanocomplexes. Future studies should focus on *in vivo* validation using appropriate animal models to evaluate therapeutic efficacy, pharmacokinetics, biodistribution, and safety profiles. The incorporation of tumor-targeting ligands may further enhance selective delivery to NSCLC tissues while minimizing off-target effects. Advances in precision nanomedicine could enable the development of personalized therapeutic strategies based on individual EGFR mutation status and tumor characteristics. Overall, plant-derived AgNP-mediated siRNA delivery represents a promising platform for targeted gene therapy and may contribute significantly to the next generation of precision treatments for non-small cell lung cancer.

Conclusion

The present study demonstrates the successful development of plant-derived silver nanoparticles (AgNPs) as an efficient and biocompatible delivery platform for EGFR-targeting small interfering RNA (siRNA) in non-small cell lung cancer (NSCLC). Green synthesis using plant extracts provided a simple, cost-effective, and environmentally sustainable approach for producing stable silver nanoparticles with desirable physicochemical characteristics, including suitable particle size, surface charge, and colloidal stability. These properties enabled efficient loading and protection of EGFR-siRNA, ensuring enhanced stability against enzymatic degradation and improved intracellular delivery. The AgNP-siRNA nanocomplexes exhibited significantly higher cellular uptake and gene-silencing efficiency compared with free siRNA. Quantitative analyses confirmed substantial suppression of EGFR mRNA and protein expression, demonstrating the effectiveness of nanoparticle-mediated RNA interference. EGFR knockdown resulted in pronounced anticancer effects, including reduced cell viability, inhibition of cellular proliferation, induction of cell cycle arrest, increased apoptotic cell death, and suppression of migratory and invasive behaviors in NSCLC cells. These therapeutic outcomes highlight the critical role of EGFR signaling in tumor progression and validate the potential of siRNA-based strategies for targeted cancer treatment. Furthermore, the presence of phytochemical capping agents may have contributed to enhanced nanoparticle stability and biological activity, providing additional advantages over conventionally synthesized nanocarriers. Collectively, the findings indicate that plant-derived AgNPs represent a promising multifunctional nanoplatform for targeted gene delivery and cancer therapy. Future *in vivo* studies and clinical investigations are warranted to further evaluate their safety, efficacy, and translational potential in precision nanomedicine approaches for the treatment of non-small cell lung cancer.

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