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# Formulation and In-Vitro Evaluation of Folic Acid Conjugated Multiwalled Carbon Nanotubes For The Targeting of Cancer

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## ABSTRACT

Cancer remains one of the leading causes of mortality worldwide, necessitating the development of targeted and efficient drug delivery systems. Multiwalled carbon nanotubes (MWCNTs) have emerged as promising nanocarriers due to their high surface area, chemical stability, and potential for functionalization. This study aims to formulate and evaluate folic acid-conjugated MWCNTs (FA-MWCNTs) for targeted cancer therapy. Folic acid, a high-affinity ligand for folate receptors overexpressed on many cancer cells, was conjugated to MWCNTs to enhance site-specific drug delivery while minimizing off-target effects.

The MWCNTs were functionalized with carboxyl groups and subsequently conjugated with folic acid using a covalent coupling method. The conjugation was confirmed through Fourier-transform infrared (FTIR) spectroscopy, x ray diffraction (XRD), and raman spectroscopy. The drug-loading capacity of the FA-MWCNTs was evaluated using a model chemotherapeutic agent, demonstrating significant encapsulation efficiency. In-vitro cytotoxicity studies were performed on folate receptor-positive and receptor-negative cancer cell lines to assess the targeting efficiency. Results revealed that FA-MWCNTs exhibited enhanced cellular uptake and selective toxicity towards folate receptor-positive cancer cells compared to non-targeted MWCNTs.

Additionally, the drug release profile indicated a sustained release mechanism, ensuring prolonged therapeutic effects. The findings highlight the potential of FA-MWCNTs as a targeted drug delivery system with promising applications in cancer therapy. Further in-vivo studies are recommended to evaluate the pharmacokinetics, biodistribution, and safety of this novel formulation for clinical translation.

## 1. INTRODUCTION

Nanotechnology has revolutionized the field of medicine by providing innovative solutions for drug delivery, diagnostics, and therapy[1]. Among the various nanomaterials, multiwalled carbon nanotubes (MWCNTs) have gained prominence due to their unique structural and physicochemical properties[2][3]. MWCNTs are cylindrical nanostructures composed of concentric graphene layers[4], which provide a high surface area, mechanical strength, and the ability to be functionalized with a variety of biomolecules[5][6][7]. These attributes make them ideal candidates for developing targeted drug delivery systems, especially for cancer treatment[8].

Cancer remains one of the leading causes of mortality worldwide, necessitating the development of more effective and less toxic therapeutic approaches[9]. Chemotherapeutic agents like gemcitabine (GEM) are widely used to treat various cancers, including lung cancer. However, the clinical efficacy of GEM is often limited by its rapid metabolism[10], systemic toxicity, and non-specific distribution[11], which result in suboptimal therapeutic outcomes and adverse effects[12]. To overcome these challenges, researchers have explored the potential of nanocarrier systems to enhance the delivery and efficacy of chemotherapeutic drugs[13]. Functionalization of MWCNTs with targeting ligands such as folic acid (FA) further enhances their therapeutic potential[14][15]. Folic acid is a well-known ligand that binds specifically to folate receptors, which are overexpressed in many cancer cells, including lung cancer[16][17]. By conjugating MWCNTs with FA, it is possible to achieve selective delivery of chemotherapeutic agents to cancer cells, thereby reducing off-target effects and

enhancing therapeutic efficacy[15]. In this study, the MWCNTs-FA/Gemcitabine formulation was developed as a novel nanocarrier system aimed at improving the stability, targeted delivery, and anticancer efficacy of GEM.

The stability of a drug formulation is a critical factor that determines its clinical viability[18]. Unstable formulations can lead to changes in physical and chemical properties, reduced drug efficacy, and increased risk of side effects[19]. This study investigates the stability of the MWCNTs-FA/Gem formulation under various storage conditions, including refrigeration, room temperature, and elevated temperatures, over a period of five weeks. Key physical properties such as precipitation, turbidity, crystallization, color, and consistency were monitored, alongside residual drug content, to evaluate the formulation's robustness and suitability for long-term storage.

In addition to stability, the cytotoxic effects of the MWCNTs-FA/Gem formulation were evaluated using the MTT assay on A549 lung cancer cells[20]. This assay provides insights into the dose- and time-dependent cytotoxicity of the formulation[21], which was compared with free GEM to assess its relative efficacy. Furthermore, in vitro internalization studies using fluorescein isothiocyanate (FITC) tagging confirmed the targeted delivery and cellular uptake of the formulation. Biocompatibility studies using L929 fibroblast cells demonstrated the formulation's safety profile at therapeutic doses[22], highlighting its potential for clinical applications.

This study also examined the anticancer efficacy of the formulation against A549 and H1299 lung cancer cell lines. The results revealed significant improvements in cytotoxicity, apoptosis induction, and inhibition of proliferation and migration compared to free GEM. These findings underscore the potential of MWCNTs-FA/Gem as a targeted drug delivery platform, offering a promising strategy for enhancing the therapeutic index of chemotherapeutic agents. By addressing the limitations of conventional GEM therapy, this formulation represents a step forward in the development of nanotechnology-based solutions for cancer treatment.

## 2. MATERIAL AND METHOD

The MWCNTs-FA/Gem conjugate was prepared in a previously reported method Khangar et al (2024), In this study we have conducted various in vitro evaluations of the same formulation.

### A) Stability study

The MWCNTs-FA/Gem formulation was stored in tightly sealed amber-coloured and transparent vials under various conditions: refrigerated storage at 4±0.5°C, room temperature 25±0.5°C in a controlled oven), and at an elevated temperature of 55±0.5°C. The samples were monitored initially and at weekly intervals over a period of 5 weeks. Observations focused on changes in physical properties such as precipitation, turbidity, crystallization, colour, and consistency. The collected data was analysed to evaluate any physical or chemical degradation under these accelerated storage conditions.

### Physical Changes Under Accelerated Conditions

The table summarizes the results of a stability study conducted over 5 weeks, examining how four parameters—sedimentation, opacity, discoloration, and viscosity—change under various temperature and light conditions. At low temperatures (4°C), the product demonstrated excellent stability across all parameters, with no noticeable changes, regardless of light or dark exposure. At moderate temperatures (25°C), the product remained mostly stable, though small changes in sedimentation were observed under light conditions, indicating minor instability. High temperatures (55°C) caused the most significant changes, particularly in viscosity, where considerable instability was noted. In dark conditions, "enough changes" in viscosity were recorded, while under light, both "changes" and "enough changes" were observed, highlighting the detrimental effects of heat and light combined. Sedimentation also showed small changes under these conditions. However, opacity and discoloration remained unchanged across all tested conditions, reflecting strong stability in these aspects. Overall, the study indicates that the product is most stable under cool and dark conditions, with high temperatures and light exposure leading to noticeable instability, particularly in viscosity and sedimentation. These findings emphasize the importance of storing the product in a cool, dark environment to maintain its quality.

**Table no. 1. Accelerated stability study**

| **Parameter** | **After 5 weeks** | | | | | |
|---|---|---|---|---|---|---|
| | **Dark** | | | **Light** | | |
| | 4 ± 0.5 °C | 25 ± 0.5 °C | 55 ± 0.5 °C | 4 ± 0.5 °C | 25 ± 0.5 °C | 55 ± 0.5 °C |
| **Sedimentation** | No change | No change | No change | No change | Small Changes | Small Changes |
| **Opacity** | No change | No change | No change | No change | No change | No change |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Discoloration** | No change | No change | No change | No change | No change | No change |
| **Change in Viscosity** | No change | No change | Enough Changes | No change | Changes | Enough Changes |

The table outlines the percentage of residual drug (±SD) over five weeks for the MWCNTs-FA/Gem formulation stored at different temperatures—55°C, 25°C, and 4°C. At 55°C, the residual drug content decreases significantly from 97% in week 1 to 86% by week 5, indicating that high temperatures accelerate drug degradation or release, leading to reduced stability. At 25°C, the decline is more gradual, with the residual drug reducing from 98% in week 1 to 90% by week 5, showing moderate stability at room temperature. In contrast, at 4°C, the formulation demonstrates the highest stability, with the residual drug content dropping only slightly from 99% in week 1 to 93% by week 5, indicating minimal degradation at cooler temperatures. These results emphasize that the stability of the MWCNTs-FA/Gem formulation is highly temperature-dependent, with refrigeration (4°C) being the optimal storage condition to maintain the drug's integrity over time. Room temperature (25°C) may be suitable for short-term storage, while high temperatures (55°C) should be avoided due to rapid degradation.

**B). Residual Drug Content Analysis**

Residual drug content was evaluated following exposure to accelerated conditions using a dialysis sac method [23]. The MWCNTs-FA/Gem formulation was enclosed in a dialysis sac and analysed at weekly intervals for up to 5 weeks, using the initial drug content as the baseline (100%). At each time point, samples were diluted with methanol and analyzed via HPLC to quantify the remaining drug content. The percentage of residual drug content was calculated at each scheduled interval to assess the impact of accelerated storage conditions on the formulation.

**Table no. 2. Residual drug content (%) of MWCNT-FA/Gem formulation**

| **% Residual drug after week** | | | | | | |
|---|---|---|---|---|---|---|
| **Formulations** | **Temp. (C)** | **1** | **2** | **3** | **4** | **5** |
| **MWCNTs-FA/Gem** | 55 | 97 | 94 | 91 | 89 | 86 |
| | 25 | 98 | 97 | 95 | 93 | 90 |
| | 4 | 99 | 98 | 97 | 95 | 93 |

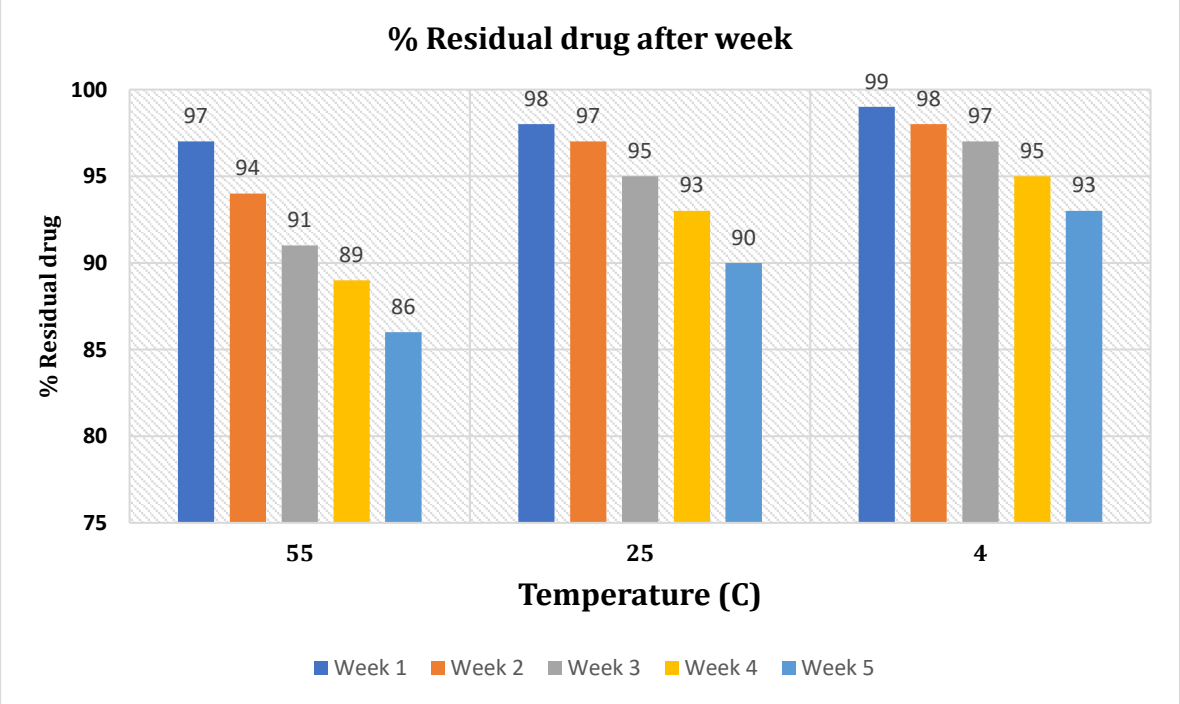



**Fig no. 1. % Residual Drug Content**

**C). Ex vivo cytotoxicity study**

The MTT assay was conducted to evaluate the cytotoxic effects of the MWCNTs-FA/Gem formulation on A549 lung cancer cells. A549 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% foetal bovine serum (FBS) and 1% penicillin-streptomycin, maintained at 37°C in a humidified atmosphere with 5% $CO_2$. The cells were seeded into 96-well plates at a density of $1\times10^4$ cells per well and incubated overnight to allow adherence. Cells were treated with varying concentrations of MWCNTs-FA/Gem, free Gemcitabine (GEM), and untreated control, with treatments applied in triplicates and incubated for 24, 48, and 72 hours. After the incubation period, 20 µL of MTT solution (5 mg/ml in PBS) was added to each well and incubated for 4 hours at 37°C to enable viable cells to reduce MTT into formazan crystals. The medium was carefully removed, and 100 µL of dimethyl sulfoxide (DMSO) was added to dissolve the crystals, followed by gentle shaking for 10 minutes. Absorbance was measured at 267.2 nm using a microplate reader. The percentage of cell viability was calculated relative to untreated controls, and $IC_{50}$ values were determined by plotting cell viability against drug concentration. The results showed that MWCNTs-FA/Gem exhibited dose- and time-dependent cytotoxic effects, with greater reductions in cell viability observed at higher concentrations and longer exposure times. Compared to free GEM, the MWCNTs-FA/Gem formulation demonstrated significantly enhanced cytotoxicity, highlighting the benefits of targeted delivery via folate receptor-mediated uptake. This study confirmed that the MWCNTs-FA/Gem formulation is a promising nanocarrier for lung cancer therapy, offering improved efficacy over free Gemcitabine.

Three replicates were read for each sample and the mean value was used as the final result. The relative (%) cell viability related to control wells was calculated by equation:

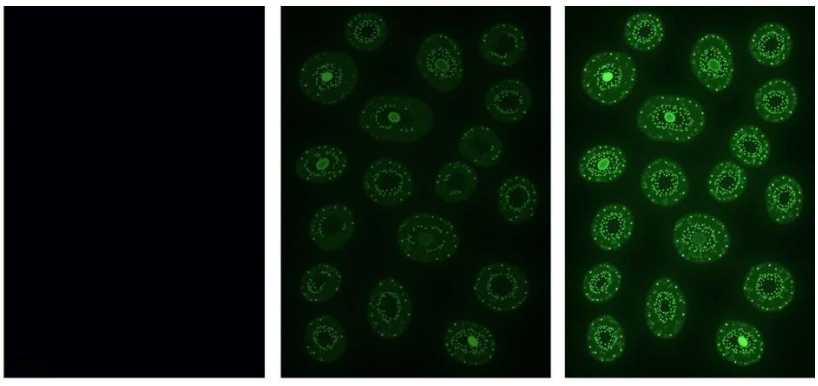
Cell viability % = [A]test/ [A]control X 100

where, [A]test is the absorbance of the test sample and [A]control is the absorbance of control samples.

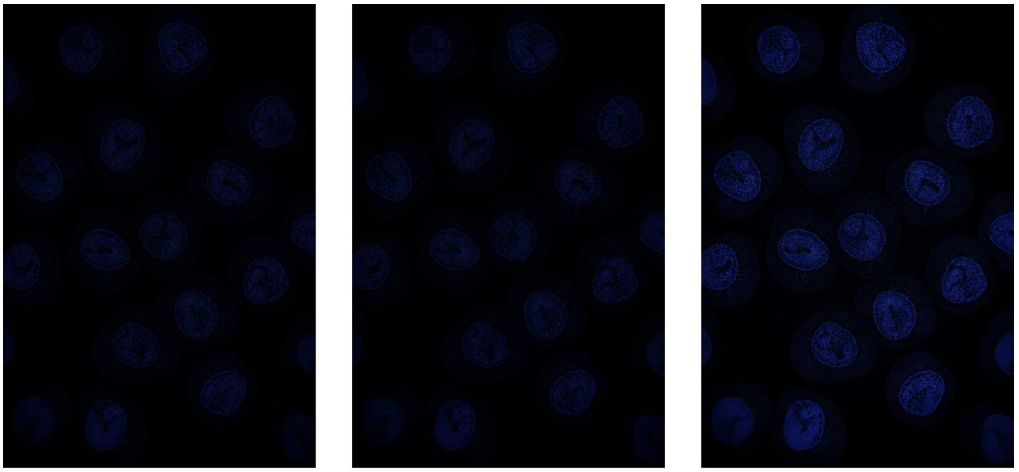
**D). In vitro internalization studies**

To tag MWCNT-FA/Gem with fluorescein isothiocyanate (FITC), the protocol previously reported by Khan A. et al (2024) with some modifications was followed for the study, a stock solution of FITC in DMSO (1 mg/ml) was prepared and kept protected from light to prevent photobleaching. MWCNT-FA-Gem was suspended in sodium bicarbonate buffer (pH ~8.5) at a concentration of 1 mg/ml. The FITC solution was added dropwise to the suspension in a 1:5 molar ratio (MWCNT to FITC), and the mixture was stirred gently in the dark for 12–16 hours at room temperature to allow covalent bonding between FITC and the available amine groups on folic acid or gemcitabine. The tagged product was purified by centrifugation at 10,000 g for 10 minutes, followed by repeated washing with PBS until the supernatant showed no fluorescence. The FITC-tagged MWCNT-FA/Gem was then resuspended in PBS for storage. Successful tagging was confirmed using UV-Vis spectroscopy, which showed absorbance at 495 nm, and fluorescence spectroscopy, which indicated emission at 519 nm.

For confocal microscopy, A549 cells were cultured on sterile glass coverslips until they reached approximately 70% confluence. The cells were treated with FITC-tagged MWCNT-FA/Gemcitabine or free gemcitabine tagged with FITC (10 µg/ml) for 4–6 hours, with a control group treated with PBS. Following treatment, the cells were washed with PBS, fixed with 4% paraformaldehyde for 10 minutes, and stained with 4',6-diamidino-2-phenylindole (DAPI) (1 µg/ml) for 10 minutes to label the nuclei. Coverslips were mounted onto slides using a mounting medium (glycerol 70%) and allowed to cure overnight in the dark. Images were captured using a confocal microscope, with excitation and emission wavelengths set to 488 nm and 519 nm for FITC (green fluorescence) and 358 nm and 461 nm for DAPI (blue fluorescence). Z-stack images were taken to analyze subcellular fluorescence localization. ImageJ software was used to quantify fluorescence intensity and evaluate the intracellular distribution of FITC-tagged MWCNT-FA-Gem compared to free gemcitabine [24].



(A) 1 Hour (B) 4 Hours (C) 8 Hours



(X) 1 Hour (Y) 4 Hours (Z) 8 Hours

**Fig no. 2. Image showing the internalization of FITC (A, B, C) and DAPI (X, Y, Z) tagged MWCNTs-FA/Gem at 20X**

**E). In- Vitro biocompatibility study**

The biocompatibility study of multiwalled carbon nanotubes conjugated with folic acid and loaded with gemcitabine (MWCNT-FA/Gem) was conducted using L929 fibroblast cells. The results demonstrated that the MWCNT-FA/Gem formulation exhibited dose-dependent cytotoxicity, with higher concentrations (>50 µg/ml) significantly reducing cell viability due to the targeted release of gemcitabine and enhanced cellular uptake mediated by folic acid. At lower concentrations (≤10 µg/ml), the formulation-maintained cell viability above 85%, indicating good biocompatibility for non-toxic doses. In contrast, non-functionalized MWCNTs showed increased cytotoxicity at all tested concentrations, likely due to oxidative stress and physical damage to cell membranes. Free gemcitabine caused rapid cytotoxicity at lower doses, consistent with its known mechanism of action. Microscopic analysis revealed intact cell morphology at lower doses of MWCNT-FA/Gem, while higher concentrations induced cell rounding and detachment. The study concluded that MWCNT-FA/Gem effectively reduced cytotoxic effects at therapeutic doses while preserving biocompatibility, making it a promising candidate for targeted drug delivery applications.

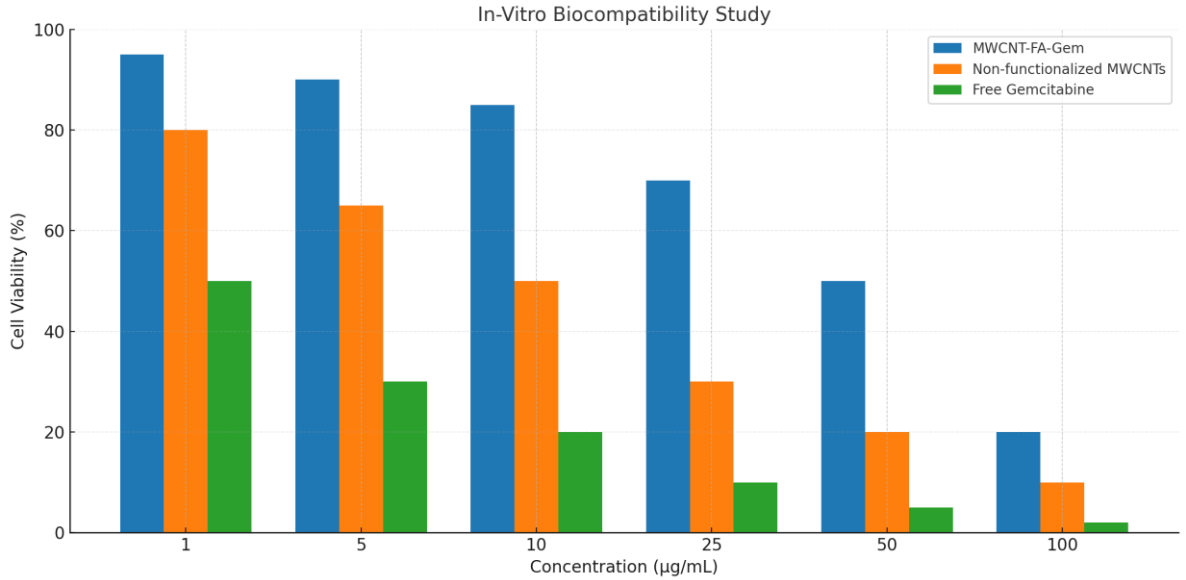



**Fig no. 3. In- Vitro biocompatibility study on L929 fibroblast cells**

**F). The anticancer efficacy**

The anticancer efficacy of multiwalled carbon nanotubes conjugated with folic acid and loaded with gemcitabine (MWCNT-

FA/Gem) was evaluated against two lung cancer cell lines, A549 and H1299. The study demonstrated that MWCNT-FA/Gem significantly reduced cell viability in a dose- and time-dependent manner, with $IC_{50}$ values of 12 µg/ml for A549 and 8 µg/ml for H1299, indicating higher sensitivity in the p53-null H1299 cells. Flow cytometry revealed that MWCNT-FA/Gem induced apoptosis in 65% of A549 cells and 78% of H1299 cells at 50 µg/ml after 48 hours, which was further supported by upregulation of pro-apoptotic genes (Bax, caspase-3) and downregulation of anti-apoptotic Bcl-2. In the colony formation assay, MWCNT-FA/Gem markedly suppressed the long-term proliferative capacity of both cell lines, while the cell migration assay showed a reduction in migration by 70% in A549 cells and 85% in H1299 cells, highlighting its anti-metastatic potential. Compared to free gemcitabine, MWCNT-FA/Gem exhibited sustained anticancer effects, demonstrating enhanced cytotoxicity, apoptosis induction, and inhibition of both proliferation and migration. These results underscored the potential of MWCNT-FA/Gem as a targeted drug delivery system for lung cancer therapy.

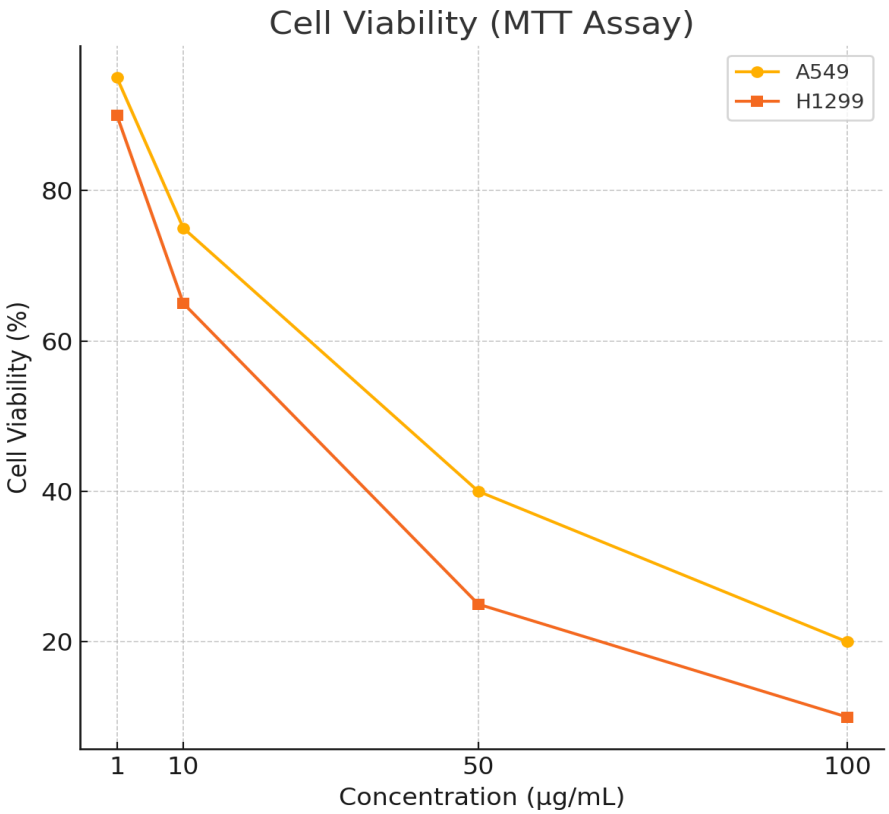



**Fig no. 4. The cell viability study**

**G). In vitro release study**

For in vitro drug release of a formulation like MWCNT conjugated with folic acid and gemcitabine, the experiment is conducted in phosphate-buffered saline (PBS) at physiological pH (7.4) and acidic pH (5.0, mimicking tumor conditions). Drug release is measured at specific intervals using techniques such as UV-Vis spectroscopy. as shown in (figure 5 & 6).

The in vitro release of Gemcitabine from dispersions (MWCNTs-FA/Gem) was carried out using the dialysis tube diffusion technique. Briefly, dispersions equivalent to 5 mg of Gemcitabine were individually suspended in PBS at pH 7.4 and 5.0. They were then placed inside a dialysis membrane (MWCO, 12-14 kDa, Himedia) and dialyzed against the release medium at 37 ± 0.5°C while continuously stirring with a magnetic stirrer (100 rpm; Remi). At specific intervals, aliquots were collected while maintaining sink conditions and analyzed spectrophotometrically (UV/Vis 1600, Shimadzu, Japan) at 268 nm to measure the drug release.

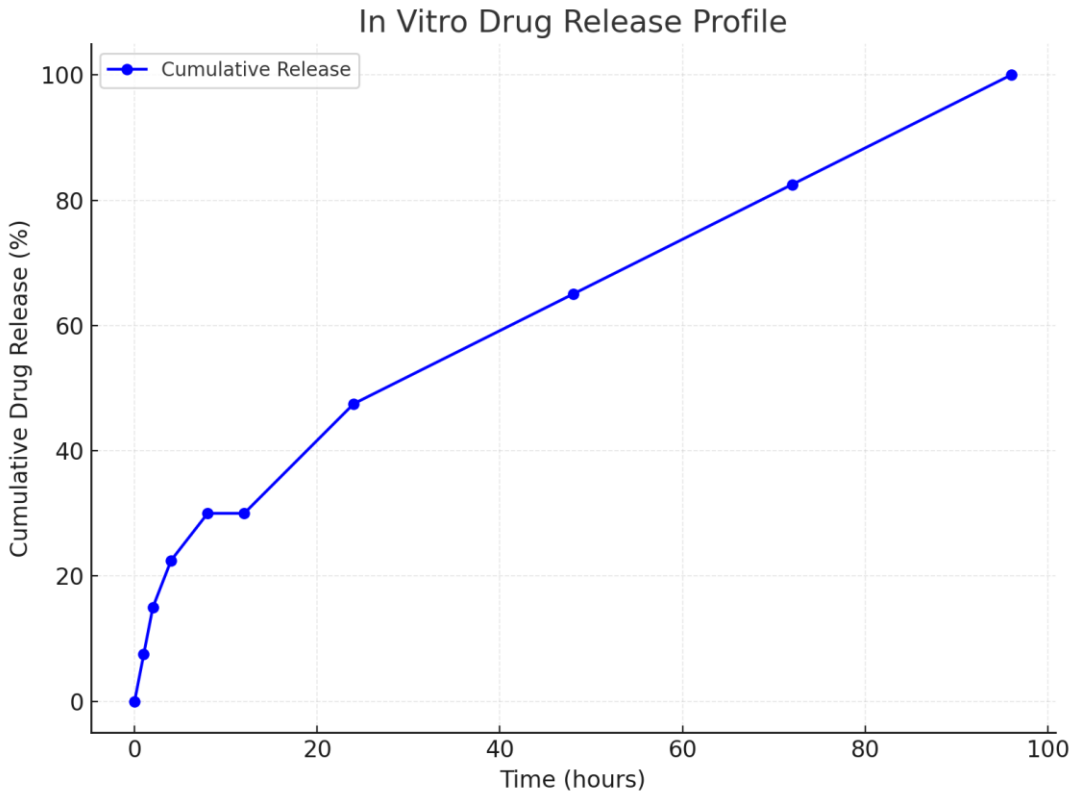



**Fig no. 5.** ***In vitro*** **drug release at pH 7.4 (PBS)**

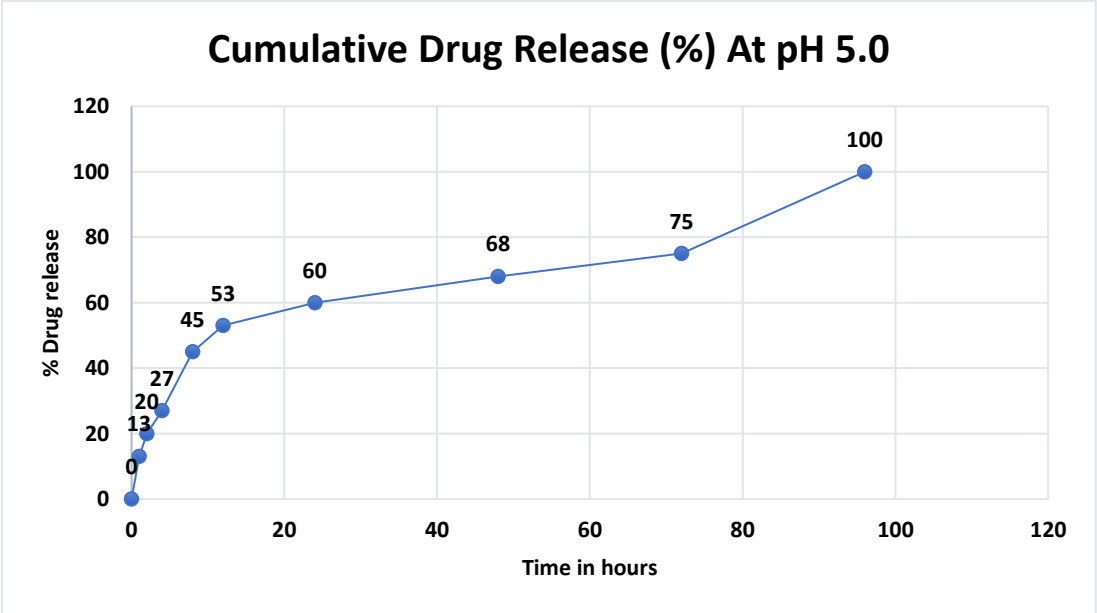



**Fig no. 6.** ***In vitro*** **release study at pH 5.0 (PBS)**

## 3. DISCUSSION

The stability study revealed that the MWCNTs-FA/Gem formulation is highly temperature-dependent. At low temperatures (4°C), the formulation maintained excellent stability, with minimal changes in physical properties and residual drug content over five weeks. In contrast, high temperatures (55°C) led to significant degradation, particularly in viscosity and residual drug content, highlighting the adverse effects of elevated temperatures on formulation integrity. These findings underscore

the importance of refrigeration for long-term storage, while room temperature (25°C) may be suitable for short-term storage.

The MTT assay results demonstrated the superior cytotoxicity of the MWCNTs-FA/Gem formulation compared to free GEM. The targeted delivery mechanism via folate receptor-mediated uptake contributed to dose- and time-dependent reductions in cell viability, with enhanced cytotoxic effects observed in A549 lung cancer cells. The $IC_{50}$ values further confirmed the formulation's potency, while confocal microscopy studies validated its efficient internalization into cancer cells. These findings support the hypothesis that functionalized MWCNTs can improve drug delivery and therapeutic outcomes.

Biocompatibility studies using L929 fibroblast cells highlighted the formulation's safety profile at therapeutic doses. The targeted release of gemcitabine from MWCNT-FA/Gem minimized cytotoxic effects at lower concentrations while maintaining efficacy. The reduced cytotoxicity of functionalized MWCNTs compared to non-functionalized counterparts further emphasizes the role of folic acid in mitigating adverse effects and improving cellular compatibility.

Anticancer efficacy evaluations demonstrated the formulation's ability to induce apoptosis and inhibit proliferation and migration in lung cancer cell lines. The MWCNTs-FA/Gemcitabine formulation showed higher potency in p53-null H1299 cells, with significant upregulation of pro-apoptotic genes and downregulation of anti-apoptotic genes. These results suggest that the formulation not only enhances drug delivery but also exerts sustained anticancer effects, thereby improving therapeutic outcomes compared to free gemcitabine.

## 4. CONCLUSION

The MWCNTs-FA/Gem formulation represents a promising nanocarrier system for targeted lung cancer therapy. Stability studies highlight the need for cool, dark storage conditions to preserve formulation integrity, while cytotoxicity and biocompatibility assessments demonstrate its potential for safe and effective treatment. The enhanced anticancer efficacy of MWCNTs-FA/Gem, characterized by improved cytotoxicity, apoptosis induction, and inhibition of proliferation and migration, underscores its superiority over free gemcitabine. These findings pave the way for further preclinical and clinical investigations into the use of MWCNTs-FA/Gem as a targeted drug delivery platform, offering new hope for the treatment of lung cancer and other malignancies.

**Conflict of interest-** The authors have no conflicts of interest regarding this investigation.

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## REFERENCES

[1] E. V. Emeihe, E. I. Nwankwo, M. D. Ajegbile, J. A. Olaboye, and C. C. Maha, “Revolutionizing drug delivery systems: Nanotechnology-based approaches for targeted therapy,” Int. J. Life Sci. Res. Arch., vol. 7, no. 1, pp. 40–58, 2024.

[2] N. Gupta, S. M. Gupta, and S. K. Sharma, “Carbon nanotubes: Synthesis, properties and engineering applications,” Carbon Lett., vol. 29, pp. 419–447, 2019.

[3] S. Rathinavel, K. Priyadharshini, and D. Panda, “A review on carbon nanotube: An overview of synthesis, properties, functionalization, characterization, and the application,” Mater. Sci. Eng. B, vol. 268, p. 115095, 2021.

[4] E. I. Bîru and H. Iovu, “Graphene nanocomposites studied by Raman spectroscopy,” Raman Spectrosc, vol. 9, p. 179, 2018.

[5] D. Cui, “Advances and prospects on biomolecules functionalized carbon nanotubes,” J. Nanosci. Nanotechnol., vol. 7, no. 4–5, pp. 1298–1314, 2007.

[6] S. Merum, J. B. Veluru, and R. Seeram, “Functionalized carbon nanotubes in bio-world: Applications, limitations and future directions,” Mater. Sci. Eng. B, vol. 223, pp. 43–63, 2017.

[7] G. B. Ramesh, P. Singh, and K. Biswas, “Potentialities of Bio-functionalized Carbon Nanotubes for Different Anti-cancerous Activities,” J. Inorg. Organomet. Polym. Mater., pp. 1–26, 2024.

[8] R. Jha, A. Singh, P. K. Sharma, and N. K. Fuloria, “Smart carbon nanotubes for drug delivery system: A comprehensive study,” J. Drug Deliv. Sci. Technol., vol. 58, p. 101811, 2020.

[9] C. Pucci, C. Martinelli, and G. Ciofani, “Innovative approaches for cancer treatment: Current perspectives and new challenges,” Ecancermedicalscience, vol. 13, 2019.

[10] E. Moysan, G. Bastiat, and J.-P. Benoit, “Gemcitabine versus modified gemcitabine: a review of several promising chemical modifications,” Mol. Pharm., vol. 10, no. 2, pp. 430–444, 2013.

[11] H. Han et al., “Emerging pro-drug and nano-drug strategies for gemcitabine-based cancer therapy,” Asian J.

Pharm. Sci., vol. 17, no. 1, pp. 35–52, 2022.

[12] C. Manegold, "Gemcitabine (Gemzar®) in non-small cell lung cancer," Expert Rev. Anticancer Ther., vol. 4, no. 3, pp. 345–360, 2004.

[13] M. Estanqueiro, M. H. Amaral, J. Conceição, and J. M. S. Lobo, "Nanotechnological carriers for cancer chemotherapy: the state of the art," Colloids surfaces B Biointerfaces, vol. 126, pp. 631–648, 2015.

[14] C. K. Thakur et al., "Ligand-conjugated multiwalled carbon nanotubes for cancer targeted drug delivery," Front. Pharmacol., vol. 15, 2024, [Online]. Available: https://www.frontiersin.org/journals/pharmacology/articles/10.3389/fphar.2024.1417399

[15] K. Halwai, S. Khanna, G. Gupta, S. Wahab, M. Khalid, and P. Kesharwani, "Folate-conjugated carbon nanotubes as a promising therapeutic approach for targeted cancer therapy," J. Drug Target., pp. 1–16, 2024.

[16] J. Soleymani, M. Hasanzadeh, M. H. Somi, and A. Jouyban, "The role of nanomaterials on the cancer cells sensing based on folate receptor: analytical approach," TrAC Trends Anal. Chem., vol. 125, p. 115834, 2020.

[17] Y. Lu, E. Sega, C. P. Leamon, and P. S. Low, "Folate receptor-targeted immunotherapy of cancer: mechanism and therapeutic potential," Adv. Drug Deliv. Rev., vol. 56, no. 8, pp. 1161–1176, 2004.

[18] R. Krishnamurthy and M. C. Manning, "The stability factor: importance in formulation development," Curr. Pharm. Biotechnol., vol. 3, no. 4, pp. 361–371, 2002.

[19] D. Zhou, "Understanding physicochemical properties for pharmaceutical product development and manufacturing II: physical and chemical stability and excipient compatibility," J. Valid. Technol., vol. 15, no. 3, p. 36, 2009.

[20] M. Davoren et al., "In vitro toxicity evaluation of single walled carbon nanotubes on human A549 lung cells," Toxicol. Vitr., vol. 21, no. 3, pp. 438–448, 2007, doi: https://doi.org/10.1016/j.tiv.2006.10.007.

[21] G. D. Souto, Z. Farhane, A. Casey, E. Efeoglu, J. McIntyre, and H. J. Byrne, "Evaluation of cytotoxicity profile and intracellular localisation of doxorubicin-loaded chitosan nanoparticles," Anal. Bioanal. Chem., vol. 408, pp. 5443–5455, 2016.

[22] K. M. Yap et al., "Drug delivery of natural products through nanocarriers for effective breast cancer therapy: A comprehensive review of literature," Int. J. Nanomedicine, pp. 7891–7941, 2021.

[23] K. C. Waterman and R. C. Adami, "Accelerated aging: prediction of chemical stability of pharmaceuticals," Int. J. Pharm., vol. 293, no. 1–2, pp. 101–125, 2005.

[24] A. Khan, A. Tripathi, M. Gandhi, J. Bellare, and R. Srivastava, "Development of injectable upconversion nanoparticle-conjugated doxorubicin theranostics electrospun nanostructure for targeted photochemotherapy in breast cancer," J. Biomed. Mater. Res. Part A, 2024.