

Masked Therapeutic Activity of *Tridax* in Silk Fibroin Nanoparticles: A Critical Evaluation of SFNPs as Drug Carriers

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ABSTRACT:

Targeted drug delivery systems have gained significant attention for improving therapeutic specificity and minimizing systemic toxicity in cancer treatment. In the present study, silk-based nanoparticles were developed as carrier systems for the targeted delivery of and *Tridax procumbens* leaf extract to breast cancer cells in a murine model. Epigallocatechin gallate (EGCG) was conjugated to the nanoparticle surface to function as a targeting ligand, with the aim of enhancing selective interaction with malignant cells. Drug-loaded nanoparticles were prepared using a co-precipitation technique to ensure uniform encapsulation and stability. The therapeutic efficacy of the formulated nanocarriers was evaluated through in vivo assessment of cancerous cells. However, experimental findings indicated no significant inhibition of cell growth. These results suggest that, under the conditions employed in this study, the silk nanoparticle-based delivery system did not enhance the anticancer activity of *Tridax procumbens* extract against breast cancer cells in mice. The findings highlight the need for further optimization of nanoparticle functionalization, drug loading strategies, and ligand conjugation approaches to improve targeted delivery outcomes in breast cancer therapy.

INTRODUCTION:

Nanoparticles have emerged as highly effective drug delivery systems due to their ability to protect therapeutic agents from degradation, enhance biological stability, and improve tissue-specific targeting.^[5] They also contribute to increased bioavailability, prolonged circulation time, and efficient intracellular uptake. Furthermore, nanoparticle-based systems can reduce systemic toxicity and overall treatment costs. Over the past few decades, a wide range of nanoparticle platforms has been developed using materials such as liposomes, ceramics, carbon-based structures, metals, micelles, dendrimers, and polymers.^[2]

Among these, biodegradable polymeric nanoparticles are particularly advantageous because of their excellent biocompatibility, high drug-loading efficiency, and capacity for controlled and sustained drug release.^[1] These systems can be synthesized using synthetic polymers such as poly(lactic acid) (PLA), poly(ϵ -caprolactone) (PCL), and poly(glycolic acid) (PGA), as well as natural polymers including polysaccharides (e.g., cellulose, chitosan, hyaluronic acid, alginate, dextran, and starch) and proteins such as collagen, gelatine, elastin, albumin, and silk fibroin.^[12]

Protein-based nanoparticles have gained increasing attention owing to their biodegradability, minimal immunogenicity, and excellent compatibility with biological systems. The presence of diverse functional groups on protein surfaces further enables chemical modifications, including the covalent attachment of drugs and targeting ligands, thereby improving therapeutic specificity and delivery efficiency.^[6]

Silk fibroin, a structural protein primarily derived from the cocoon of *Bombyx mori*, has emerged as a promising natural biomaterial due to its biocompatibility, tuneable mechanical properties, and environmentally favourable degradation profile.^[2] Structurally, silk fibroin consists of large, repetitive hydrophobic domains interrupted by short, non-repetitive hydrophilic segments. Its primary composition—dominated by glycine (~43%), alanine (~30%), and serine (~12%)—contributes to its unique structural stability and physicochemical behaviour. The short hydrophilic linkers between hydrophobic regions promote a highly ordered molecular arrangement.^[10]

Owing to these characteristics, silk fibroin has been extensively explored for various biomedical applications. It can be processed into multiple material formats, including films, hydrogels, three-dimensional scaffolds, electro spun fibres, and nanoparticles. In nanoparticulate form, silk fibroin has demonstrated potential in drug delivery, antimicrobial applications, tissue engineering, and biosensing.^[2]

Despite these advantages, certain challenges limit the broader application of nanoparticles. One major issue is their tendency to aggregate, which can reduce their functional efficiency and compromise nanoscale properties. Additionally, their small size and high surface area may result in unintended interactions with biological systems, raising concerns about cytotoxicity and long-term safety.^[11]

To overcome these limitations, embedding nanoparticles within polymer matrices has been proposed as an effective strategy to enhance stability and reduce toxicity. Such polymer–nanoparticle composites can be engineered to achieve desired physicochemical and biological properties.^[10] In this context, silk fibroin-based nanocomposites have shown excellent interfacial compatibility along with improved structural integrity, mechanical performance, and biological response.^[10]

Recent studies have highlighted the potential of fibroin-based nanocarriers in targeted drug delivery. For instance, fibroin–folate nanoparticles have been reported to be non-toxic, efficiently internalized by cells, and capable of sustained drug release. Similarly, silk fibroin nanoparticles loaded with chemotherapeutic agents such as doxorubicin have demonstrated enhanced targeting efficiency toward cancer cells, underscoring their promise in oncology.^[3]

A drug delivery system typically involves a carrier matrix in which the active pharmaceutical ingredient is dissolved, encapsulated, dispersed, or surface-bound. The selection of an appropriate carrier material is critical, as it influences drug stability, release kinetics, and therapeutic

effectiveness. Among various delivery platforms, nanoparticles have attracted significant interest due to their superior performance and versatility.^[8]

At the nanoscale, silk fibroin nanoparticles (SFNPs) are particularly promising for anticancer drug delivery.^[8] & ^[7] Their small size, combined with inherent biocompatibility and biodegradability, enables improved permeability, prolonged systemic circulation, and controlled drug release. Surface functionalization, such as the incorporation of tumour-targeting peptides (e.g., cRGD), can further enhance selective uptake by cancer cells while minimizing off-target effects.^[7]

Recent investigations have also demonstrated the capability of SFNPs to encapsulate poorly soluble anticancer drugs. For example, formulations containing auranofin have shown sustained drug release over extended periods and significant activity against three-dimensional cancer spheroid models.^[8] Additionally, SFNPs exhibit favourable physicochemical stability in biological environments and improved pharmacokinetic behaviour, making them strong candidates for advanced drug delivery applications.^[10]

Beyond drug delivery, silk fibroin has also contributed to regenerative medicine. For instance, electro spun fibroin–gelatine matrices have been explored for corneal tissue engineering, demonstrating suitable optical transparency, mechanical strength, and biocompatibility.^[1]

In the present study, silk fibroin nanoparticles were utilized as carriers for *Tridax procumbens* ^[9] to evaluate their potential in anticancer therapy and targeted drug delivery. Although *Tridax* is known for its anticancer properties, particularly at higher concentrations; the study highlights a reduction or masking of its biological activity when encapsulated within SFNPs. This finding raises important concerns regarding the effectiveness of silk fibroin nanoparticles as delivery vehicles for certain bioactive compounds and highlights the need for further investigation into their interaction mechanisms and optimization for targeted therapy.

MATERIALS AND METHODOLOGY:

A) Preparation of *Tridax* leaf extract:

The leaves of *Tridax Procumbens* were collected and washed thoroughly. The leaves were then dried and grinded into a fine powder. This powder was soaked in methanol for 24 to 48 hrs. and this mixture was separated using a Soxhlet apparatus. The prepared solution was filtered using Whatman filter paper.^[9]

B) Preparation of SFNPs:

Silk fibroin nanoparticles (SFNPs) were synthesized and characterized according to previously established protocols. In brief, silk fibroin (SF) was dissolved in an appropriate solvent system,

followed by dialysis to remove residual salts and low-molecular-weight impurities. The centrifuged silk solution was mixed with the *Tridax Procumbens* in separate tubes. These both solutions were co-precipitated with acetone. And the tubes were further centrifuged at 5000rpm for 30 mins. This was followed by two sonication cycles and vortexing the solution for about 1

min. The solution was reacted with the EGCG solution which was extracted from the green tea and hence the nanoparticles are synthesized. The synthesized SFNPs were employed in the following experimental applications:

C) Anti- Cancerous Assay:

MBA-MB231 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) with Non-Essential Amino Acids (NEAA), supplemented with 10% (v/v) fetal bovine serum (FBS). Cells were maintained at 37°C in a 5% CO₂ incubator, and the complete medium was replaced every 2 to 3 days. Cells were seeded in 96-well plates at a density of 100,000 cells per well (100 µL/well). Plates were incubated at 37°C in a 5% CO₂ atmosphere for 24 hours to allow cell attachment and growth. After 24 hours, cells were observed for a half-confluent monolayer under an inverted microscope. The culture medium was carefully removed, and cells were treated with 6 different concentrations of the test item i.e. 1,5,10,50,100,200 (µg/ml) with each concentration assayed in triplicate. Control wells contained cells in culture medium without any test item, incubated under the same conditions. Negative control contains the DMSO only. Plates were incubated for another 24 hours at 37°C in 5% CO₂. After 24 hours, cells were examined under an inverted microscope for any morphological changes or signs of cell death. The culture medium was carefully removed. 100 µL of fresh medium was added to each well, followed by 10 µL of MTT reagent. Plates were incubated for 4 hours at 37°C in a 5% CO₂ incubator. Formazan Solubilization & Absorbance Measurement. Cell viability (%) was calculated relative to the untreated control (100%). Statistical analysis was performed using Student's unpaired t-test to compare control and treatment groups at each concentration. The half-maximal inhibitory concentration (IC₅₀) value was determined from the concentration– response curve. IC₅₀ values were determined by plotting a logarithmic graph of test item concentration vs. % cell survival. Percentage Cell Survival was calculated using the formula:

$$\text{Parent Cell Survival (\%)} = \frac{\text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

RESULTS AND DISCUSSION:

Anti-Cancerous: The cytotoxic potential of the synthesized nanoparticle formulations was evaluated using the MTT assay against the MDA-MB-231 human breast cancer cell line. The study included nanoparticles loaded with *Tridax procumbens* extract, along with its control, to assess and compare its anticancer efficacy.

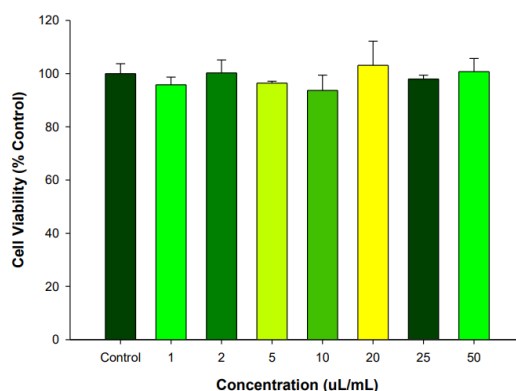
1. Sample N (Nanoparticles – *Tridax* loaded):

The cytotoxic effect of Sample N-1 was evaluated over a concentration range of 1–50 µg/ml. The results demonstrated that cell viability remained consistently high across all tested concentrations, ranging approximately between 93% and 103%. At lower concentrations (1–10 µg/ml), the viability values were close to control levels, indicating negligible cytotoxicity. Even at intermediate concentrations such as 20 and 25 µg/ml, the cells showed no significant reduction in viability, and in some cases, values exceeded 100%, suggesting enhanced metabolic activity rather

than cell death. At the highest tested concentration (50 $\mu\text{g/ml}$), the viability still remained around ~98%, confirming the absence of cytotoxic effects. Statistical analysis further supported these observations, as all p-values were greater than 0.05, indicating no significant difference from control. Importantly, the IC_{50} value was not achieved within the tested range ($\text{IC}_{50} > 50 \mu\text{g/ml}$), confirming that the formulation exhibits very low or negligible anticancer activity.

Concentration ($\mu\text{g/ml}$)	Absorbance			Average	% Cell Survival			Mean % $\pm$ SD
	R1	R2	R3		R1	R2	R3	
Control	0.49	0.53	0.56	0.53 ± 0.04	93.13	100.95	105.92	100.00 ± 6.45
1	0.53	0.56	0.48	0.50 ± 0.03	101.34	94.47	91.60	95.80 ± 5.01
2	0.56	0.50	0.58	0.53 ± 0.04	96.37	94.47	109.92	100.25 ± 8.43
5	0.51	0.50	0.51	0.51 ± 0.01	96.95	95.04	97.33	96.44 ± 1.23
10	0.54	0.49	0.44	0.49 ± 0.05	103.63	93.70	83.78	93.70 ± 9.93
20	0.46	0.54	0.62	0.54 ± 0.08	87.60	102.67	119.08	103.12 ± 15.74
25	0.53	0.52	0.50	0.52 ± 0.02	100.19	98.47	95.23	97.96 ± 2.52
50	0.50	0.51	0.58	0.53 ± 0.04	94.47	97.33	110.50	100.77 ± 8.55
Log IC_{50} value	> 1.70							
IC_{50} value	$> 50 \mu\text{g/ml}$							

Table 2. Effect of Sample ID: N-1 on Cell Viability in MDA-MB-231 Cells (MTT Assay, 24 h)



2. Sample C (Control for Tridax)

Sample C-1 showed a markedly different and significant cytotoxic profile. At lower concentrations (1–10 $\mu\text{g/ml}$), cell viability remained high (~98–117%), indicating no toxicity initially. However, beyond 20 $\mu\text{g/ml}$, a sharp decline in cell viability was observed:

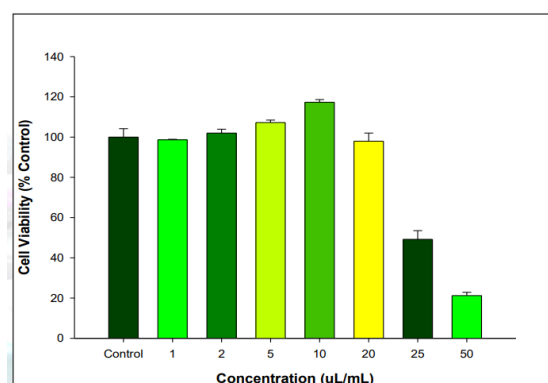
At 25 $\mu\text{g/ml}$ → ~49% viability (significant)

At 50 $\mu\text{g/ml}$ → ~21% viability (highly significant)

This clearly indicates a strong dose-dependent cytotoxic effect. Statistical analysis confirmed significance ($p < 0.05$ and $p < 0.01$). The IC_{50} value was calculated as $24.86 \mu\text{g/ml}$, placing this sample in the category of moderately cytotoxic compounds. This suggests that the active component in this control sample is highly effective in inhibiting cancer cell growth when not encapsulated.

Concentration ($\mu\text{g/ml}$)	Absorbance			Average	% Cell Survival			Mean % $\pm$ SD
	R1	R2	R3		R1	R2	R3	
Control	0.57	0.50	0.55	0.54 ± 0.04	105.38	91.84	102.78	100.00 ± 6.90
1	0.53	0.53	0.53	0.53 ± 0.00	99.07	98.14	98.70	98.64 ± 0.46
2	0.54	0.57	0.57	0.55 ± 0.02	100.74	105.75	99.44	101.98 ± 3.25
5	0.57	0.58	0.58	0.58 ± 0.01	105.38	106.86	109.46	107.23 ± 2.11
10	0.63	0.65	0.62	0.63 ± 0.02	117.07	119.67	115.21	117.32 ± 2.23
20	0.49	0.52	0.57	0.53 ± 0.04	91.65	96.85	105.38	97.96 ± 6.83
25	0.30	0.22	0.27	0.26 ± 0.04	56.03	41.19	50.28	49.17 ± 7.46
50	0.12	0.10	0.13	0.12 ± 0.02	21.52	18.18	23.93	21.21 ± 2.88
Log IC_{50} value	1.3955							
IC_{50} value	$24.86 \mu\text{g/ml}$							

Table 4. Effect of Sample ID: C-1 on Cell Viability in MDA-MB-231 Cells (MTT Assay, 24 h)



COMPARATIVE DISCUSSION: A comparison of all two samples reveals clear differences in cytotoxic behaviour between nanoparticle-loaded systems and their respective controls.

***Tridax* Nanoparticles (N-1) vs Control (C-1)-**

Interpretation: Encapsulation of *Tridax* extract into nanoparticles significantly reduces its cytotoxic activity. This may be due to:

- Controlled/slow release of active compounds
- Reduced direct interaction with cells
- Limited cellular uptake
- Masking of the *Tridax*'s anti-cancerous activity by the SFNPs.

Thus, while free extract (C-1) shows moderate anticancer activity, nanoparticle formulation (N-1) masks its effect and reduces efficacy.

N-1 (<i>Tridax</i> NP):	C-1 (Control):
Very high cell viability (~95–103%)	Strong dose-dependent cytotoxicity
No cytotoxicity	IC ₅₀ = 24.86 µg/ml
IC ₅₀ > 50 µg/mL	Significant cell death at higher concentrations

CONCLUSION:

The SFNP formulations exhibited negligible cytotoxicity against MDA-MB-231 cells under the tested conditions. However, despite the known bioactivity of *Tridax procumbens*, its incorporation within the SFNP system did not translate into significant anticancer activity. This suggests a possible masking or attenuation of the intrinsic therapeutic effects of *Tridax* when encapsulated within the fibroin matrix. Such an outcome indicates that the nanoparticle system may be limiting the effective release, bioavailability, or interaction of the active phytoconstituents with target cells. This observation raises critical questions regarding the efficiency of SFNPs as targeted drug delivery carriers, particularly in the context of phytochemical-loaded systems. Collectively, these results highlight that therapeutic efficacy of the SFNPs requires considerate optimization including drug loading efficiency, release kinetics, and surface modification strategies.

REFERENCES:

- 1) Ahangar, P., Cooke, M. E., Weber, M. H., & Rosenzweig, D. H. (2021). Current biomedical applications of silk fibroin. *International Journal of Molecular Sciences*, 22(3), 1499.
- 2) Altman, G. H., Diaz, F., Jakuba, C., Calabro, T., Horan, R. L., Chen, J., ... & Kaplan, D. L. (2003). Silk-based biomaterials. *Biomaterials*, 24(3), 401–416.
- 3) Bano Subia, Sourov Chandra, Sarmistha Talukdar, Subhas C. Kundu, Folate conjugated silk fibroin nanocarriers for targeted drug delivery, *Integrative Biology*, Volume 6, Issue 2, February 2014, Pages 203-214
- 4) Das, S., Chatterjee, K., & Naskar, D. (2025). An alcohol-addition–freezing method to modulate pore size and stiffness of silk fibroin scaffolds. *Journal of Materials Chemistry B*, 13, 580–593.

- 5) Gopal, S., & Dutta, P. (2024). Silk fibroin nanoparticles as emerging drug delivery vehicles. *Colloids and Surfaces B: Biointerfaces*, 237, 113445.
- 6) Mottaghitalab, F., Farokhi, M., Shokrgozar, M. A., Atyabi, F., & Hosseinkhani, H. (2015). Silk fibroin nanoparticles as a novel drug delivery platform. *Journal of Controlled Release*, 206, 161–176.
- 7) Qin, Z., Wang, X., & Liu, X. (2023). Anti-CEA nanobody modified silk fibroin nanoparticles for active colorectal cancer targeting. *ACS Biomaterials Science & Engineering*, 9(3), 1549–1562.
- 8) Reddy, N., Jiang, Q., Yang, Y., & Liu, Y. (2024). Silk fibroin nanoparticles in cancer drug delivery: A review. *Cancers*, 15(6), 1725.
- 9) Sujitha, R., & Sharmila, R. (2018). Phytochemical analysis and in vitro anticancer activity of *Tridax procumbens* Linn. *World J Pharmaceut Res*, 7, 867-878.
- 10) Shao, Z., Vollrath, F., & Yang, Y. (2022). Physicochemical properties of silk fibroin: A multifunctional biopolymer. *Frontiers in Chemical Engineering*, 4, 1044431.
- 11) Sun, W., Zheng, J., & Xu, L. (2025). Stability and release kinetics of silk fibroin nanoparticles for therapeutic delivery. *Colloids and Surfaces B: Biointerfaces*, 228, 113372.
- 12) Zhao, Z., Li, Y., & Xie, M.-B. (2015). Silk Fibroin-Based Nanoparticles for Drug Delivery. *International Journal of Molecular Sciences*, 16(3), 4880-4903.