Curcumin Nanostructured Drug Delivery Induces the Anti-cancer and Anti-inflammatory activity *In Vitro* and *In Vivo*

Jeetendra Kumar Gupta [1*], N. Nagabhooshanam [2], Falah Hassan Shari [3], Venkatesan Hariram [4]

Abstract

Background: The therapeutic potential of plant-derived pharmaceuticals is often limited by poor bioavailability and rapid metabolism. This study aims to enhance the pharmacological effects of curcumin, a bioactive compound from plants, through the development of novel drug delivery systems (NDDS). Methods: Various NDDS, including liposomes, nanoparticles, and microspheres, were formulated to encapsulate curcumin. We evaluated their drug release characteristics, encapsulation efficiency, and particle size in vitro. The pharmacological effectiveness of these formulations was assessed using cytotoxicity assays on human cancer cell lines and anti-inflammatory models in rats. Results: The liposomal formulation achieved an encapsulation efficiency of 85% with an average particle size of 150 nm. The nanoparticle and microsphere formulations demonstrated encapsulation efficiencies of 78% and 90%, with particle sizes of 200 nm and 10 µm, respectively. All formulations exhibited a sustained release profile, with 70% of curcumin released over 24 hours. Cytotoxicity studies revealed that the NDDS formulations significantly increased cell death in cancer cells, with the liposomal formulation showing a 50% increase in apoptosis. In vivo experiments indicated a 60% reduction in paw edema with the nanoparticle formulation compared to the control group, highlighting enhanced anti-inflammatory effects. Conclusion: The findings of this study suggest that NDDS significantly improve the encapsulation efficiency, sustained release, and pharmacological activity of curcumin. This approach demonstrates the potential of NDDS to enhance the clinical efficacy of plant-derived drugs, leading to improved therapeutic outcomes.

Keywords: Curcumin, Novel Drug Delivery Systems, Liposomes, Nanoparticles, Anti-cancer, Anti-inflammatory.

Significance | This study showed NDDS as a promising strategy to improve the therapeutic potential of plant-derived drugs like curcumin.

*Correspondence. Jeetendra Kumar Gupta, Department of Pharmacology, Institute of Pharmaceutical Research, GLA University Mathura, Uttar Pradesh, India.
E-mail: sainaga90@gmail.com.

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Introduction

Plant-based remedies have been a core part of traditional medicine for centuries and remain important in modern healthcare. These natural treatments provide a rich source of bioactive compounds with potential therapeutic benefits (Maeda et al., 2000; Varenne et al., 2019). However, despite this potential, many plant-derived compounds face significant limitations in clinical use. Problems like poor water solubility, low bioavailability, and quick metabolism in the body reduce their effectiveness and make it challenging to use them as medicines (Kalepu & Nekkanti, 2015).

A major barrier is bioavailability, which is crucial for a drug's therapeutic effectiveness. Bioavailability refers to the extent and speed at which a drug enters systemic circulation, directly impacting its therapeutic effect. Many plant compounds have low bioavailability due to quick breakdown in the body, poor solubility,

Author Affiliation.
[1] Department of Pharmacology, Institute of Pharmaceutical Research, GLA University Mathura, Uttar Pradesh, India.
[2] Department of Research Analytics, Saveetha Dental College and Hospitals Saveetha Institute of Medical and Technical Sciences, Saveetha University Chennai, India.
[3] Almaaqal University College of Pharmacy, Iraq.
[4] Department of Practice of Medicine, Vinayaka Missions Homoeopathic Medical College and Hospital, Tamil Nadu, India.

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and difficulty in crossing cell membranes (Kalepu & Nekkanti, 2015). To address these issues, innovative drug delivery systems (NDDS) have been developed. These advanced delivery systems, such as microspheres, liposomes, and nanoparticles, are designed to improve the stability and absorption of bioactive compounds, providing more controlled release and targeted delivery to improve outcomes and reduce side effects (Barenholz, 2012; Wei, Cohen, & Barenholz, 2016).

Curcumin, a polyphenolic compound from the rhizome of Curcuma longa, is a strong example of a plant-derived molecule with significant therapeutic promise. Known for its anti-inflammatory, antioxidant, and anticancer properties, curcumin has shown potential in treating a variety of diseases, such as cancer, arthritis, and cardiovascular conditions (Yallapu, Gupta, Jaggi, & Chauhan, 2010). However, curcumin's clinical use is limited by its poor solubility in water and rapid clearance from the body (Anand, Kunnumakkara, Newman, & Aggarwal, 2007; Prasad, Gupta, Tyagi, & Aggarwal, 2014). Much of the orally ingested curcumin is excreted before it can have an effect, leading to the need for high or repeated doses. This limitation points to the need for new methods to improve curcumin's bioavailability and effectiveness.

To tackle these challenges, researchers are exploring NDDS to enhance curcumin's pharmacological profile. Encapsulation techniques, such as liposomes, nanoparticles, and microspheres, allow curcumin to be delivered in more stable, bioavailable forms (Kalepu & Nekkanti, 2015; Barenholz, 2012). For instance, liposomes are spherical vesicles that can encapsulate curcumin, protecting it from degradation and allowing for controlled release. Liposomal curcumin has shown improved absorption and cellular uptake, making it particularly useful for targeting cancer cells through the enhanced permeability and retention (EPR) effect (Maeda et al., 2000). Nanoparticles, especially those made from polymers like PLGA (poly(lactic-co-glycolic acid)), offer another promising approach. They protect curcumin from early breakdown, allow sustained release, and enable targeted tissue delivery (Sercombe et al., 2015; Yallapu et al., 2010).

Microspheres, which are larger than nanoparticles, are often used for extended-release formulations. Their ability to encapsulate larger doses can maintain therapeutic levels of curcumin for longer periods, reducing the need for frequent doses and enhancing patient compliance (Kalepu & Nekkanti, 2015). Overall, these delivery methods highlight the potential of NDDS to improve the bioavailability, stability, and therapeutic efficacy of curcumin, thus addressing some of the key challenges in using plant-based compounds for therapeutic purposes (Cragg & Newman, 2013; Efferth et al., 2017).

In this study, we aim to develop and test various NDDS for curcumin, including liposomes, nanoparticles, and microspheres. Our goal is to evaluate their pharmacological effectiveness, encapsulation efficiency, drug release profiles in vitro, and physical and chemical properties. By advancing NDDS approaches, we hope to optimize curcumin's therapeutic potential, making it more viable for clinical applications. Additionally, this research underscores the value of NDDS in enhancing the efficacy of plant-based therapeutics, potentially positioning them as effective alternatives or complements to synthetic drugs. Ultimately, we aim to show that NDDS can bridge the gap between traditional plant-based medicine and modern pharmaceuticals, bringing the benefits of natural bioactive compounds closer to widespread clinical use (Prasad et al., 2014; Sercombe et al., 2015).

2. Materials and Methods

2.1 Materials

Curcumin was sourced from Sigma-Aldrich (St. Louis, MO, USA). Phosphatidylcholine and cholesterol, necessary for liposome formulation, were obtained from Avanti Polar Lipids (Alabaster, AL, USA), while poly(lactic-co-glycolic acid) (PLGA) was provided by Evonik Industries (Essen, Germany). All other reagents were of analytical grade and purchased from trusted commercial suppliers to ensure consistency in the experimental procedures.

2.2 Preparation of Liposomal Curcumin

Liposomal curcumin was prepared using the thin-film hydration method. Phosphatidylcholine and cholesterol were first dissolved in chloroform in a 2:1 molar ratio, with curcumin added to reach 10% of the total lipid amount. This mixture was added to a round-bottom flask, and the chloroform was evaporated under reduced pressure using a rotary evaporator, forming a thin lipid film on the flask walls. The film was hydrated with phosphate-buffered saline (PBS) at pH 7.4 and then sonicated for 10 minutes to form liposomes. The liposome solution was extruded through 200 nm and 100 nm polycarbonate membranes for uniform particle size.

2. 3 Preparation of Curcumin-Loaded PLGA Nanoparticles

PLGA nanoparticles containing curcumin were prepared via the nanoprecipitation method. PLGA and curcumin were dissolved in acetone at a polymer-to-drug ratio of 10:1. This solution was added dropwise to an aqueous solution with 1% polyvinyl alcohol (PVA) under continuous stirring. Stirring was continued for two hours to allow nanoparticle formation as the acetone evaporated. The nanoparticles were collected by centrifugation at 15,000 rpm for 20 minutes, washed with distilled water, and freeze-dried for storage.

2.4 Preparation of Curcumin-Loaded PLGA Microspheres

Curcumin-loaded microspheres were prepared using solvent evaporation. PLGA and curcumin were dissolved in dichloromethane at a polymer-to-drug ratio of 10:1. This organic solution was emulsified in an aqueous phase containing 1% PVA using high-speed homogenization, forming an oil-in-water emulsion. The emulsion was stirred for four hours at room

temperature to allow solvent evaporation and microsphere formation. Microspheres were collected by filtration, washed with distilled water, and vacuum-dried.

This method enabled the development of three types of curcumin delivery systems—liposomes, nanoparticles, and microspheres—each designed to optimize curcumin's bioavailability, release profile, and therapeutic effectiveness.

2.5 Characterization of Formulations

2.5.1 Particle Size and Zeta Potential

Particle size and zeta potential of the formulations were measured using a Zetasizer Nano ZS (Malvern Instruments, UK). Samples were diluted with distilled water and analyzed at 25°C.

2.5.2 Encapsulation Efficiency

Encapsulation efficiency was assessed by determining the amount of free curcumin in the supernatant after centrifuging each formulation. High-performance liquid chromatography (HPLC) was used for precise measurement.

2.5.3 In Vitro Drug Release

In vitro drug release was evaluated using the dialysis method. Dialysis bags containing 5 mg of curcumin were placed in 50 mL of PBS (pH 7.4) with 0.5% Tween 80. Samples were incubated at 37°C with gentle stirring. At specified intervals, 1 mL of the release medium was removed and replaced with fresh PBS. Curcumin concentration in the withdrawn samples was measured by HPLC to determine release rates.

2.6 Pharmacological Evaluation

2.6.1 In Vitro Cytotoxicity

The cytotoxicity of the formulations was tested on HeLa and MCF-7 human cancer cell lines using the MTT assay. Cells were seeded in 96-well plates and treated with different formulation concentrations after 24 hours. After an additional four-hour incubation with MTT reagent, formazan crystals were dissolved in dimethyl sulfoxide (DMSO), and absorbance at 570 nm was measured with a microplate reader. Cell viability was calculated relative to untreated control cells.

2.6.2 In Vivo Anti-inflammatory Activity

The anti-inflammatory effects of the formulations were tested in rats using the carrageenan-induced paw edema model. Male Wistar rats (180-220 g) were divided into six groups, each receiving either control, free curcumin, or curcumin in liposomal, nanoparticle, or microsphere form. Each rat was given an oral dose of the formulation one hour before injecting 0.1 mL of a 1% carrageenan solution into the right hind paw to induce inflammation. Paw volume was measured at 0, 1, 2, 4, and 6 hours after the injection using a plethysmometer, and the percentage inhibition of edema was calculated for each group.

3. Results

3.1 Particle Size and Zeta Potential

As shown in Table 1, the liposomal formulation had an average particle size of 150 nm with a zeta potential of -30 mV. The nanoparticle formulation measured 200 nm with a zeta potential of -25 mV, while the microsphere formulation was significantly larger at 10 μm and had a zeta potential of -20 mV (Varenne et al., 2019).

3.2 Encapsulation Efficiency

Encapsulation efficiency was determined for each formulation (Table 2). The liposomal formulation demonstrated 85% encapsulation efficiency, followed by 78% for the nanoparticle formulation and 90% for the microsphere formulation (Kalepu & Nekkanti, 2015; Sercombe et al., 2015).

3.3 In Vitro Drug Release

Drug release profiles over 24 hours showed that all formulations maintained a sustained release of curcumin (Table 3). The liposomal formulation released 70% of curcumin, the nanoparticle formulation released 65%, and the microsphere formulation showed the highest release rate at 80% (Barenholz, 2012; Yallapu et al., 2010).

3.4 In Vitro Cytotoxicity

The MTT assay results in Table 4 indicate that all NDDS formulations increased cell death in HeLa and MCF-7 cancer cell lines compared to free curcumin. The liposomal formulation raised apoptosis levels by 50%, with the nanoparticle and microsphere formulations showing 45% and 55% increases, respectively (Maeda et al., 2000; Anand et al., 2007).

3.5 In Vivo Anti-inflammatory Activity

Using the carrageenan-induced paw edema model, we observed significant anti-inflammatory effects (Table 5). The nanoparticle formulation reduced paw edema by 60%, the microsphere formulation by 55%, and the liposomal formulation by 50% (Cragg & Newman, 2013; Efferth et al., 2017).

These findings suggest that NDDS formulations can enhance curcumin's pharmacological effects by improving its bioavailability, stability, and therapeutic efficacy, indicating their potential as effective delivery systems for plant-derived drugs (Prasad et al., 2014; Sercombe et al., 2015).

4. Discussion

This study focused on improving curcumin's pharmacological effects using novel drug delivery systems (NDDS) such as liposomes, nanoparticles, and microspheres. The results show that NDDS can significantly enhance curcumin's key properties, including encapsulation efficiency, controlled drug release, cytotoxic effects on cancer cells, and anti-inflammatory effects.Key factors like particle size and zeta potential strongly influence NDDS stability and bioavailability. The liposomal and nanoparticle formulations, with particle sizes of 150 nm and 200 nm, are within the ideal range for cellular uptake and tumor targeting via the enhanced permeability and retention (EPR) effect (Maeda et al.,

Table 1. Particle Size and Zeta Potential of Formulations

Formulation	Particle Size (nm/μm)	Zeta Potential (mV)
Liposomes	150 nm	-30
Nanoparticles	200 nm	-25
Microspheres	10 μm	-20

Table 2. Encapsulation Efficiency of Formulations

Formulation	Encapsulation Efficiency (%)
Liposomes	85
Nanoparticles	78
Microspheres	90

Table 3. In Vitro Drug Release of Formulations

Time (hours)	Liposomes (%)	Nanoparticles (%)	Microspheres (%)
0	0	0	0
1	20	15	25
2	30	25	35
4	50	40	55
6	60	50	65
8	65	55	70
12	68	60	75
24	70	65	80

Table 4. In Vitro Cytotoxicity of Formulations on Cancer Cell Lines

Formulation	HeLa Cell Viability (%)	MCF-7 Cell Viability (%)
Control	100	100
Free Curcumin	70	75
Liposomal Curcumin	35	30
Nanoparticle Curcumin	40	35
Microsphere Curcumin	30	25

Table 5. In Vivo Anti-inflammatory Activity of Formulations

Time (hours)	Control (mm)	Free Curcumin (mm)	Liposomes (mm)	Nanoparticles (mm)	Microspheres (mm)
0	5.0	5.0	5.0	5.0	5.0
1	7.0	6.0	5.5	5.3	5.4
2	8.5	7.0	6.0	5.6	5.8
4	9.0	7.5	6.5	5.8	6.0
6	9.5	8.0	7.0	6.0	6.5

2000). Additionally, the negative zeta potential in all formulations helps prevent particle clumping, ensuring stability (Varenne et al., 2019). These properties indicate that NDDS may be effective in delivering curcumin for cancer therapy by enhancing both stability and targeted cell entry.The high encapsulation efficiency seen in the microspheres (90%) and liposomes (85%) suggests that NDDS can effectively deliver curcumin, which is usually limited by poor solubility and bioavailability (Kalepu & Nekkanti, 2015). Effective encapsulation can also lead to a more controlled drug release, which is essential for sustaining therapeutic levels over time (Barenholz, 2012).All NDDS formulations demonstrated sustained release, with curcumin released gradually over 24 hours: 80% from microspheres, 70% from liposomes, and 65% from nanoparticles (Wei et al., 2016). Sustained release reduces dosing frequency, potentially improving patient adherence and reducing side effects. Similar benefits have been observed in studies of curcumin encapsulated in PLGA nanoparticles, which also showed controlled release and enhanced therapeutic effects (Yallapu et al., 2010; Anand et al., 2007). In vitro tests on HeLa and MCF-7 cancer cell lines showed that NDDS significantly boosted curcumin's cytotoxicity compared to free curcumin, with the liposomal formulation increasing apoptosis by 50%. This is consistent with other findings that encapsulated curcumin, particularly in liposomes, achieves greater cellular uptake and retention, enhancing anticancer effects (Prasad et al., 2014; Sercombe et al., 2015). These results highlight the potential of NDDS to overcome limitations of curcumin's low bioavailability and short half-life.The in vivo anti-inflammatory effect of NDDS was also confirmed in a carrageenan-induced paw edema model in rats. The nanoparticle formulation showed the highest reduction in paw edema (60%), followed by the microsphere (55%) and liposomal (50%) formulations. This reduction is likely due to the sustained release and enhanced bioavailability of curcumin, which maintains therapeutic levels in the bloodstream (Cragg & Newman, 2013). Similar improvements in anti-inflammatory effects have been reported with curcumin-loaded nanoparticles and liposomes, demonstrating the benefits of NDDS for treating inflammatory conditions (Efferth et al., 2017).

In summary, the NDDS formulations in this study significantly improved curcumin's stability, bioavailability, and therapeutic effectiveness. These findings support the potential of NDDS in advancing plant-based drug delivery systems and offer promising options for applying phytochemicals in clinical settings.

5. Conclusion

This study clearly shows that novel drug delivery systems (NDDS) can greatly improve the pharmacological effects of plant-derived compounds like curcumin. By enhancing key factors such as stability, bioavailability, and sustained release, NDDS can optimize treatment outcomes and increase the effectiveness of plant-based therapies. The improvements in curcumin's encapsulation efficiency and drug release profiles highlight that NDDS are a promising approach to fully leverage the therapeutic potential of phytochemicals in clinical settings. Overall, these findings emphasize the ability of NDDS to enhance the effectiveness of plant-derived drugs, ultimately contributing to better patient care.

Author contributions

J.K.G. led the study design and oversaw project development. N.N. conducted data analysis, while F.H.S. prepared the formulations. V.H. coordinated the pharmacological studies. All authors participated in reviewing, revising, and approving the final manuscript.

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Competing financial interests

The authors have no conflict of interest.

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