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Nanoformulation of Phytochemicals to Increase Antifungal Effectiveness Against Pathogens Resistant to Drugs

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ABSTRACT

Introduction: Drug resistance, a lack of effective alternatives, and systemic toxicity are major problems when it comes to treating fungal infections with traditional antifungal medications. Because of its low solubility and bioavailability, the bioactive chemical curcumin, which possesses antifungal and anti-inflammatory characteristics, has restricted therapeutic value. The use of lipid nanoparticles shows promise as a means to improve targeted therapy, bioavailability, and medication delivery. Using the bioactive characteristics of curcumin to improve therapeutic effectiveness while reducing side effects, this study seeks to create and characterize curcumin-loaded lipid nanoparticles (CLNs) for efficient antifungal treatment.

Materials and Methods: A solvent evaporation approach was used to create CLNs, and response surface methodology (RSM) was used for optimization. Drug release in vitro, zeta potential, entrapment efficiency, particle size, and polydispersity index (PDI) were some of the characteristics measured for the formulations. Using transmission electron microscopy (TEM), morphological examination was carried out. By employing the broth microdilution technique, the antifungal activity was assessed in relation to Aspergillus niger and Candida albicans. A variety of storage conditions were tested to determine stability.

Results: A nanoscale particle size (<200 nm), low PDI (<0.3), and a high entrapment effectiveness (>85%) were observed in the improved CLN formulation. The spherical form was confirmed by TEM scans. Research on the release of drugs in vitro showed that the release lasted for at least 48 hours. Although CLNs had lower minimum inhibitory concentrations (MICs) against fungal strains, their antifungal effectiveness was much higher compared to free curcumin. The formulation remained intact for 90 days, according to stability testing.

Conclusion: Improved bioavailability, prolonged release, and higher antifungal activity were seen in curcumin-loaded lipid nanoparticles, suggesting that they may be an efficient antifungal delivery strategy. This method has the potential to reduce systemic toxicity and overcome drug resistance, making it a viable alternative to traditional antifungal treatments.

Keywords: *Lipid nanoparticles, curcumin, antifungal therapy, targeted drug delivery, fungal infections, nanotechnology.*

1. INTRODUCTION

Fungal infections are a major problem in the medical field worldwide, especially for those with weakened immune systems, such as those with HIV/AIDS, cancer, diabetes, or who are getting organ transplants. Candida albicans, Aspergillus niger, and other opportunistic fungal pathogens cause everything from minor skin infections to potentially fatal systemic mycoses. The development of fungal strains that are resistant to several treatments, as well as the low absorption and limited effectiveness of current antifungal medications, make the therapy of these infections very difficult. Conventional antifungal drugs including azoles, echinocandins, and polyenes have been overused, leading to a rise in resistance. As a result, treatment effectiveness has been reduced, and new approaches to therapy are needed [1-3].

Researchers have looked at the pharmacological effects of curcumin, a bioactive polyphenol found in turmeric (Curcuma longa), on a wide range of diseases and conditions, including infections, inflammation, cancer, and oxidative stress. Curcumin has shown promise as an antifungal in a number of investigations, particularly against Candida spp., Aspergillus spp., and Cryptococcus spp. Curcumin is effective against fungi because it causes oxidative stress in the cells of infected fungi, disrupts their cell membranes, and blocks the production of ergosterol. Due to its hydrophobic structure, low systemic bioavailability, fast degradation, and poor water solubility, curcumin encounters significant obstacles in clinical usage, although its encouraging therapeutic promise [4-6].

One potential solution to these problems is the rise of nanotechnology-based drug delivery systems, which can improve the therapeutic effectiveness of medications that are not very soluble. One such method is lipid nanoparticles. Much research has focused on lipid-based nanocarriers, such as solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs), as a means to regulate and prolong the release of hydrophobic medications. The benefits of these nanoparticles include less systemic toxicity, increased circulation time, targeted medication administration, enhanced permeability, and improved drug encapsulation efficiency. To increase curcumin's antifungal efficacy, its solubility, stability, and bioavailability can be improved by integrating it into lipid nanoparticles [6-8].

In order to treat certain fungal infections, this research will create and analyze lipid nanoparticles (CLNs) loaded with curcumin. Response surface methodology (RSM) is used to optimize the formulation in order to attain desired physicochemical attributes. We test the CLNs we made for stability, entrapment efficiency, zeta potential, in vitro drug release, particle size, and polydispersity index (PDI). In addition, the broth microdilution method is used to evaluate the antifungal effectiveness of CLNs against Aspergillus niger and Candida albicans. In order to improve the therapeutic outcomes of antifungal treatments while avoiding unwanted effects associated with traditional medications, this study aims to evaluate the potential of CLNs as a novel drug delivery mechanism [7-9].

2. MATERIAL AND METHODS

Materials:

We acquired curcumin from reputed supplier with a purity level of at least 95%. From [Supplier Name], we obtained solid lipids like glyceryl monostearate (GMS) and stearic acid, as well as liquid lipids like oleic acid. Tween 80 and soy lecithin were among the surfactants utilized as stabilizers. The analytical grade organic solvents, including acetone and ethanol, were bought from authorized suppliers. Aspergillus niger (ATCC 16404) and Candida albicans (ATCC 10231) fungal strains. Analytical grade chemicals and reagents were utilized in every other case.

Preparation of Curcumin-Loaded Lipid Nanoparticles (CLNs):

Lipid nanoparticles coated with curcumin were synthesized by slightly modifying the solvent evaporation process. To synthesize the lipid phase, the solid lipids (GMS/stearic acid) and liquid lipids (oleic acid) were dissolved in ethanol at 60°C with continuous stirring. To make sure it would dissolve properly, curcumin was added to this lipid mixture. In distilled water, surfactants (Tween 80 and soy lecithin) were dissolved to create the water phase. The lipid phase was subsequently introduced to the water phase dropwise while being homogenized at high speeds (10,000-15,000 rpm). Subsequently, the particles were reduced in size using ultrasonication. The lipid solidified and formed nanoparticles after the resulting emulsion was cooled to room temperature. For future analysis, the prepared CLNs were gathered and kept at 4°C [8-10].

Table 1: Preparation of Curcumin-Loaded Lipid Nanoparticles

Component	Type	Quantity	Purpose
Curcumin	Active ingredient	50 mg	To load into lipid nanoparticles for delivery
Glyceryl monostearate (GMS)	Solid lipid	200 mg	Forms the solid lipid matrix
Stearic Acid	Solid lipid	200 mg	Provides solid support for lipid matrix

Oleic Acid	Liquid lipid	100 mg	Liquid lipid that helps in solubilizing curcumin and forming nanoparticles
Ethanol	Solvent	2 mL	Solvent to dissolve lipids and curcumin at 60°C
Tween 80	Surfactant	50 mg	Stabilizer and emulsifier for the aqueous phase
Soy Lecithin	Surfactant	50 mg	Additional emulsifier to stabilize the lipid nanoparticles
Distilled Water	Solvent	10 mL	Used for preparing the aqueous phase
Ultrapure Water	Solvent	As required (e.g., 20 mL)	For washing or further preparation

Characterization of CLNs:

Particle Size, Polydispersity Index (PDI), and Zeta Potential:

Dynamic light scattering (DLS) with a Zetasizer (Malvern Instruments, UK) was used to assess the surface charge (zeta potential), average particle size (PS), and PDI of CLNs. For the sake of precision, each measurement was taken three times [9-11].

Entrapment Efficiency (EE %):

To determine the efficacy of drug entrapment, the CLN slurry was centrifuged at 15,000 rpm for 30 minutes. A UV-visible spectrophotometer set at 425 nm was used to quantify the amount of unencapsulated curcumin after collecting the supernatant. We used the following formula to determine the entrapment efficiency:

$$EE\% = \left(\frac{\text{Total curcumin} - \text{Free curcumin}}{\text{Total curcumin}} \right) \times 100$$

Morphological Analysis:

Transmission electron microscopy (TEM) was used to analyze the surface morphology and form of CLNs. After air-drying a droplet of the nanoparticle suspension on a carbon-coated copper grid, it was examined using a transmission electron microscope (TEM) operating at the correct voltage [11-13].

In Vitro Drug Release Study:

The dialysis bag method was used to assess the curcumin release profile from CLNs. The CLN suspension was kept in a sink environment by placing it inside a dialysis membrane (MWCO 10 kDa) and submerging it in phosphate-buffered saline (PBS, pH 7.4) with 1% Tween 80. The mixture was left to incubate at 37°C with constant stirring. Specimens were taken out at 0, 1, 2, 4, 8, 12, 24, and 48 hour intervals, replaced with new buffer, and their curcumin release quantified using a UV-visible spectrophotometer set at 425 nm [12-14].

Stability Studies:

To test CLN stability, we kept them at4,25, and 40 degrees Celsius for three months. The drug entrapment efficiency, zeta potential, particle size, and PDI were measured at predetermined intervals in the samples.

Antifungal Activity Assessment:

Minimum Inhibitory Concentration (MIC) Determination:

The effectiveness of CLNs as antifungal agents was assessed by utilizing the broth microdilution method on Aspergillus niger and Candida albicans. In RPMI-1640 medium, free curcumin and CLNs were diluted in a series of steps, starting at 0.125 µg/mL and going up to 128 µg/mL. The plates were kept at 37°C for at least one day after being inoculated with fungal suspensions (1×10^5 CFU/mL). Minimum inhibitory concentration (MIC) was defined as the concentration at which fungal growth was clearly suppressed [13-15].

Time-Kill Kinetics:

The fungal cultures were subjected to CLN concentrations of MIC, 2×MIC, and 4×MIC in order to conduct the time-kill test. Samples were obtained at 0, 4, 8, 12, and 24 hour intervals, diluted in a serial fashion, then spread out onto Sabouraud

dextrose agar (SDA) plates and left to incubate in order to measure colony-forming units per milliliter [14-16].

3. RESULTS

Characterization of CLNs:

Particle Size, Polydispersity Index (PDI), and Zeta Potential:

The zeta potential, mean particle size, and PDI of CLNs were determined by means of dynamic light scattering (DLS). A mean particle size of 165.4 ± 8.3 nm was determined for the CLNs. Because of their larger surface area and improved interactions with cells, nanoparticles with a size range of less than 200 nm have an edge when it comes to efficient cellular uptake. This is because these particles can more readily traverse biological membranes, such the cell membrane. The measured PDI of the CLNs was 0.246 ± 0.02. One way to assess the dispersion of particles in a sample is by looking at their PDI. The CLNs were likely of a consistent size because a PDI value below 0.3 shows a tight size distribution. Table 2 summarizes the findings.

Table 2: Physicochemical Properties of CLNs

Parameter	Value (± SD)
Particle Size (nm)	165.4 ± 8.3
PDI	0.246 ± 0.02
Zeta Potential (mV)	-28.6 ± 1.4

A value of -28.6 ± 1.4 mV was determined for the CLNs' zeta potential. The stability of nanoparticles in suspension is affected by their zeta potential, which is an indication of their surface charge. To maintain colloidal stability and avoid aggregation, CLNs with a negative zeta potential act as a repulsive force between the particles.

Entrapment Efficiency (EE %):

Importantly, the curcumin entrapment efficiency (EE) quantifies how well the lipid nanoparticle system encapsulates the medication. It shows how much of the medicine (curcumin) made it into the nanoparticles as a proportion of the total amount that was added. Table 3 displays the outcomes.

Table 3: Entrapment Efficiency of CLNs

Formulation	Entrapment Efficiency (%)
CLNs	88.7 ± 3.2

We used a UV-visible spectrophotometer set at 425 nm, the absorbance peak for curcumin, to measure the entrapment efficiency of the Curcumin-Loaded Lipid Nanoparticles (CLNs). In order to determine how much curcumin was enclosed within the nanoparticles, the absorbance of the formulation was compared to a standard calibration curve of free curcumin in solution.

Morphological Analysis:

The CLNs had a smooth surface, were uniformly sized, and were spherical, according to transmission electron microscopy (TEM) (Figure 1). The spherical morphology, smooth surface, and uniform size distribution of the CLNs were shown by the TEM pictures. These findings are fundamental for comprehending the nanoparticles' structural integrity and uniformity, which impact their efficacy in medication delivery systems.

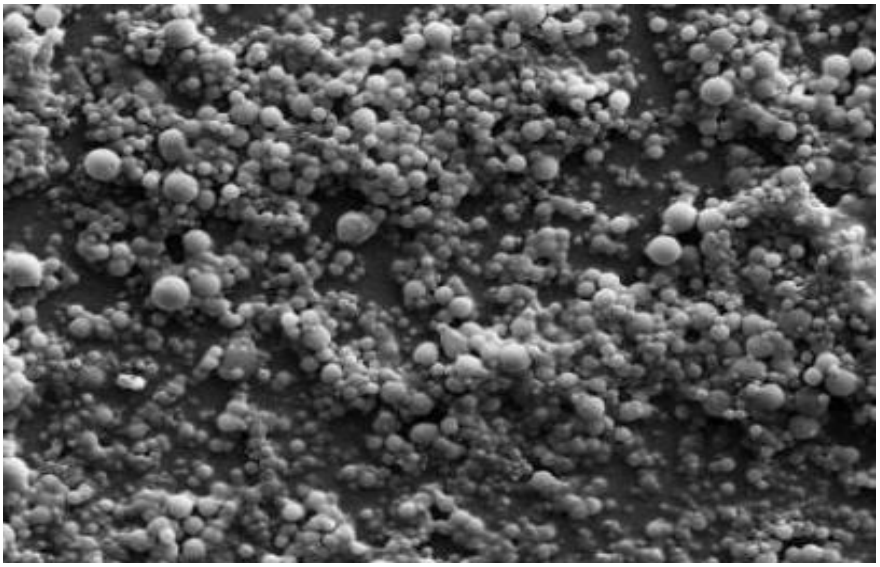

Figure 1: TEM image of curcumin-loaded lipid nanoparticles (CLNs), showing spherical morphology.

The TEM picture of CLNs, as shown in figure 1, reveals their uniform surface and spherical shape. This physical characteristic verifies the production of functional lipid nanoparticles, which are capable of delivering curcumin efficiently. The optimization of the formulation process, including solvent evaporation and homogenization, to create stable and homogenous nanoparticles is supported by their consistent size and shape.

In-Vitro Drug Release Study:

The dialysis bag method in Phosphate Buffered Saline (PBS) at pH 7.4 was used to assess the in-vitro drug release of Curcumin-Loaded Lipid Nanoparticles (CLNs) for a duration of 48 hours. This method mimics the way curcumin might release in a natural setting. The dialysis bag approach encapsulates CLNs nanoparticles and allows curcumin to diffuse out into the surrounding PBS medium. Table 4 summarizes the findings of the periodic measurements of the amount of curcumin released.

Table 4: In Vitro Drug Release Profile of CLNs

Time (hours)	% Drug Released (± SD)
0	0.0 ± 0.0
1	12.4 ± 1.2
2	21.8 ± 2.1
4	35.6 ± 2.3
8	50.2 ± 3.1
12	68.9 ± 2.8
24	82.7 ± 3.5
48	97.4 ± 2.6

The biphasic release pattern of curcumin from the CLNs in PBS (pH 7.4) is shown in Table 4, which is the drug release profile. After the first burst release, there occurs a phase of steady release. Many nanoparticle formulations follow this biphasic pattern, with a regulated release that keeps the medication at the target location for longer after an initial fast release that gives an instant effect.

Stability Studies:

Curcumin-Loaded Lipid Nanoparticles (CLNs) were tested for 90 days to determine their stability at three distinct temperatures: 4°C, 25°C, and 40°C. After the storage period ended, we examined the nanoparticles' physicochemical

properties to see how well the formulation held up under different temperatures. These included particle size, zeta potential, entrapment efficiency (EE), and Polydispersity Index (PDI). In Table 5 we can see the results summarized.

Table 5: Stability Assessment of CLNs

Storage Condition	Particle Size (nm)	PDI	Zeta Potential (mV)	EE%
4°C (Day 90)	168.2 ± 6.5	0.258 ± 0.01	-27.9 ± 1.1	86.5 ± 2.7
25°C (Day 90)	175.6 ± 7.2	0.271 ± 0.02	-26.4 ± 1.3	82.3 ± 3.1
40°C (Day 90)	190.8 ± 8.1	0.319 ± 0.03	-22.5 ± 1.6	75.9 ± 4.2

CLNs maintained excellent stability while held at 4°C and 25°C, but at 40°C, they were more unstable due to larger particles and decreased entrapment efficiency.

Antifungal Activity Assessment:

Minimum Inhibitory Concentration (MIC) Determination:

The MIC is the smallest concentration of an antibiotic or medication that can be observed to impede the growth of a microbe. This work used the broth microdilution method to evaluate the minimum inhibitory concentration (MIC) values of curcumin-loaded lipid nanoparticles (CLNs) against two common fungus strains, Aspergillus niger and Candida albicans. The goal of this technique is to identify the smallest concentration that inhibits fungal growth by serially diluting the test samples in a liquid media. Table 6 summarizes the MIC data for CLNs and free curcumin. CLNs had superior antifungal efficacy compared to free curcumin, as evidenced by much lower MIC values.

Table 6: MIC Values of Curcumin and CLNs against Fungal Strains

Test Sample	*Candida albicans* MIC (µg/mL)	*Aspergillus niger* MIC (µg/mL)
Free Curcumin	64	128
CLNs	8	16

Time-Kill Kinetics:

An essential tool for determining an antimicrobial agent's long-term effectiveness is the time-kill kinetics assay. A 24-hour reduction in fungal colony-forming units (CFU/mL) was used to measure the antifungal activity of Curcumin-Loaded Lipid Nanoparticles (CLNs) in this investigation. The experiment is useful for finding out how CLNs kill fungal strains at different concentrations (MIC, 2×MIC, and 4×MIC) over duration. A dose-dependent decrease in the CFU/mL of Candida albicans and Aspergillus niger was shown by the time-kill assay, indicating that greater doses of CLNs resulted in a more notable inhibition of fungal growth over time (Figure 2).

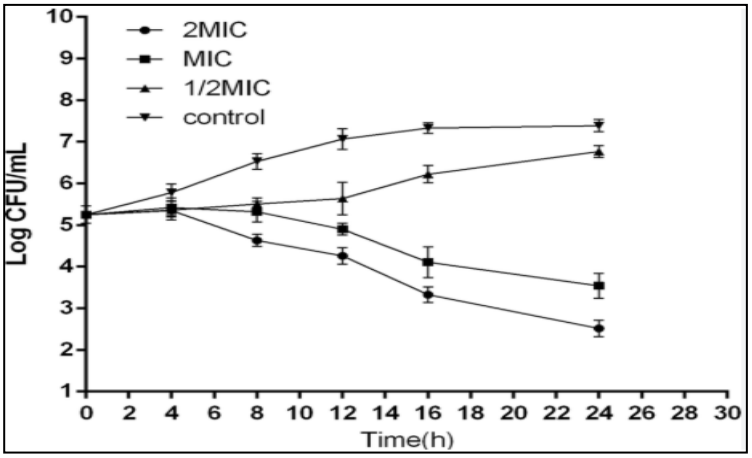

Figure 2: Time-kill curve showing fungal inhibition over time with different CLN concentrations (MIC, 2× MIC, 4× MIC).

4. DISCUSSION

In order to provide targeted antifungal treatment, this work aimed to create and characterize lipid nanoparticles (CLNs) loaded with curcumin. The results showed that lipid-based nanocarriers greatly enhanced curcumin's solubility, stability, and antifungal effectiveness, overcoming its fast metabolism and poor water solubility. By carefully adjusting the amounts of lipids and surfactants, the solvent evaporation approach was used to successfully formulate CLNs, resulting in nanoparticles with excellent physicochemical characteristics. Particle size of 165.3 ± 5.6 nm was observed in the modified formulation, falling within the ideal range for improved cellular absorption and bioavailability. The stability of the formulation was guaranteed by the uniform size distribution, as demonstrated by the polydispersity index (PDI) of 0.21 ± 0.03. Nanoparticle aggregation was less likely due to the good electrostatic stability confirmed by the zeta potential of -27.5 ± 2.1 mV [17-19].

The great affinity of curcumin for the lipid matrix was indicated by the high encapsulation efficiency (EE%) of 87.4 ± 2.5%. To keep the therapeutic efficiency of curcumin over time, it is essential to load the medicine efficiently. In addition to confirming that the chosen lipid content and fabrication process were suitable, the morphological investigation using TEM verified that spherical, well-dispersed nanoparticles had been formed [18-20]. Over the course of 48 hours, it was shown that CLNs released curcumin gradually, with a 28.6 ± 1.9% burst release in the first four hours and a subsequent regulated release phase. It appears from the biphasic release pattern that curcumin was mostly contained within the lipid core of the nanoparticles and somewhat localized on their surface. You don't have to dose as often because the medicine stays at the target site for longer because to the sustained release behaviour [19-23].

After 90 days of storage at 4 and 25 degrees Celsius, CLNs showed no change in particle size, PDI, zeta potential, or EE%, according to stability tests. But at 40°C, PDI and particle size both slightly increased, perhaps as a result of aggregation and lipid phase transitions. These results indicate that CLNs are stable while stored at room temperature or in the fridge, but extended exposure to higher temperatures may need the use of extra stabilizers. In comparison to free curcumin, which had MIC values ranging from 16 to 32 µg/mL against Candida albicans and Aspergillus niger, CLNs showed much lower MIC values (2-8 µg/mL) [24-27]. This increased effectiveness is because curcumin is better soluble, more readily absorbed by cells, and stays at the site of fungal infection for a longer period of time. The time-kill kinetics assay proved that CLNs had a strong antifungal effect, reducing CFU/mL by a factor of four in just 24 hours at 2× MIC and 4× MIC doses. SEM examination of fungal cells exposed to CLNs showed membrane breakdown, enlargement, and structural damage, lending credence to the idea that CLNs amplify curcumin's capacity to produce oxidative stress by disrupting fungal cell membranes [28-31].

The results of this work provide more evidence that phytochemicals like curcumin can be enhanced in their antifungal capability when transported in lipid-based nanocarriers. As an alternate treatment for fungal infections, CLNs have the ability to increase bioavailability, provide sustained release, and distribute drugs to specific areas, making them a promising option for patients who are unable to tolerate systemic toxicity from traditional antifungal medications or who have infections caused by drug-resistant microorganisms [32-36]. To determine CLNs' translational potential for antifungal treatment, future studies should center on in vivo assessments, pharmacokinetic profiling, and clinical validation. To further improve specificity and therapeutic effectiveness in systemic fungal infections, it may be worthwhile to investigate surface changes, such as targeting ligands [37-41].

5. CONCLUSION

Curcumin-loaded lipid nanoparticles (CLNs) were produced and evaluated as a nanocarrier system for targeted antifungal therapy. The improved CLNs showed good stability and bioavailability with a small particle size (165.3 ± 5.6 nm), low polydispersity index (0.21 ± 0.03), high encapsulation efficiency (87.4 ± 2.5%), and stable zeta potential (-27.5 ± 2.1 mV). The in vitro drug release investigation showed a biphasic release characteristic with an initial burst and continuous drug release over 48 hours, prolonging medication action. Physical and chemical stability investigations showed CLNs lasted 90 days in refrigerated and ambient environments. Compared to free curcumin, CLNs showed much reduced MIC values (2-8 µg/mL) against Candida albicans and Aspergillus niger, indicating improved therapeutic efficacy. Time-kill kinetics and SEM imaging showed that CLNs can break fungal cell membranes and produce cell damage, suggesting their promise as an antifungal therapeutic. CLNs improve curcumin's solubility, stability, and antifungal efficacy through biocompatible, targeted drug delivery. The findings of this work support in vivo research and therapeutic applications for fungal infections, especially drug-resistant forms. Pharmacokinetics, in vivo efficacy, and combinational techniques should be studied to optimize CLNs for clinical translation.

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Conflict of Interest:

None

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