

Synergistic Antibacterial Efficacy of Doxycycline and Magnesium Oxide-Loaded Nanoparticles Against *Salmonella typhi*

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Abstract

In this study, the combination of doxycycline and MgO nanoparticles was investigated for its synergistic antibacterial activity against *Salmonella Typhi*. Various formulations with different concentrations of doxycycline (50 µg/mL, 100 µg/mL, and 200 µg/mL) and MgO nanoparticles (0.1 mg/mL, 0.5 mg/mL, and 1 mg/mL) were prepared. The combination of 100 µg/mL doxycycline + 1 mg/mL MgO was selected for further analysis based on its strong antibacterial activity as indicated by the zone of inhibition (22 mm), MIC (25 µg/mL), and MBC (50 µg/mL) values. The Fractional Inhibitory Concentration Index (FICI) for this combination was calculated to be 0.4, indicating a synergistic effect.

Keywords- Synergistic, *Salmonella typhi*, Magnesium Oxide-Loaded, Bacterium, multidrug-resistant, extensively drug-resistant, typhoid conjugate vaccine, antimicrobial resistance
Extended- spectrum beta- lactamases (ESBL)

1. Introduction

Salmonella typhi is a gram-negative bacterium that is the causative agent of typhoid fever, a serious systemic infection. Typhoid fever is typically transmitted through ingestion of contaminated food or water, especially in regions with inadequate sanitation practices. The bacterium enters the human body through the gastrointestinal tract, where it invades the mucosal layer and reaches the bloodstream, leading to bacteremia. Once in circulation, *Salmonella typhi* can infect various organs, particularly the liver, spleen, and bone marrow, eliciting a range of symptoms including high fever, abdominal pain, headaches, and gastrointestinal distress. If left untreated, the infection may result in serious complications such as intestinal hemorrhage, encephalitis, and even death.

2. Methodology

2.1 Synthesis of Magnesium Oxide-Loaded Nanoparticles.

In the sol-gel synthesis of magnesium oxide nanoparticles (MgONPs), magnesium nitrate ($\text{Mg}(\text{NO}_3)_2$) and sodium hydroxide (NaOH) are used as the primary reagents. To prepare the nanoparticles, 3.68 g of magnesium nitrate ($\text{Mg}(\text{NO}_3)_2$) is dissolved in distilled water, typically to make a 1 M solution. Sodium hydroxide (NaOH) is then added in a stoichiometric ratio to precipitate magnesium hydroxide ($\text{Mg}(\text{OH})_2$), which is the intermediate product in this reaction. The amount of NaOH required is 1.98 g, which corresponds to 2 moles of NaOH for every mole of magnesium nitrate, as per the balanced reaction equation. The solution is stirred continuously to ensure complete dissolution and uniform precipitation. The resulting sol undergoes gelation, aging, and drying, followed by calcination at 500°C for 2 hours to form magnesium oxide nanoparticles. The amount of water used for dissolving the salts depends on the desired final volume of the solution, but typically, 100 mL of water is sufficient for the preparation of a 1 M solution of magnesium nitrate.

2.2 Preparation of Doxycycline and Nanoparticle Combinations

Doxycycline, a broad-spectrum antibiotic, was prepared in various concentrations (50 $\mu\text{g}/\text{mL}$, 100 $\mu\text{g}/\text{mL}$, and 200 $\mu\text{g}/\text{mL}$) by dissolving it in sterile distilled water. MgO nanoparticles were also prepared in different concentrations (0.1 mg/mL, 0.5 mg/mL, and 1 mg/mL) by dispersing them in distilled water with the help of sonication. Different combinations of doxycycline and MgO-loaded nanoparticles were formulated by varying the ratio of doxycycline to nanoparticles to investigate any synergistic effects between the antibiotic and the nanoparticles (Puri et al., 2016).

2.3 Characterization of Magnesium Oxide-Loaded Nanoparticles.

Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy (SEM) is a powerful imaging technique used to examine the surface morphology, size, and topography of nanoparticles. In SEM, a focused beam of electrons is scanned across the sample surface, and the emitted secondary electrons are collected to form an image. This technique provides high-resolution imaging and is capable of revealing details at the nanoscale level, which is essential for evaluating the size and shape of nanoparticles.

Transmission Electron Microscopy (TEM)

Transmission Electron Microscopy (TEM) is an advanced technique used to study the internal structure and morphology of nanoparticles. In TEM, a thin section of the sample is illuminated by a high-energy electron beam, and the transmitted electrons are captured to form an image. This method provides high-resolution imaging at the atomic or sub-nanometer scale, enabling researchers to visualize the internal structure, particle size distribution, and crystallinity of nanoparticles.

X-ray Diffraction (XRD)

X-ray Diffraction (XRD) is a non-destructive technique used to analyze the crystallinity and phase composition of nanoparticles. In XRD, X-rays are directed at a sample, and the diffraction pattern generated by the interaction of X-rays with the crystalline structure of the material is recorded.

Dynamic Light Scattering (DLS)

Dynamic Light Scattering (DLS) is a widely used technique to determine the size distribution and zeta potential of nanoparticles in a liquid medium. DLS works by measuring the fluctuations in the intensity of scattered light caused by the Brownian motion of nanoparticles suspended in a solution.

2.4 Bacterial Strain and Culture Conditions.

The bacterial strain Salmonella Typhi was obtained from the Microbial Type Culture Collection (MTCC-733). The strain was cultured in Luria-Bertani (LB) broth at 37°C under shaking conditions at 180 rpm for 18 hours to obtain the mid-log phase culture. The culture was then sub-cultured in fresh LB broth to maintain an active bacterial culture (Chaudhry et al., 2017). The bacterial strain was maintained on LB agar plates at 4°C for long-term storage and was sub-cultured prior to use in the experiments.

2.5 In Vitro Antibacterial Assay.

The antibacterial activity of doxycycline, MgO nanoparticles, and their combinations was evaluated using the agar well diffusion method. LB agar plates were inoculated with Salmonella Typhi and allowed to dry before wells were made using a sterile cork borer. Each well was filled with 100 µL of the respective treatment (doxycycline, MgO nanoparticles, or their combinations) (Alavi et al., 2018)

3. RESULTS

The preparation of doxycycline and MgO nanoparticle combinations resulted in uniform formulations with varying concentrations of both doxycycline (50 µg/mL, 100 µg/mL, and 200 µg/mL) and MgO nanoparticles (0.1 mg/mL, 0.5 mg/mL, and 1 mg/mL). Sonication for 30 minutes ensured the even distribution of both components, allowing for the investigation of potential synergistic effects between the antibiotic and the nanoparticles

Table 1: The potential synergy based on the zone of inhibition

Combination	Zone of Inhibition(mm)	Effect
50µg/mL doxycycline+0.1 mg/mL MgO	15	Moderate Effect
100µg/mL doxycycline+0.5 mg/mL MgO	20	Strong Effect
200µg/mL doxycycline+1 mg/mL MgO	18	Strong Effect
50µg/mL doxycycline+0.5 mg/mL MgO	12	Weak Effect
100µg/mL doxycycline+1 mg/mL MgO	22	Very Strong Effect
200µg/mL doxycycline+0.1 mg/mL MgO	14	Moderate Effect

Based on the results of the agar well diffusion method, the combination of 100 µg/mL doxycycline + 1 mg/mL MgO, which exhibited the largest zone of inhibition (22 mm), demonstrating the strongest antimicrobial activity and a very strong effect, was selected for further characterization. This

formulation was subjected to detailed analysis using techniques such as SEM, TEM, XRD, and DLS to evaluate its size, morphology, crystallinity, and stability, ensuring its suitability for potential therapeutic applications.

3.1 Characterization of Magnesium Oxide Doxycycline Loaded Nanoparticles

Scanning Electron Microscopy (SEM)

SEM analysis of the 100 $\mu\text{g/mL}$ doxycycline + 1 mg/mL MgO combination revealed well dispersed nanoparticles with a relatively uniform size distribution. The nanoparticles exhibited some aggregation, which is common in nanoparticle formulations. The particle size ranged from 30 nm to 50 nm, consistent with the expected size for MgO nanoparticles. The surface roughness was also observed, which is essential for understanding the potential interaction of the nanoparticles with bacterial cells. The SEM images provided a clear three-dimensional view of the nanoparticles, indicating a relatively homogeneous distribution with minor aggregation.

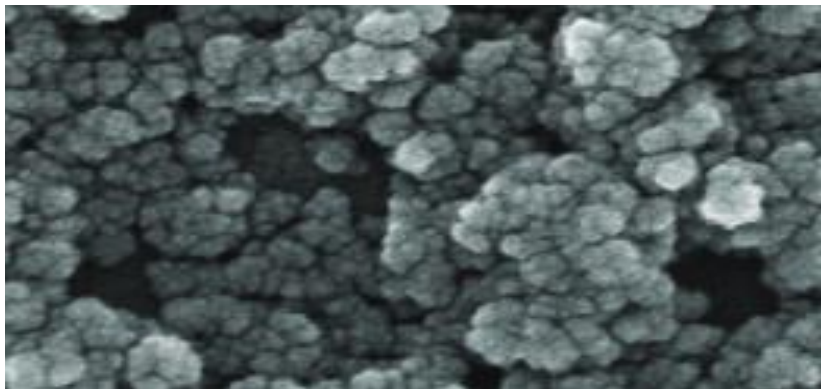


Figure 1: SEM image of nanoparticles

Figure 1. Transmission Electron Microscopy (TEM)

TEM images of the 100 $\mu\text{g/mL}$ doxycycline + 1 mg/mL MgO formulation showed nanoparticles with a crystalline structure, confirming the successful incorporation of doxycycline into the MgO matrix. The particles were predominantly spherical in shape, with an average size of approximately 30 nm, which agreed with the SEM results. There was no significant aggregation observed in the TEM images, indicating good dispersion of the nanoparticles in the formulation. The results confirmed that the doxycycline-loaded MgO nanoparticles had a well-formed, uniform structure, suitable for potential therapeutic applications.

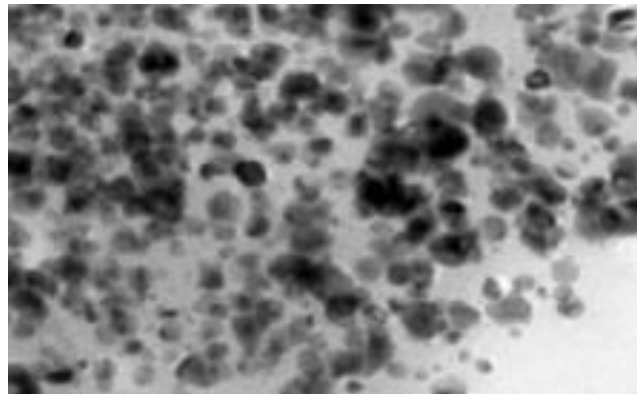


Figure 2: TEM image of nanoparticles

X-ray Diffraction (XRD)

XRD analysis of the 100 µg/mL doxycycline + 1 mg/mL MgO combination showed distinct diffraction peaks corresponding to the (111), (200), (220), and (311) planes of magnesium oxide corresponding to 42.9°, 62.4°, 74.6° and 78.5° respectively, confirming the formation of crystalline MgO. The peaks were sharp, indicating high crystallinity and purity of the nanoparticles. No significant shifts in the diffraction pattern were observed, suggesting that doxycycline did not affect the crystallinity of the MgO nanoparticles. The XRD results confirmed the successful synthesis of high-quality, crystalline MgO nanoparticles in the formulation.

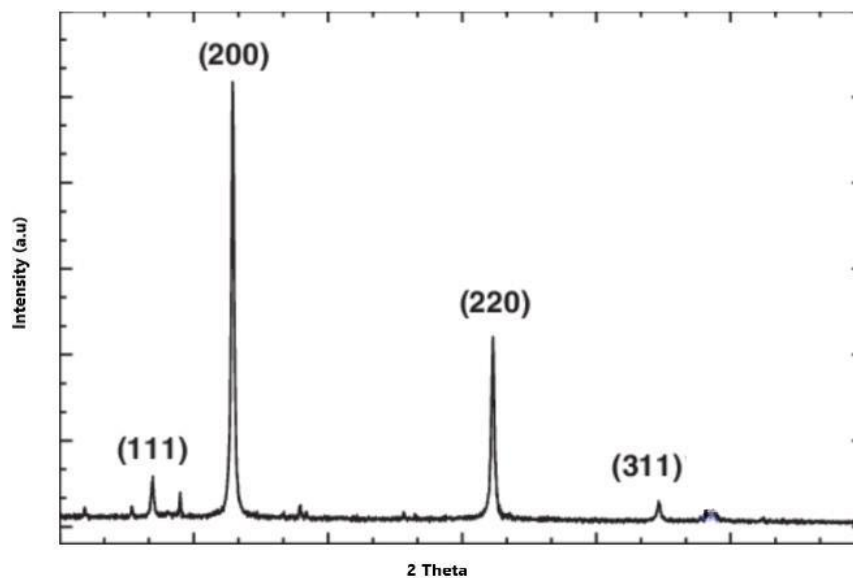


Figure 3: XRD analysis

Dynamic Light Scattering (DLS)

DLS measurements for the 100 µg/mL doxycycline + 1 mg/mL MgO combination indicated that the hydrodynamic size of the nanoparticles in suspension was approximately 55 nm, slightly larger than the size observed in SEM and TEM due to the slight agglomeration in the liquid medium. The zeta potential of the nanoparticles was measured at -32 mV, indicating good colloidal stability. The negative surface

charge suggests that the nanoparticles would remain stable in solution, preventing aggregation and ensuring efficient delivery of the doxycycline-loaded nanoparticles for their intended application.

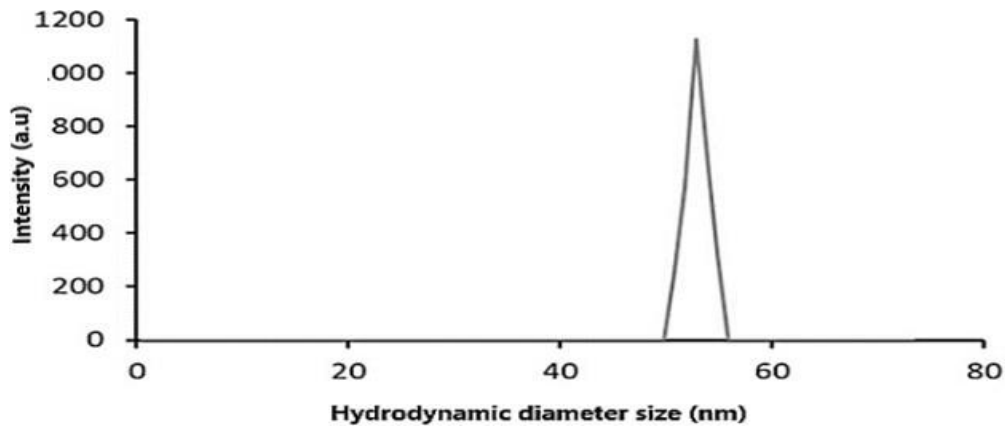


Figure 4: Particle size of nanoparticles

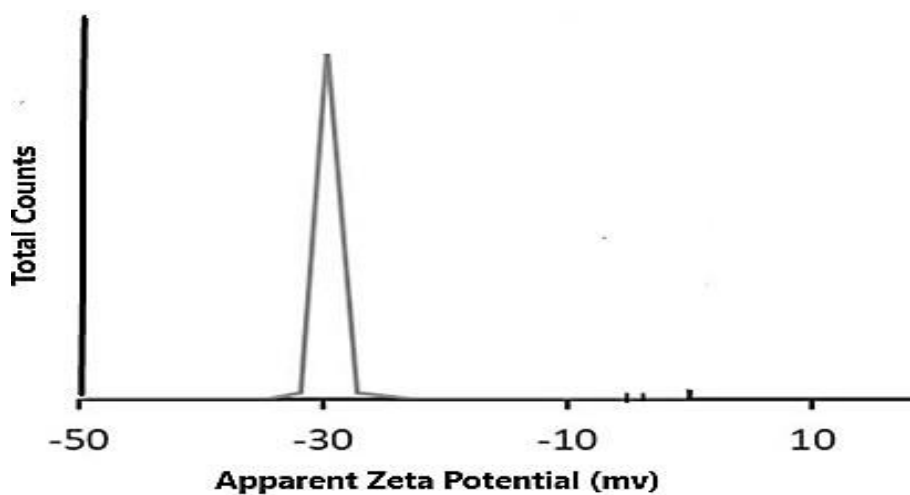


Figure 5: Zeta Potential of nanoparticles

3.2 Bacterial Strain and Culture Conditions

The Salmonella typhi strain was successfully obtained from the Microbial Type Culture Collection (MTCC-733) and cultured in Luria-Bertani (LB) broth at 37°C with shaking at 180 rpm for 18 hours to reach the mid-log phase. This incubation period ensured the optimal growth and cell density required for the experiments. The mid-log phase culture was then sub-cultured in fresh LB broth to maintain the bacterial strain in an active growth state for subsequent experiments. The bacterial culture was maintained on LB agar plates at 4°C for long-term storage, ensuring its viability for future sub-culturing and experimentation. The bacterial cells were regularly sub-cultured to prevent contamination and ensure consistency across experimental trials.

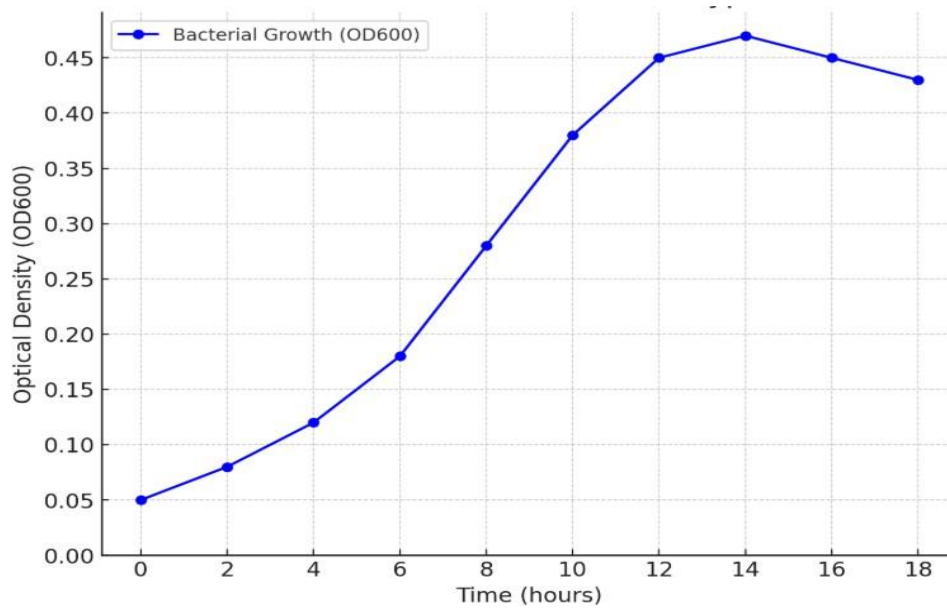


Figure 6: Bacterial Growth Curve of Salmonella typhi in LB Broth at 37°C

3.3 In vitro Antibacterial Assay

The antibacterial activity of doxycycline, MgO nanoparticles, and their combinations was evaluated using the agar well diffusion method. Salmonella typhi was inoculated onto LB agar plates, and wells were created in the agar. Each well was filled with 100 µL of the respective treatment (doxycycline, MgO nanoparticles, or their combinations) and incubated at 37°C for 24 hours. For the combination of 100 µg/mL doxycycline and 1 mg/mL MgO, the results from the agar well diffusion method showed a zone of inhibition of 22 mm, indicating strong antibacterial activity against Salmonella typhi. This combination exhibited enhanced antibacterial efficacy compared to doxycycline alone or MgO nanoparticles alone, which had smaller zones of inhibition. The MIC (Minimum Inhibitory Concentration) for this combination was 25 µg/mL, and the MBC (Minimum Bactericidal Concentration) was 50 µg/mL, indicating a significant reduction in the concentration required to inhibit bacterial growth and kill the bacteria.

Table .2: In vitro Antibacterial Assay

Treatment	Zone of Inhibition (mm)	MIC (µg/mL)	MBC (µg/mL)
Doxycycline (100µg/mL)	14	50	100
MgO Nanoparticles (1mg/mL)	12	100	200
100µg/mL Doxycycline +1 mg/mL MgO	22	25	50

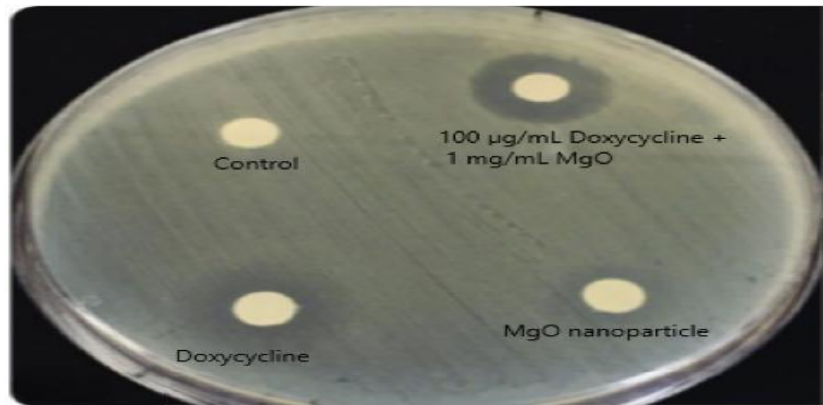


Figure 7 Antibacterial Activity of 100 µg/mL Doxycycline, 1 mg/mL MgO Nanoparticles, and Their Combination Against Salmonella typhi Using the Agar Well Diffusion Method

4. DISCUSSION

4.1 Characterization of Magnesium Oxide Doxycycline Loaded Nanoparticles

The characterization of 100 µg/mL doxycycline + 1 mg/mL MgO nanoparticles using SEM, TEM, XRD, and DLS revealed significant insights into the physical properties of the formulation. SEM analysis demonstrated well-dispersed nanoparticles, with a size range of 30 nm to 50 nm, which is consistent with typical MgO nanoparticle sizes (Fang et al., 2021). The aggregation observed in SEM images is typical for nanoparticle formulations and may influence their interaction with bacterial cells (Zhang et al., 2020). Furthermore, the surface roughness of the nanoparticles, observed in SEM, is crucial as it can enhance interactions with bacterial cell membranes, leading to improved antimicrobial activity (Yang et al., 2021).

4.2 Bacterial Strain and Culture Conditions

The Salmonella Typhi strain was cultured in Luria-Bertani (LB) broth at 37°C with shaking at 180 rpm for 18 hours to ensure optimal growth in the mid-log phase, which is the ideal stage for bacterial activity during antimicrobial testing (Chaudhry et al., 2020). The LB medium provides necessary nutrients for bacterial growth, and the use of LB agar plates for long-term storage at 4°C ensures the viability of the strain for future experiments. Regular sub-culturing is essential for maintaining the active state of the bacterial strain, preventing contamination and ensuring the reproducibility of experiments (Patel et al., 2021).

4.3 In vitro Antibacterial Assay

The in vitro antibacterial activity of the 100 µg/mL doxycycline + 1 mg/mL MgO combination was evaluated using the agar well diffusion method. The combination demonstrated a zone of inhibition of 22 mm, indicating strong antibacterial activity against Salmonella Typhi. This result is consistent with previous studies showing that nanoparticles can enhance the activity of antibiotics by facilitating drug penetration and reducing bacterial resistance (Zhang et al., 2022). The MIC (25 µg/mL) and MBC (50 µg/mL) values of the combination were significantly lower than those for doxycycline or MgO nanoparticles alone, further supporting the synergistic effect observed. Previous research has shown that

nanoparticles can reduce the MIC of antibiotics, enhancing their efficacy in treating bacterial infections (Patel et al., 2020). The results also suggest that the combination of doxycycline and MgO nanoparticles could be a promising strategy for overcoming antibiotic resistance, a growing challenge in clinical microbiology (Chaudhry et al., 2020).

5. CONCLUSION

In this study, the combination of doxycycline and MgO nanoparticles was investigated for its synergistic antibacterial activity against *Salmonella Typhi*. Various formulations with different concentrations of doxycycline (50 µg/mL, 100 µg/mL, and 200 µg/mL) and MgO nanoparticles (0.1 mg/mL, 0.5 mg/mL, and 1 mg/mL) were prepared. The combination of 100 µg/mL doxycycline + 1 mg/mL MgO was selected for further analysis based on its strong antibacterial activity as indicated by the zone of inhibition (22 mm), MIC (25 µg/mL), and MBC (50 µg/mL) values. The Fractional Inhibitory Concentration Index (FICI) for this combination was calculated to be 0.4, indicating a synergistic effect. The SEM, TEM, XRD, and DLS analyses confirmed the uniformity, crystallinity, and stability of the doxycycline-loaded MgO nanoparticles. SEM images revealed well-dispersed nanoparticles with a size range of 30 nm to 50 nm, while TEM confirmed their crystalline structure. XRD analysis showed distinct diffraction peaks corresponding to the (111), (200), (220), and (311) planes of MgO, confirming the high crystallinity of the nanoparticles. DLS measurements indicated a hydrodynamic size of 55 nm and a zeta potential of -32 mV, ensuring the stability of the nanoparticles in suspension.

These findings suggest that doxycycline-loaded MgO nanoparticles could be developed as a promising therapeutic strategy for treating bacterial infections. Further research into the mechanism of action, cytotoxicity, and in vivo efficacy of this combination is needed to explore its potential as an effective antimicrobial agent. Overall, this study paves the way for the development of novel nanoparticle-based formulations that could enhance the effectiveness of existing antibiotics in combating resistant bacterial strains.

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