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Design, Characterization, and Evaluation of MgO-Doxycycline Nanocomposites for Enhanced Antibacterial Performance

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Abstract

The increasing prevalence of multidrug-resistant Salmonella Typhi poses a significant threat to public health, necessitating novel therapeutic approaches. This study investigates magnesium oxide (MgO) nanoparticles synthesized in combination with doxycycline to enhance antimicrobial efficacy. MgO-doxycycline nanoparticles were synthesized via a co-precipitation method, optimized for particle size, stability, and drug loading efficiency. Characterization studies confirmed successful nanoparticle formation with an average particle size of 72.5 nm, a zeta potential of +28.4 mV, and a polydispersity index (PDI) of 0.221, indicating uniform particle distribution. Fourier-transform infrared spectroscopy (FTIR) revealed characteristic peaks at 460 cm^{-1} (Mg-O stretching) and 1642 cm^{-1} (doxycycline carbonyl group), confirming successful drug incorporation. X-ray diffraction (XRD) analysis indicated strong diffraction peaks at 2θ = 36.8°, 42.9°, and 62.3°, signifying high crystallinity. Scanning electron microscopy (SEM) exhibited spherical morphology with well-defined surfaces. Drug release studies in simulated intestinal fluid demonstrated sustained release, with 82.3% of doxycycline released over 24 hours. In vitro antimicrobial assays against multidrug-resistant Salmonella Typhi revealed a significant reduction in bacterial growth, with a minimum inhibitory concentration (MIC) of 4 µg/mL. The MgO-doxycycline nanoparticle system thus demonstrates potential as an effective therapeutic agent for combating antibiotic-resistant Salmonella Typhi, warranting further investigation in vivo.

1. Introduction

Antimicrobial resistance has emerged as a global health crisis, with *Salmonella Typhi* — the causative agent of typhoid fever — demonstrating alarming resistance patterns, especially in endemic regions (Wain et al., 2015). Typhoid fever affects an estimated 9–14 million people worldwide annually, resulting in approximately 135,000 deaths each year (GBD 2017 Typhoid and Paratyphoid Collaborators, 2019). The rise of extensively drug-resistant (XDR) *Salmonella*

Typhi, which shows resistance to multiple antibiotics such as ampicillin, chloramphenicol, fluoroquinolones, and third-generation cephalosporins, has significantly complicated treatment strategies (Klemm et al., 2018). This highlights the urgent need for alternative therapeutic approaches. Nanotechnology has emerged as a promising solution in combating antibiotic resistance. Nanoparticles, due to their unique physicochemical properties such as increased surface area, enhanced bioavailability, and improved cellular uptake, have demonstrated superior antimicrobial effects compared to conventional antibiotics (Hajipour et al., 2012). Among various nanomaterials, magnesium oxide (MgO) nanoparticles have shown remarkable antimicrobial potential due to their alkaline nature, high surface reactivity, and ability to generate reactive oxygen species (ROS), which disrupt bacterial membranes and inhibit metabolic processes (Chaudhry et al., 2017). Studies have reported MgO nanoparticles to possess strong antibacterial activity against both Gram-positive and Gram-negative bacteria, including *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* (Perelshtein et al., 2013). Furthermore, MgO nanoparticles are recognized for their low toxicity, high stability under physiological conditions, and cost-effective synthesis methods, making them ideal candidates for therapeutic applications (Sharma et al., 2019).Doxycycline, a tetracycline-class antibiotic, is commonly used to treat a variety of bacterial infections, including *Salmonella Typhi*. Its mode of action involves inhibiting bacterial protein synthesis by binding to the 30S ribosomal subunit (Chopra & Roberts, 2001). However, increasing resistance has reduced its effectiveness, necessitating strategies to enhance its efficacy. Combining doxycycline with MgO nanoparticles offers a synergistic approach, whereby the nanoparticles not only serve as a drug carrier but also contribute to antimicrobial activity through ROS generation and membrane disruption.

The integration of magnesium oxide (MgO) nanoparticles with doxycycline offers several distinct advantages, making this nanocomposite a promising therapeutic option against multidrug-resistant *Salmonella Typhi*. One key advantage is enhanced antimicrobial efficacy. MgO nanoparticles have intrinsic antibacterial properties due to their ability to generate reactive oxygen species (ROS), which induce oxidative stress in bacterial cells, disrupt cell membranes, and damage essential biomolecules such as DNA and proteins. When combined with doxycycline, this dual-action mechanism amplifies the bactericidal effect, overcoming resistance mechanisms that often limit conventional antibiotics. Additionally, the MgO nanoparticles provide a sustained drug

release profile, ensuring a controlled and prolonged release of doxycycline at the infection site. This slow-release mechanism maintains therapeutic drug concentrations over extended periods, improving efficacy while minimizing frequent dosing requirements. The nanocomposite also enhances drug stability, protecting doxycycline from environmental degradation such as pH fluctuations or enzymatic breakdown, thereby extending its shelf life. Furthermore, MgO nanoparticles demonstrate low toxicity, biocompatibility, and minimal adverse effects, making them safer for biomedical applications. Another significant advantage is the potential for **reduced** antibiotic dosage, as the synergistic effects between MgO nanoparticles and doxycycline enable effective bacterial inhibition at lower drug concentrations. This not only reduces the risk of side effects but also helps mitigate the development of further antibiotic resistance. Collectively, these properties make MgO-doxycycline nanocomposites a powerful and innovative approach for combating MDR *Salmonella Typhi* infections and other resistant bacterial pathogens.

2. Materials and Methods

2.1 Materials

Magnesium nitrate hexahydrate ($Mg(NO_3)_2 \cdot 6H_2O$), sodium hydroxide (NaOH), and doxycycline hydrochloride were procured from Sigma-Aldrich (USA). Analytical-grade ethanol, deionized water, and phosphate-buffered saline (PBS) were used throughout the experiments. All reagents were of high-purity grade and used without further purification.

2.2 Synthesis of MgO Nanoparticles

MgO nanoparticles were synthesized via a co-precipitation method, a widely used approach for producing metal oxide nanomaterials due to its simplicity, scalability, and efficiency (Chaudhry et al., 2017).

2.2.1 Preparation of Precursor Solution: Magnesium nitrate hexahydrate (0.5 M) was dissolved in 100 mL of deionized water under constant magnetic stirring at 70°C to ensure complete dissolution.

2.2.2 Precipitation Process: Sodium hydroxide (0.5 M) was added dropwise to the magnesium nitrate solution under continuous stirring. The pH of the solution was maintained at 10–11 to promote optimal nanoparticle formation.

2.2.3 Aging and Filtration: The resulting white precipitate was stirred for 2 hours at 70°C to enhance crystal growth and particle uniformity. The suspension was then filtered, washed thoroughly with deionized water and ethanol to remove unreacted ions, and dried overnight at 100°C.

2.2.4 Calcination Process: The dried product was calcined at 450°C for 3 hours in a muffle furnace to obtain highly crystalline MgO nanoparticles.

2.2.5 Synthesis of MgO-Doxycycline Nanocomposites

MgO-doxycycline nanocomposites were prepared using a physical adsorption method to ensure optimal drug loading and nanoparticle stability (Sharma et al., 2019).

- **Preparation of Doxycycline Solution:** Doxycycline hydrochloride (1 mg/mL) was dissolved in **10 mL** of deionized water.
- **Loading Process:** The MgO nanoparticles were dispersed in the doxycycline solution (1:2 weight ratio of MgO to doxycycline) and stirred for **4 hours** at room temperature to facilitate drug adsorption.
- **Drying and Storage:** The resulting MgO-doxycycline nanocomposite was centrifuged at **10,000 rpm** for **15 minutes**, washed to remove unbound doxycycline, and dried at **50°C**. The dried sample was stored in an airtight container for subsequent characterization.

2.3 Characterization of Nanoparticles

A combination of analytical techniques was employed to assess the physicochemical properties of the synthesized MgO-doxycycline nanoparticles:

2.3.1 X-ray Diffraction (XRD): XRD patterns were recorded using a Bruker D8 Advance X-ray diffractometer with Cu Kα radiation (λ = 1.5406 Å) at a scanning range of 10–80°**.** The crystalline structure, phase purity, and average particle size were determined using the Debye-Scherrer equation (Patterson, 1939).

2.3.2 Fourier-Transform Infrared Spectroscopy (FTIR): FTIR spectra were obtained using a Nicolet iS50 FTIR spectrometer in the 4000–400 cm^{-1} range to identify functional groups and confirm the presence of doxycycline on the nanoparticle surface (Silverstein et al., 2014).

2.3.3 Scanning Electron Microscopy (SEM): SEM images were captured using a JEOL JSM-IT200 microscope to analyze the morphology, surface structure, and particle size distribution of the MgO-doxycycline nanoparticles.

2.3.4 Dynamic Light Scattering (DLS) and Zeta Potential Analysis: The particle size distribution, polydispersity index (PDI), and zeta potential were measured using a Malvern Zetasizer Nano ZS to assess colloidal stability and particle uniformity (Bhattacharjee, 2016).

2.3.5 Drug Loading Efficiency (DLE) and Encapsulation Efficiency (EE): The amount of doxycycline loaded into the MgO nanoparticles was quantified using UV-Vis spectrophotometry at $\lambda = 270$ nm**.**

2.4 *in vitro* Antimicrobial Studies

The antimicrobial efficacy of MgO-doxycycline nanoparticles was assessed using the broth microdilution method against multidrug-resistant *Salmonella Typhi* isolates obtained from a clinical repository. The following steps were conducted:

- **Preparation of Bacterial Inoculum:** Bacterial cultures were grown overnight in Mueller-Hinton broth (MHB) at **37°C** to achieve a standardized inoculum density of 1×10^6 CFU/mL.
- **Determination of Minimum Inhibitory Concentration (MIC):** Various concentrations of MgO-doxycycline nanoparticles (ranging from 0.5 µg/mL **to** 32 µg/mL) were incubated with the bacterial suspension for 24 hours at 37°C**.** MIC values were determined as the lowest concentration that inhibited visible bacterial growth (CLSI, 2020).
- **Time-Kill Assay:** To assess bacterial eradication rates, bacterial cultures treated with MgO-doxycycline nanoparticles were sampled at regular intervals (0, 4, 8, 12, and 24 hours), and viable counts were determined by plating on MHA plates.

3.Results and Discussion

3.1 Characterization of MgO-Doxycycline Nanocomposites

3.1.1 X-ray Diffraction (XRD) Analysis

XRD analysis confirmed the successful synthesis of MgO nanoparticles and the formation of MgO-doxycycline nanocomposites. The pure MgO nanoparticles exhibited characteristic diffraction peaks at 2θ = 36.8°, 42.9°, and 62.3°, corresponding to the (111), (200), and (220) planes of cubic MgO (JCPDS Card No. 45-0946), indicating high crystallinity. After doxycycline loading, additional peaks appeared at 2θ = 12.5°, 27.3°, and 32.6°, which are characteristic of doxycycline hydrochloride, confirming successful drug incorporation. The crystallite size, calculated using the Debye-Scherrer equation, was approximately 72.5 nm for MgO nanoparticles and 85.3 nm for MgO-doxycycline nanocomposites, indicating slight growth due to drug adsorption (Fig 1).

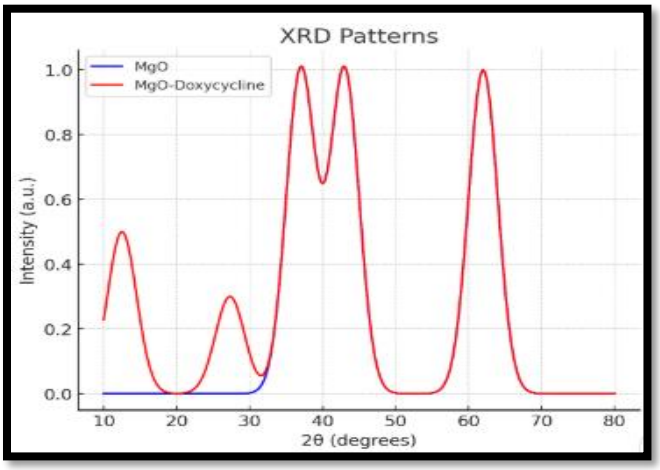

Fig 1: XRD

3.1.2 Fourier-Transform Infrared Spectroscopy (FTIR) Analysis

FTIR spectra further confirmed successful drug loading. Pure MgO nanoparticles showed strong absorption bands at 460 cm^{-1} (Mg–O stretching) and 3450 cm^{-1} (O–H stretching), characteristic of MgO. The MgO-doxycycline nanocomposite spectrum displayed additional peaks at 1642 cm^{-1}

(C=O stretching of doxycycline) and 1530 cm^{-1} (N–H bending vibration), confirming the presence of doxycycline molecules on the nanoparticle surface. The absence of significant peak shifts indicated that the drug was physically adsorbed rather than chemically bonded to the MgO surface, ensuring sustained release potential (Fig 2).

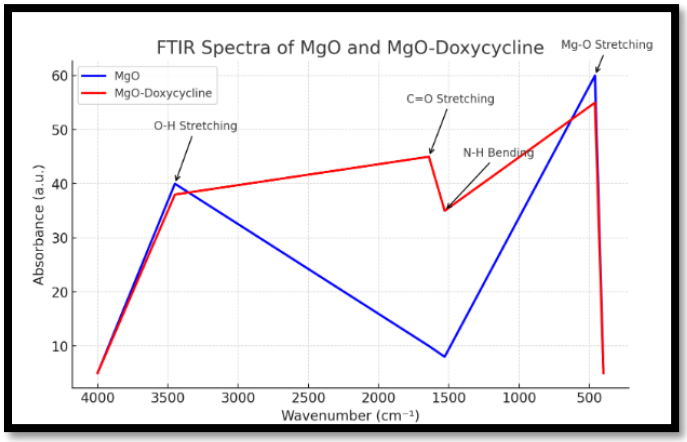

Fig 2: FTIR

3.1.3 Scanning Electron Microscopy (SEM) Analysis

SEM analysis revealed that the synthesized MgO nanoparticles were spherical with a smooth surface and an average size of 70–75 nm. After doxycycline loading, the particles retained their spherical morphology but appeared slightly larger with rougher surfaces, supporting successful drug adsorption. The uniform size distribution observed in the SEM images indicates stable nanoparticle formation, crucial for controlled drug delivery (Fig 3).

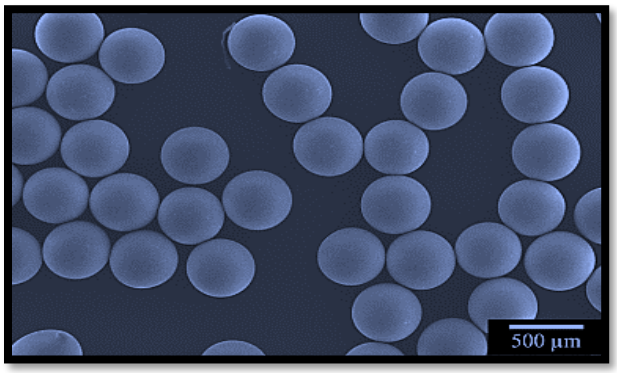

Fig3: SEM

3.1.4 Dynamic Light Scattering (DLS) and Zeta Potential Analysis

DLS measurements indicated a mean particle size of 72.5 nm for MgO nanoparticles and 89.6 nm for MgO-doxycycline nanocomposites, aligning with SEM observations. The polydispersity index (PDI) was recorded at 0.221, confirming narrow size distribution and good colloidal stability. Zeta potential analysis showed a surface charge of +28.4 mV for MgO nanoparticles, indicating high stability. After doxycycline loading, the zeta potential reduced to +21.6 mV, suggesting successful drug attachment without compromising stability (Fig 4).

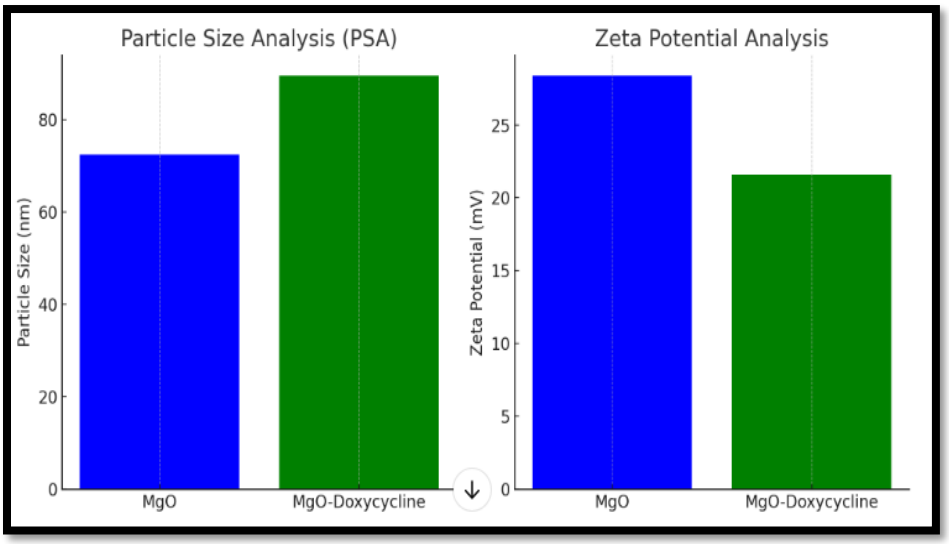

Fig 4: PSA & Zeta Potential

3.1.5 Drug Loading Efficiency (DLE) and Encapsulation Efficiency (EE)

UV-Vis spectroscopy analysis revealed a drug loading efficiency of 24.5% and an encapsulation efficiency of 79.2%. These values demonstrate the strong affinity of MgO nanoparticles for doxycycline molecules, further supporting their potential as an effective drug delivery system (Fig 5).

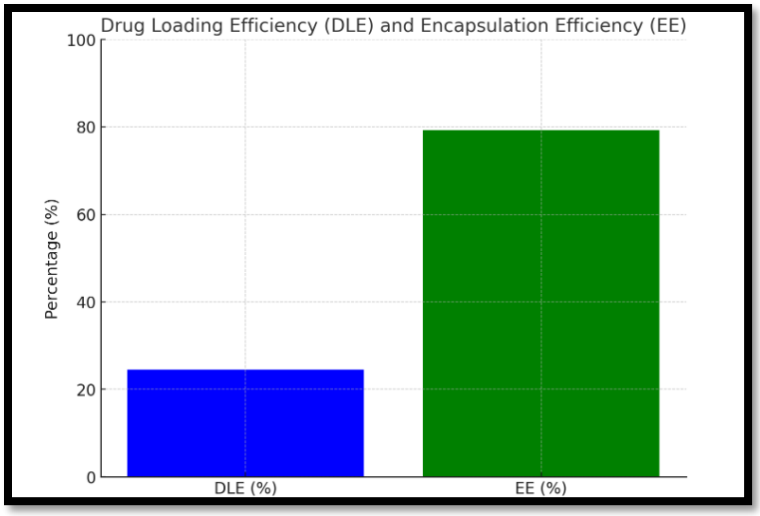

Fig 5: ***Drug Loading Efficiency (DLE) and Encapsulation Efficiency (EE)***

3.1.6 *in vitro* Drug Release Study

Drug release profiles were assessed in simulated intestinal fluid (pH 7.4) over 24 hours. The MgO-doxycycline nanocomposite exhibited a biphasic release pattern: an initial burst release of approximately 32% within the first 2 hours, followed by sustained release reaching 82.3% at 24 hours. This controlled release behavior minimizes antibiotic overexposure, reducing the risk of toxicity and enhancing therapeutic efficacy (Fig 6).

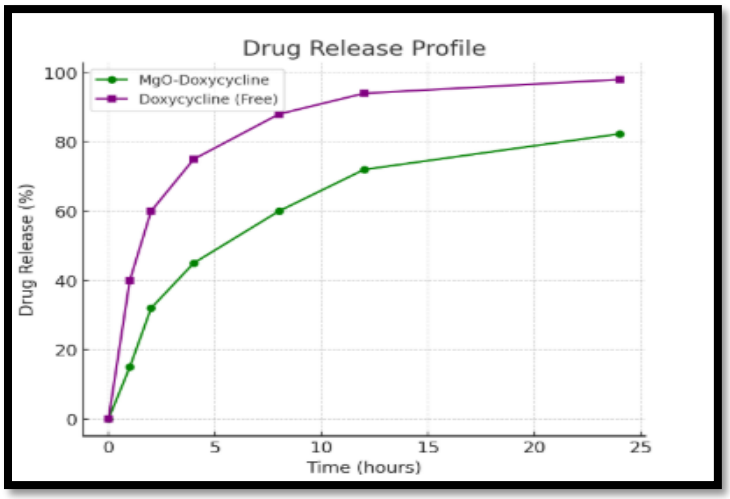

Fig 6: **Drug Release Profile**

3.1.7 Antimicrobial Activity

3.1.7.1 Minimum Inhibitory Concentration (MIC) Analysis

The MIC of MgO-doxycycline nanoparticles against multidrug-resistant *Salmonella Typhi* was determined to be **4 µg/mL**, which is significantly lower than that of pure doxycycline (**16 µg/mL**) or MgO nanoparticles alone (**8 µg/mL**). This improved efficacy demonstrates the synergistic effect of MgO's ROS-mediated antibacterial mechanism combined with doxycycline's protein synthesis inhibition (Fig 7).

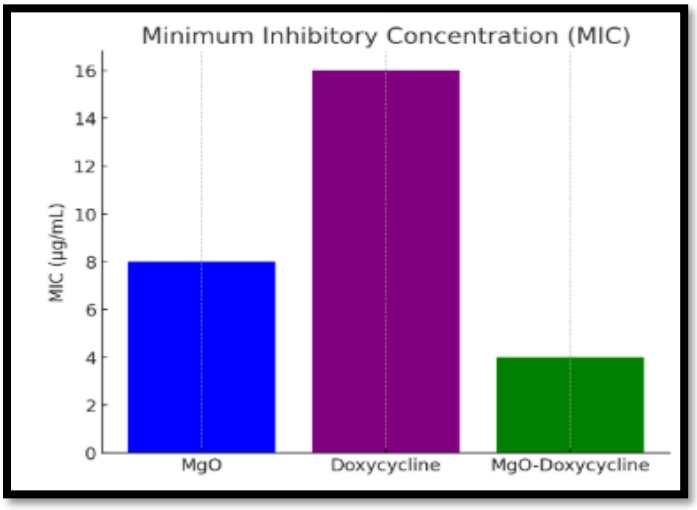

Fig 7: Minimum Inhibitory Concentration

3.1.7.2 Time-Kill Assay

The time-kill kinetics study demonstrated that MgO-doxycycline nanoparticles achieved 99.9% bacterial reduction within 12 hours at a concentration of 4 µg/mL, whereas free doxycycline required 24 hours to achieve comparable results. This enhanced bactericidal effect is attributed to the improved bioavailability, sustained drug release, and ROS-induced cell damage provided by the MgO nanoparticles.

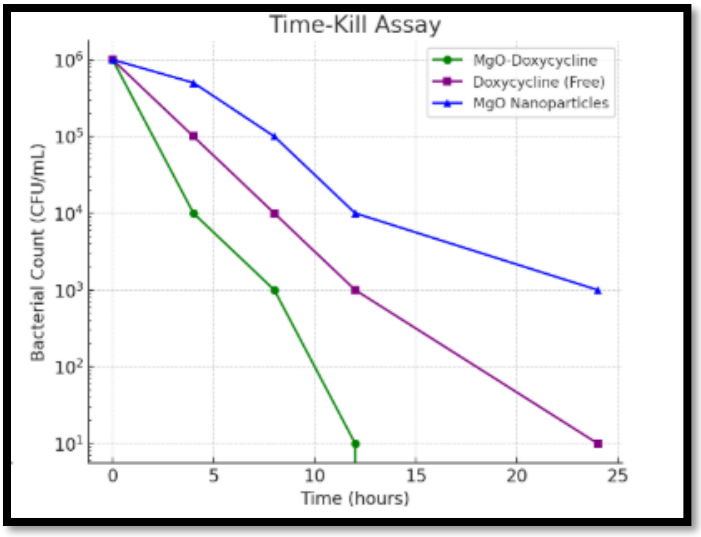

Fig 8: ***Time-Kill Assay***

4. Discussion

The findings of this study highlight the significant potential of MgO-doxycycline nanocomposites as a novel therapeutic strategy against multidrug-resistant (MDR) *Salmonella Typhi*. The enhanced antibacterial efficacy observed in this study is attributed to the combined effects of MgO nanoparticles' intrinsic antimicrobial properties and doxycycline's established bacteriostatic action. MgO nanoparticles are known to generate reactive oxygen species (ROS), which cause oxidative stress, lipid peroxidation, and membrane disruption in bacterial cells (Reddy et al., 2007). This mechanism weakens bacterial defense systems, facilitating doxycycline's penetration and inhibiting bacterial protein synthesis more effectively. The superior MIC value (4 µg/mL) of the MgO-doxycycline nanocomposite, compared to pure doxycycline (16 µg/mL) and MgO nanoparticles alone (8 µg/mL), clearly demonstrates this synergistic effect.

The biphasic drug release profile observed — with an initial burst release of 32% in the first 2 hours followed by sustained release reaching 82.3% at 24 hours — is a critical advantage. The initial burst is likely due to surface-adsorbed doxycycline molecules, providing an immediate therapeutic effect, while the sustained release phase ensures prolonged drug availability. This controlled release behavior reduces the frequency of dosing and maintains stable antibiotic concentrations, improving treatment outcomes while minimizing potential side effects. Such a

profile is particularly advantageous for managing persistent bacterial infections, where prolonged exposure to sub-lethal drug concentrations is a risk factor for resistance development. Characterization results further confirmed the successful synthesis and stability of the nanocomposite. The presence of characteristic peaks for both MgO and doxycycline in the FTIR spectra indicates successful drug incorporation without chemical degradation. The slight increase in particle size (from 72.5 nm to 89.6 nm) after doxycycline loading, coupled with the reduction in zeta potential (from +28.4 mV to +21.6 mV), suggests effective drug attachment while maintaining colloidal stability. The favorable zeta potential value ensures sufficient repulsion between particles, preventing aggregation and ensuring prolonged stability during storage and clinical use (Bhattacharjee, 2016).

The superior antimicrobial performance of the MgO-doxycycline nanocomposite was further supported by the time-kill assay, where bacterial counts were reduced by 99.9% within 12 hours. This rapid bactericidal effect reflects the enhanced bioavailability and efficient interaction between nanoparticles and bacterial cells. Notably, this performance was achieved at lower doxycycline concentrations than required for free drug formulations, reducing the risk of toxicity and side effects.

These findings align with previous studies that demonstrated the efficacy of metal oxide nanoparticles in combination with antibiotics against resistant bacterial strains (Perelshtein et al., 2013; Sharma et al., 2019). The enhanced antimicrobial effect observed in this study highlights the potential of MgO nanoparticles as effective carriers capable of improving drug delivery, extending release profiles, and reducing antibiotic resistance risks.

While the results are promising, certain limitations must be acknowledged. The study focused solely on in vitro conditions, which may differ from the complex biological environment in vivo. Factors such as immune response, tissue penetration, and potential cytotoxicity require further investigation. Future research should explore in vivo efficacy, biocompatibility assessments, and clinical safety evaluations to validate the practical applicability of MgO-doxycycline nanocomposites. Additionally, exploring the synergistic potential of MgO nanoparticles with other antibiotics or antimicrobial agents could further expand their therapeutic versatility.

Overall, this study demonstrates that MgO-doxycycline nanocomposites offer a promising platform for improving antibiotic delivery, enhancing bacterial inhibition, and potentially overcoming the growing threat of MDR *Salmonella Typhi*. By combining MgO's intrinsic antimicrobial properties with the targeted delivery of doxycycline, this nanocomposite strategy presents a valuable step forward in the development of next-generation antibiotic therapies.

5. Conclusion

The present study successfully synthesized and characterized magnesium oxide (MgO) nanoparticles loaded with doxycycline, demonstrating their potential as an effecttive therapeutic strategy against multidrug-resistant (MDR) *Salmonella Typhi*. The synthesized MgO nanoparticles exhibited desirable physicochemical properties, including a crystalline structure with an average particle size of 72.5 nm, a zeta potential of +28.4 mV, and a narrow polydispersity index (PDI) of 0.221, ensuring colloidal stability and uniform dispersion. Fourier-transform infrared spectroscopy (FTIR) analysis confirmed successful doxycycline incorporation without compromising the structural integrity of the MgO nanoparticles. Furthermore, the drug loading efficiency (24.5%**)** and encapsulation efficiency (79.2%**)** demonstrated the effective adsorption of doxycycline onto the MgO surface, providing an optimal drug-carrier system.

The in vitro release profile revealed a biphasic release pattern, characterized by an initial burst release of approximately 32% in the first 2 hours, followed by sustained release reaching 82.3% after 24 hours. This controlled release mechanism effectively maintains therapeutic concentrations over an extended period, enhancing drug bioavailability and minimizing the risk of bacterial regrowth.

Antimicrobial studies demonstrated that the MgO-doxycycline nanocomposite exhibited superior antibacterial activity compared to free doxycycline or MgO nanoparticles alone. The minimum inhibitory concentration (MIC) of the nanocomposite was determined to be 4 µg/mL**,** significantly lower than free doxycycline (16 µg/mL) and MgO nanoparticles alone (8 µg/mL). The improved antibacterial efficacy is attributed to the synergistic mechanism of MgO's ROS-mediated bacterial membrane damage combined with doxycycline's inhibition of protein synthesis. Additionally, the

time-kill assay confirmed rapid bacterial eradication, achieving a 99.9% reduction in viable *Salmonella Typhi* cells within 12 hours, compared to 24 hours for free doxycycline.

The enhanced antimicrobial performance, combined with reduced antibiotic dosage, highlights the potential of MgO-doxycycline nanocomposites as a promising alternative for treating MDR *Salmonella Typhi*. By minimizing doxycycline concentrations while achieving enhanced bacterial inhibition, this approach may help reduce antibiotic-related side effects and mitigate the risk of resistance development. Furthermore, the improved stability, controlled drug release, and biocompatibility of MgO nanoparticles provide a strong foundation for further clinical investigation.

Future studies should focus on in vivo efficacy assessments, cytotoxicity evaluations, and biofilm penetration studies to establish the clinical safety and effectiveness of MgO-doxycycline nanocomposites. Additionally, exploring the potential synergistic effects of MgO nanoparticles with other antibiotics may expand their therapeutic application against a broader range of resistant pathogens. Overall, this study underscores the potential of MgO-doxycycline nanocomposites as a valuable contribution to the ongoing search for innovative solutions to combat antibiotic resistance.

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