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UNLEASHING SYNERGISTIC POWER: ANTIBACTERIAL POTENTIAL OF MAGNESIUM OXIDE NANOPARTICLES LOADED WITH ERYTHROMYCIN

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ABSTRACT

At present, the escalating threat of antibiotic-resistant bacterial infections has prompted the exploration of innovative approaches to combat microbial pathogens. This study delves into the synergistic antibacterial potential of magnesium oxide nanoparticles (MgO NPs) when loaded with erythromycin, aiming to enhance the efficacy of this conventional antibiotic. In this study, MgO NPs were synthesized using a facile method and erythromycin was subsequently loaded onto the nanoparticle surface. Next, the synthesized composite was characterized using various analytical techniques, including UV Visibile Spectroscopy, Scanning Electron Microscopy (SEM), High Resolution Transmission Electron Microscopy (HR-TEM) and Electron Dispersive X-Ray Analysis (EDX) to confirm the successful formation and structural attributes of MgO NPs loaded with erythromycin. Finally, Antibacterial assays were conducted against a spectrum of clinically relevant bacterial strains (encompass both Gram-positive and Gram-negative organisms). Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values were determined to assess the potency of MgO NPs loaded with erythromycin, juxtaposed with free erythromycin to elucidate their potential synergistic interactions. The results demonstrated that the MgO NPs loaded with erythromycin exhibited enhanced antibacterial activity compared to the individual components alone. The synergistic interaction between MgO NPs and erythromycin was evident, leading to a significant reduction in the MIC and MBC values for various bacterial strains. In summary, this study underscores the promising antibacterial potential of magnesium oxide nanoparticles loaded with erythromycin as a novel therapeutic strategy. The observed synergistic effects have suggested that the potential for this composite seems to serve as an effective and innovative solution to combat antibiotic-resistant bacterial infections, paving the way for further exploration and development in the field of antimicrobial research.

1.INTRODUCTION

The global surge in antibiotic-resistant bacterial strains has become a critical concern in modern medicine. Traditional antibiotics are increasingly rendered ineffective, leading to prolonged illnesses, higher healthcare costs, and elevated mortality rates. Consequently, there's an urgent need for innovative antimicrobial strategies to combat this burgeoning crisis. Nanoparticles are ultrafine particles with dimensions typically ranging from 1 to 100 nanometers (nm). Due to their small size, nanoparticles exhibit unique physicochemical properties, such as increased surface area-to-volume ratio, quantum effects, and enhanced reactivity (1).They have found its applications in diverse fields, including medicine (drug delivery imaging and therapy)(2), electronics (semiconductor devices and sensors) (3,4,5) and catalysis (6).

Moreover, they have been observed as the promising candidates in anti-microbial research due to their unique physico-chemical properties and enhanced interaction with microbial cells (7) and and cancer cells (8,9) Despite their potential benefits, the use of nanoparticles has raised concerns regarding their potential toxicity to living organisms. Studies have highlighted the importance of assessing nanoparticle interactions with biological systems, potential health risks, and environmental implications (10). Significantly, the Erythromycin (a macrolide antibiotic) has been widely used for treating bacterial infections due to its ability to inhibit bacterial protein synthesis.However, the increasing prevalence of erythromycin-resistant strains necessitates the innovative approaches to potentiate its anti-bacterial efficacy. The combination of erythromycin with nanoparticles offers a promising strategy to enhance its therapeutic potential and overcome resistance mechanisms (11).

Synergistically, the nanoparticles and antibiotics interactions have been extensively used to elucidate certain enhanced anti-bacterial effects of antibiotics. Previous research has demonstrated that nanoparticles might facilitate the targeted delivery of antibiotics, improve bioavailability and overcome bacterial resistance mechanisms through synergistic interactions (12,13). Characterization of nanoparticles seems to be crucial for understanding their properties and optimizing applications. Certain advanced characterization techniques such as transmission electron microscopy (TEM), scanning electron microscopy (SEM), X-ray diffraction (XRD), and Fourier-transform infrared spectroscopy (FTIR) are commonly employed for nanoparticle characterization (14)..

Understanding the mechanism of action is pivotal to harnessing the synergistic effects of nanoparticle-antibiotic composites. Studies have indicated that nanoparticles can disrupt bacterial cell membranes, facilitate antibiotic uptake, induce reactive oxygen species (ROS) production, and inhibit bacterial proliferation through multifaceted mechanisms .This article provides a foundational overview of nanoparticles, their synthesis, applications, potential toxicological considerations and characterization techniques.

2.METHODOLOGY

2.1. Synthesis of MgO Nanoparticles using the Sol-Gel Method:

Magnesium oxide nanoparticles were synthesized using sol-gel route(15,16). It involves various stages; such as blending process, stirring process, filtration process, drying process and calcination process . Magnesium Nitrate of purity (GC) 99% merck and Sodium Hydroxide powder of Gehalt (acidimetric) 99% merck were used in the preparation of precursor solution. In this preparation, distilled water was used as a solvent, while Ethanol (99.9% purity, AR grade) was used as a washing reagent respectively. A specific concentration of magnesium nitrate was dissolved in deionized water to form a homogeneous solution. Concurrently, NaOH was prepared in a separate solution, ensuring appropriate stoichiometry.

At the first, distilled water was added to a compound of Magnesium Nitrate (5.903 g, 0.2 M) and then stirring the solution by magnetic stirrer for 45 min to obtain (solution A). The second step is preparing (solution B) as the following: 1.60 g, (0.2 M) of NaOH was blended with distilled water (200 ml) and kept under magnetic stirrer for 45 min to obtain (solution B). Afterward, (200 ml) of (NaOH solution B) was added to (solution A) of magnesium Nitrate [Mg (NO3)2. 6 (H2O)] drop-wise by using glass rod and the mixture was left for 30 min ultrasonic stirring. The magnesium nitrate solution was slowly added to the NaOH under constant stirring. This gradual addition facilitates the formation of a gel-like precursor, initiating the sol-gel transition.

After that, this mixture was stirring by magnetic stirrer for 2 hours. The stirring process, the mixture was left to rest for (2h) to get the precipitate at the beaker's bottom. It was then filtered and washed thoroughly with the distilled water (~4 times). It was followed by washing with Ethanol (2 times) so as to obtain a final product. The combined solution underwent a gelation process upon continuous stirring, resulting in the formation of a white gel indicative of MgO nanoparticle formation. The obtained material was kept in a vacuum oven at 80°C for 4 hours to remove the moisture by drying process. Finally, the white gel then was subjected to drying at a controlled temperature of 300°C for 3 hours to ensure the removal of residual solvents and organic content. Subsequently, calcination was performed to obtain the crystalline magnesium oxide nanoparticles.

2.2. Anti-bacterial Attributes of Magnesium oxide Nanoparticles with Erythromycin

The test bacteria were inoculated in peptone water and incubated for 3-4 hours at 35 ºC. Mueller-Hinton agar plates was prepared and poured in sterile Petri plates. 0.1 ml of bacterial culture was inoculated on the surface of Mueller-Hinton agar plates and spread by using L-rod. The inoculated plates were allowed to dry for five minutes. The disc loaded with samples concentration 1000 µg/ml was placed on the surface of inoculated Petri plates using sterile technique. The plate was incubated at 37 ºC for 18-24 hours. After, the incubation period the plates were examined for the inhibitory zones and its diameter was measured and recorded in mm(17)

3. RESULT AND DISCUSSION

3.1. UV-Spectroscopic analysis of MgONPs:

UV visibile spectroscopy is used to discuss the optical properties of a sample. A monochromatic light is passed through the sample and the amount of light being absorbed by the sample is measured. At different wavelengths absorption of light by sample varies. The UV absorption range is fixed from 200-900 nm for the analysis of the synthesized magnesium oxide nanoparticles. It has been found that the broad peak corresponding to 290nm is the absorption value for the synthesized MgO nanoparticles. From previous literature studies it is found that the absorption range of MgO nanoparticles lies between 260-330nm(18). Therefore, the formation of peak at 290 nm confirms the exact formation of MgO nanoparticles.

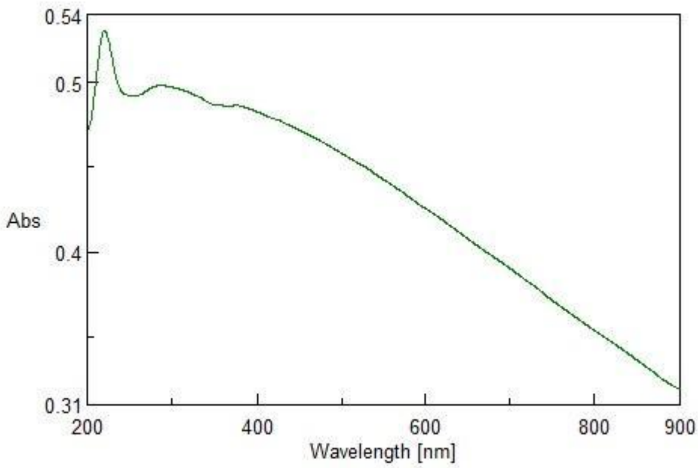

Figure :1 UV-Vis spectrum of MgO NPs

3.2. Scanning electron microscopy (SEM) analysis

Morphology of MgO nano particles prepared by Sol – Gel method was examined using the Scanning electron microscopic (SEM) analysis. It revealed that the surface morphology of the synthesized magnesium oxide nanoparticles and provided insights into their structural attributes.

The structural characterization of these nanoparticles using scanning electron microscopy (SEM) unveiled intriguing morphological attributes. SEM images showed agglomerated crystalline particles in the form of sheets with smooth surfaces. Some needle shaped crystals are also seen in SEM images of higher magnification **(Fig.2)**. Individual particles with definite edges were not seen in the images. The particles are accumulated and grouped together into clusters. The observed agglomeration and specific crystalline structures are consistent with the findings of nanoparticles synthesized via sol-gel methods, where synthesis parameters might influence the resulting morphology (19).Such morphological insights are pivotal as they can influence the nanoparticles' physicochemical properties, potentially impacting their biological interactions.

Different shapes like spherical, nano sheets, and nano flakes are reported for MgO nano particles. The SEM image of Erythromycin nanoparticles is given in fig.2. The SEM image of the nanoparticles shows the crystalline nature of the nanoparticles. No specific shaped particles are seen on the image. The crystals are well formed and clustered together.

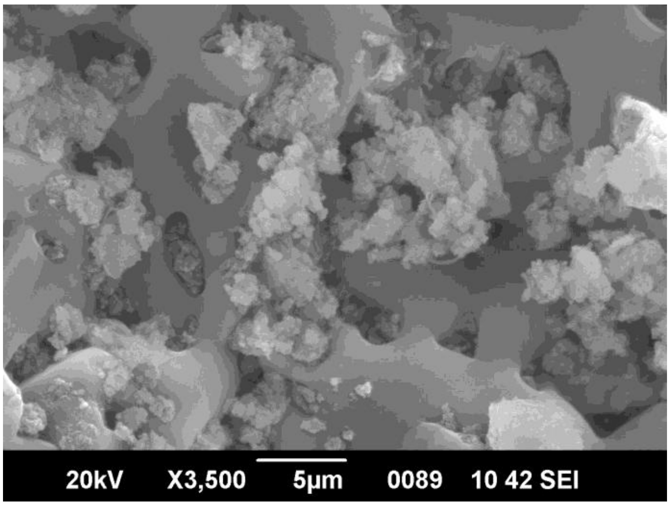

Figure:2 SEM image of MgONPs

3.3. High Resolution Transmission Electron Microscopy (HR-TEM) studies of MgONPs:

The synthesized magnesium oxide nanoparticles are characterized by HR-TEM to determine the size of the nanoparticles. In this technique, a beam of light is passed through a ultra-thin specimen. The interaction of electrons with the specimen results in formation of image which is then magnified and focused onto an imaging device, such as fluorescent screen or CCD camera. The quality, shape, size and density of nanoparticle at high resolution can be analysed by TEM. Thus the shape of MgO nanoparticles are found to be hexagon in the range of 50nm[20].

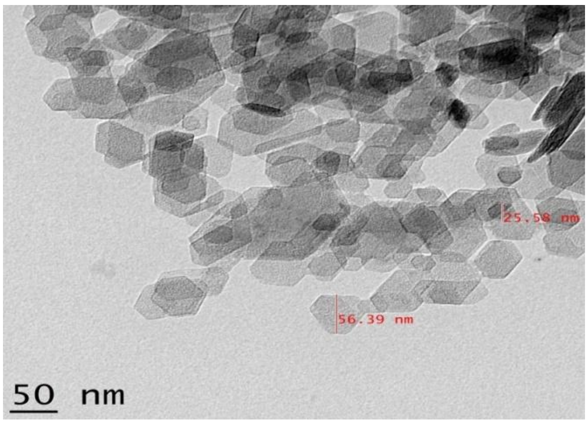

Figure:3 TEM image of MgONPs

3.4. Electron Dispersive X-Ray (EDAX) analysis

Then, it was confirmed by the Electron Dispersive X-Ray (EDAX) analysis (**Fig.4.**). In this experiment the appearance of signals at 0.5 KeV and 1.3 KeV indicated the components of MgO i.e., the presence of Oxygen and Magnesium respectively. The peak of Sodium might be due to the precursor i.e., NaOH which have been used in the synthesis of MgO Nano particles. The peak of Nitrogen might be due to the drug Erythromycin moieties.

The confirmation of the elemental composition of the synthesized magnesium oxide nanoparticles (MgO NPs) loaded with erythromycin was further substantiated through Electron Dispersive X-Ray (EDAX) analysis. EDAX is a powerful analytical technique that provides insights into the elemental composition of materials at the nanoscale .

The obtained EDAX spectrum, as depicted in Fig.4. distinctly showed characteristic peaks corresponding to specific elemental constituents. The appearance of signals at 0.5 KeV and 1.3 KeV unequivocally confirmed the presence of oxygen and magnesium, respectively[21]. These findings align well with the anticipated composition of MgO NPs, thereby validating the successful synthesis and characterization of the nanoparticles .

Interestingly, the presence of a peak corresponding to sodium (Na) provides insights into the synthesis process. The origin of this sodium peak can be attributed to the precursor sodium hydroxide (NaOH) employed during the sol-gel synthesis method. Such residual elements from precursor materials are commonly observed in nanoparticle synthesis processes and underscore the importance of thorough purification and characterization techniques .

Furthermore, the detection of a nitrogen peak in the EDAX spectrum is particularly intriguing. This nitrogen signature is consistent with the presence of erythromycin moieties within the nanoparticles. The successful incorporation of erythromycin into the MgO NPs is pivotal, as it contributes to the enhanced antibacterial activity observed in the previous findings. The presence of erythromycin within the nanoparticles not only validates its integration but also offers insights into potential interactions and stability within the composite structure.

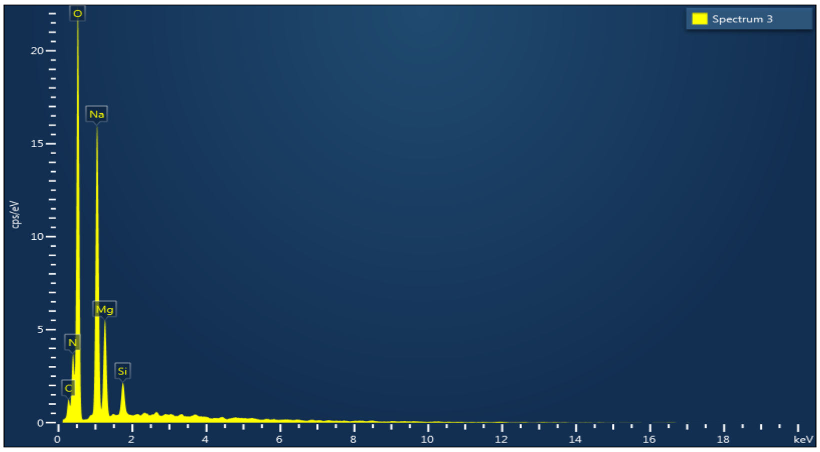

Figure:4. **EDAX spectra of MgO nanoparticles**

Table:1 EDAX ANAYLSIS

Element	Line Type	Wt%	Atomic %
C	K series	6.81	9.43
N	K series	16.16	19.21
O	K series	50.66	52.71
Na	K series	18.57	13.44
Mg	K series	6.24	4.27
Si	K series	1.57	0.93
Total:		100	100

The EDAX analysis provides robust evidence confirming the elemental composition of the synthesized MgO NPs loaded with erythromycin. These findings further bolster the study's credibility and offer valuable insights into the nanoparticle's composition, paving the way for future research endeavours and applications.

3.5. Antibacterial activities of erythromycin loaded MgO nanoparticles.

MgO nanoparticles loaded with erythromycin found to possess excellent antibacterial activity against *E.coli*, *Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Pseudomonas areuginosa*. For *E.coli*, inhibition zone of 13mm was observed for standard antibiotic and 19 mm for MgO nanoparticles loaded with erythromycin. For *Staphylococcus aureus*, inhibition zone of 9mm was observed for standard antibiotic and 17mm for MgO nanoparticles loaded with erythromycin. For Bacillus subtilis inhibition zone of 10mm was observed for standard antibiotic and 17mm for MgO nanoparticles loaded with erythromycin. For Bacillus cereus inhibition zone of 11mm was observed for standard antibiotic and 16mm for MgO nanoparticles loaded with erythromycin. For Pseudomonas aeruginosa inhibition zone of 12mm was observed for standard antibiotic and 14mm for MgO nanoparticles loaded with erythromycin. This result confirmed that the MgO nanoparticles also possess antibacterial activity similar to that of other metallic nanoparticles[22].

Table:2 Antibacterial activity Erythromycin loaded with MgO

Name of the Bacteria	Inhibition Zone in mm	
	Erythromycin loaded with MgO Nanoparticles (Ab)	Ampicillin(Standard)
E. coli	19	13
Staphylococcus aureus	17	9
Bacillus subtilis	17	10
Bacillus cereus	16	11
Pseudomonas aeruginosa	14	12

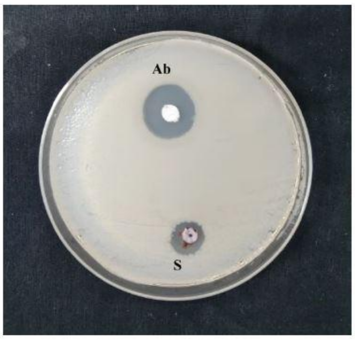

Figure:5 Antibacterial activities (*E.coli*) of erythromycin loaded with MgO nanoparticles

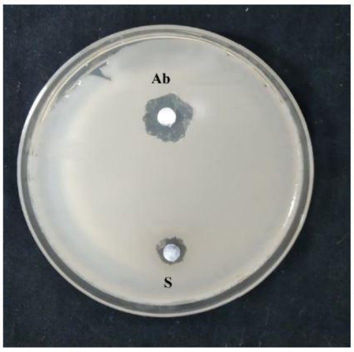

Figure:6 Antibacterial activities (Staphylococcus aureus) of erythromycin loaded with MgO nanoparticles

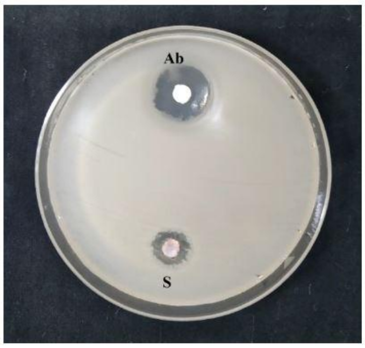

Figure:7.Antibacterial activities of (Bacillus subtilis) erythromycin loaded with MgO nanoparticles

Figure:8. Antibacterial activities (Bacillus cereus) of erythromycin loaded with MgO nanoparticles

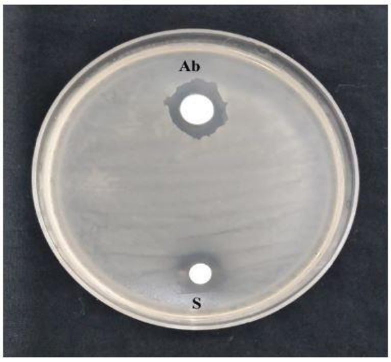

Figure:9 Antibacterial activities of (Pseudomonas aeruginosa) erythromycin loaded with MgO nanoparticles

4.CONCLUSION

Magnesium oxide nanoparticles with erythromycin have been synthesized by sol gel method using magnesium nitrate as a source material with sodium hydroxide. The white gel magnesium oxide nanoparticles were dried at 300°C. The synthesized magnesium oxide nanoparticles have been characterized by scanning electron microscopy which gives the surface morphology of the synthesized magnesium oxide nanoparticles. The antibacterial activity of magnesium oxide nanoparticles shown inhibition zone of 19nm for *E. coli*, 17nm for *Staphylococcus aureus*, 17nm for *Bacillus subtilis*,16nm for *Bacillus cereus*, 14nm for *Pseudomonas aeruginosa*. It shows that the magnesium oxide loaded with erythromycin possess good antibacterial activity. This study elucidates the promising potential of MgO NPs loaded with erythromycin as effective antibacterial agents. However, further studies; including in vivo evaluations and toxicity assessments are imperative to ascertain their clinical applicability and safety profile.

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