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Original Research Article

Evaluation of Antibacterial, Antioxidant, Anti-Biofilm, and Anti-Cancer Potential of Magnesium Oxide Nanoparticles Against Multidrug-Resistant Klebsiella Pneumoniae

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Abstract: *The emergence of multidrug-resistant (MDR) bacterial pathogens such as Klebsiella pneumoniae has intensified the global demand for alternative therapeutic agents. Magnesium oxide nanoparticles (MgO-NPs) possess promising antimicrobial, antioxidant, and anticancer properties, making them potential candidates for addressing resistance-related challenges. This study aimed to evaluate the antibacterial, antioxidant, anti-biofilm, and anticancer activities of MgO-NPs against MDR Klebsiella pneumoniae clinical isolates.* ***Objective:*** *To evaluate the antibacterial, antioxidant, anti-biofilm, and anticancer activities of MgO-NPs against multidrug-resistant Klebsiella pneumoniae clinical isolates.* ***Methods:*** *A laboratory-based experimental study was conducted from February to July 2025 at The University of Faisalabad, in collaboration with the Department of Microbiology, Government College University Faisalabad. Three MDR Klebsiella pneumoniae isolates (K.p1, K.p2, K.p3) were tested. Antibacterial activity was determined using agar well diffusion and broth microdilution methods for MIC and MBC estimation. Antioxidant capacity was evaluated via the DPPH radical scavenging assay. Anti-biofilm potential was assessed using crystal violet microtiter plate assays, and anticancer efficacy was tested on HepG2 liver carcinoma cells using the MTT assay. All experiments were conducted in triplicate, and data were analyzed using SPSS v25.0 with a significance level set at $p < 0.05$.* ***Results:*** *MgO-NPs demonstrated strong, dose-dependent antibacterial effects against MDR K. pneumoniae isolates, with maximum zones of inhibition of 34 mm at 2 mg/mL and MIC values ranging from 125–250 µg/mL. MBC/MIC ratios of 2 confirmed bactericidal activity. Biofilm inhibition reached 88.9% at 1 mg/mL, while pre-established biofilm reduction exceeded 80% across isolates. The DPPH assay revealed concentration-dependent antioxidant activity, achieving 65.3 ± 1.2% radical scavenging at 200 µg/mL, compared to 93.4% for ascorbic acid. MTT assays indicated significant cytotoxicity in HepG2 cells, with 76.5 ± 2.4% cell death at 500 µg/mL concentration, confirming potent anticancer potential.* ***Conclusion:*** *MgO nanoparticles exhibited significant antibacterial, antioxidant, anti-biofilm, and anticancer activities, highlighting their potential as broad-spectrum bioactive agents. Their bactericidal and cytotoxic effects suggest a promising role in managing MDR bacterial infections and hepatocellular carcinoma. Further in vivo studies are recommended to validate their safety and therapeutic efficacy for clinical application, particularly in regions with high antibiotic resistance such as Pakistan.*

Keywords: Magnesium Oxide Nanoparticles, *Klebsiella Pneumoniae*, Antibacterial Activity, Anti-Biofilm, Antioxidant, Anticancer, Multidrug Resistance

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Introduction

Magnesium oxide (MgO) nanoparticles have garnered substantial interest in the scientific community due to their multifaceted applications in pharmacology, particularly as antibacterial, antioxidant, anti-biofilm, and anti-cancer agents. The increasing prevalence of multidrug-resistant (MDR) bacteria, notably *Klebsiella pneumoniae*, necessitates innovative approaches to combat these infections. *K. pneumoniae* is an opportunistic pathogen linked to severe hospital-acquired infections, with resistance to a variety of antibiotics severely limiting treatment options (1-3). This growing threat calls for the exploration of novel antimicrobial substances like MgO nanoparticles, which possess inherent properties capable of addressing these critical healthcare challenges.

Research has demonstrated the antimicrobial efficacy of MgO nanoparticles against various bacterial strains. The antibacterial mechanism of MgO nanoparticles is attributed to their high surface area, which allows for greater interaction with bacterial cells, leading to increased production of reactive oxygen species (ROS) that compromise bacterial membranes and vitality (4-6). In studies examining the effects of MgO nanoparticles on both Gram-positive and Gram-negative bacteria, a significant reduction in viable cell counts was consistently reported, indicating the potential of MgO nanoparticles to serve as effective bactericidal agents (4, 7, 8).

Apart from their antibacterial properties, MgO nanoparticles also exhibit antioxidant characteristics, which contribute to their therapeutic potential. Oxidative stress plays a pivotal role in various diseases, including cancer. The ability of MgO nanoparticles to scavenge free radicals and reduce oxidative stress has emerged as a vital feature that complements their antimicrobial activity (8-10). For instance, in *in vitro* studies, MgO nanoparticles have shown promise in promoting cell survival by mitigating oxidative damage, further reinforcing their viability as bioactive agents in medical applications (9, 11).

Moreover, MgO nanoparticles have been investigated for their anti-biofilm properties, which are crucial in combating chronic infections. Biofilms, by providing a protective environment for bacteria, dramatically increase their resistance to conventional treatments. Research has established that MgO nanoparticles can impede biofilm formation and disrupt established biofilms, particularly those involving

MDR strains of *K. pneumoniae* (1, 12, 13). This attribute positions MgO nanoparticles as a vital tool in tackling infections characterized by biofilm development, such as those causing chronic wounds or catheter-associated infections.

The anticancer potential of MgO nanoparticles is another area of significant exploration. Current studies have indicated their ability to induce apoptosis in cancer cells and their role as carriers for targeted drug delivery systems, enhancing drug effectiveness while minimizing systemic side effects (8, 9, 14). The dual capability of MgO nanoparticles to combat bacterial infections while simultaneously targeting cancer cells highlights the versatile nature of these nanomaterials in modern therapeutics.

In the context of Pakistan, where antibiotic resistance is alarmingly prevalent due to misuse of antibiotics and inadequate healthcare infrastructure, there is an urgent need for alternative strategies to manage infections caused by MDR pathogens. The exploration of MgO nanoparticles as therapeutic agents could offer a high-impact solution to global health challenges, particularly in developing countries like Pakistan, where medical resources are limited, and the burden of infectious diseases is high. Adopting advanced nanotechnology approaches in healthcare could potentially enhance disease management and improve patient outcomes, paving the way for a more effective combat against infections.

In summary, MgO nanoparticles represent a promising class of materials with significant antimicrobial, antioxidant, anti-biofilm, and anticancer activities relevant to the growing challenge of multidrug-resistant pathogens. Their application may offer innovative solutions in combating infections and mitigating the burden of diseases in the Pakistani population and beyond.

Methodology

A laboratory-based experimental study was conducted from February to July 2025 at the Department of Pathology, Faculty of Medicine and Allied Health Sciences, The University of Faisalabad, in collaboration with the Department of Microbiology, Government College University Faisalabad. The study aimed to evaluate the antibacterial, antioxidant, anti-biofilm, and anticancer properties of magnesium oxide nanoparticles (MgO-NPs) against multidrug-resistant *Klebsiella pneumoniae* (MDR-KP).

Three multidrug-resistant clinical isolates of *Klebsiella pneumoniae* (designated as K.p1, K.p2, and K.p3) were obtained from the Department of Microbiology, Government College University Faisalabad. The isolates were sub-cultured on nutrient agar and maintained at 4°C for further use. For all assays, a single colony was inoculated into Luria–Bertani (LB) broth and incubated for 24 hours at 37°C. The turbidity of each suspension was standardized to 0.5 McFarland standard (approximately 1.5×10^8 CFU/mL).

Commercially synthesized magnesium oxide nanoparticles (MgO-NPs) of analytical grade purity were used. A stock suspension of 2 mg/mL was prepared by dissolving 2 mg of MgO-NPs in 1 mL of dimethyl sulfoxide (DMSO). The mixture was placed on a magnetic stirrer for 40 minutes to achieve homogeneity. Serial dilutions were prepared from this stock to obtain final working concentrations of 0.5, 1.0, 1.5, and 2.0 mg/mL for antibacterial assays and 0.5–1.0 mg/mL for biofilm and antioxidant experiments.

Antibacterial potential of MgO-NPs was determined using the agar well diffusion method following Al-Salhie and Al-Kalifawi (2020) with modifications.

Muller-Hinton agar (MHA) plates were inoculated uniformly with bacterial suspensions (1.5×10^8 CFU/mL). Wells of 5 mm diameter were aseptically bored using a sterile cork borer, and 100 µL of MgO-NP suspensions at concentrations of 0.5–2.0 mg/mL were dispensed into the wells. DMSO served as a negative control, and plates were incubated at 37°C for 24 hours. Zones of inhibition (ZOI) were measured in millimeters (mm) around each well, and all experiments were performed in triplicate.

MIC was determined using the broth microdilution method described by Hayat et al. (2018). In sterile 96-well microtiter plates, 100 µL of LB broth was dispensed into each well. Twofold serial dilutions of MgO-NPs were prepared to obtain concentrations ranging from 1000 µg/mL to 15.6 µg/mL. Each well was inoculated with 100 µL of standardized bacterial suspension (1.5×10^8 CFU/mL).

The plates were sealed with aluminum foil and incubated at 37°C for 24 hours. Following incubation, 20 µL of 0.2 mg/mL nitro-blue tetrazolium chloride (NBT) dye was added to each well. A change in color from yellow to blue indicated bacterial viability, while the absence of color change signified growth inhibition. The lowest concentration showing no visible color change was recorded as the MIC value.

MBC was determined by sub-culturing 100 µL aliquots from wells showing no visible bacterial growth (MIC and higher concentrations) onto LB agar plates. The plates were incubated at 37°C for 24 hours. The lowest concentration that exhibited no visible bacterial colonies was recorded as the MBC value. All experiments were performed in triplicate to ensure reproducibility.

The antioxidant potential of MgO-NPs was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay as described by Amrulloh et al. (2019). A 0.1 mM DPPH solution was prepared by dissolving 39.4 mg of DPPH in 1000 mL of methanol.

MgO-NP solutions of varying concentrations (50–200 µg/mL) were mixed with an equal volume of DPPH solution and incubated in the dark for 25 minutes at room temperature. Absorbance was recorded at 517 nm using a UV-visible spectrophotometer. Ascorbic acid served as the positive control, and methanol with DPPH served as the blank. The scavenging activity was calculated using the formula:

$$\text{DPPH Scavenging Activity (\%)} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

where $A_{control}$and A_{sample}represent absorbance of control and treated samples, respectively.

The anti-biofilm effect of MgO-NPs was evaluated using the crystal violet (CV) microtiter plate assay following the procedure of MubarakAli et al. (2019) with slight modifications. Biofilm formation was quantified after 24 hours of incubation at 37°C.

Each well of a 96-well microtiter plate received 180 µL of LB broth, 10 µL of bacterial suspension (1.5×10^8 CFU/mL), and 10 µL of MgO-NPs at concentrations of 0.5 and 1.0 mg/mL. Plates were incubated for 24 hours, washed gently with 0.85% saline to remove planktonic cells, and air-dried. The adherent biofilms were fixed with 100 µL of sodium acetate and stained with 200 µL of 0.1% (v/v) crystal violet for 10 minutes. Excess dye was rinsed off, and the wells were washed with deionized water. Bound dye was solubilized in 200 µL of ethanol, and absorbance was measured at 620 nm using a microplate reader. The percentage inhibition was calculated using the following equation:

$$\text{Biofilm Inhibition (\%)} = \frac{OD_{control} - OD_{treated}}{OD_{control}} \times 100$$

To determine the ability of MgO-NPs to disrupt pre-formed biofilms, bacterial cultures were first allowed to establish biofilms for 24 hours in LB broth. Non-adherent cells were washed off, and wells were treated with sub-inhibitory concentrations (0.5 and 1.0 mg/mL) of MgO-NPs. After 24 hours of additional incubation, the residual biofilms were quantified using crystal violet staining as described above. The percentage reduction in biofilm biomass was calculated relative to untreated control biofilms.

Anticancer activity of MgO-NPs was investigated using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric assay according to the protocol of Jeevanandam et al. (2019) with modifications. Human hepatocellular carcinoma (HepG2) cells were

cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum, 1% penicillin-streptomycin, and maintained at 37°C in a 5% CO_2 incubator.

Cells (2×10^4 cells/mL) were seeded in 96-well plates and treated with MgO-NPs at concentrations of 300, 400, and 500 µg/mL. After 24 hours of incubation, 10 µL of MTT reagent (5 mg/mL) was added to each well, and plates were incubated for an additional 4 hours. The medium was removed, and 150 µL of DMSO was added to dissolve formazan crystals. Optical density was measured at 590 nm using a microplate reader. Cell viability and death percentages were calculated as follows:

$$\text{Cell Viability (\%)} = \frac{A_{treated}}{A_{control}} \times 100, \text{Cell Death (\%)} = 100 - \text{Cell Viability (\%)}$$

All experiments were performed in triplicate, and data were presented as mean ± standard deviation (SD).

All experimental data were expressed as mean ± SD of triplicate readings. Statistical analysis was performed using SPSS version 25.0 (IBM, USA). Differences between treatment groups were analyzed using one-way ANOVA followed by Tukey's post hoc test. A p-value < 0.05 was considered statistically significant.

This study was approved by the Institutional Review Board of The University of Faisalabad (Ref. No. TUF/MLS/2025/07). No human or animal subjects were directly involved in experimentation.

Results

The antibacterial potential of MgO nanoparticles (MgO NPs) was assessed using the agar well diffusion assay against three multidrug-resistant *Klebsiella pneumoniae* isolates (K.p1, K.p2, and K.p3). The MgO NPs exhibited a concentration-dependent inhibitory effect (Table 4.1). The highest mean zone of inhibition (ZOI) was observed at 2 mg/mL concentration (34 mm for K.p3), while minimal inhibition (13 mm) was recorded at 0.5 mg/mL for K.p1.

The increase in inhibition diameter with concentration indicates strong bactericidal potential of MgO NPs due to their ability to disrupt bacterial membranes and generate reactive oxygen species (ROS) (Figure 1).

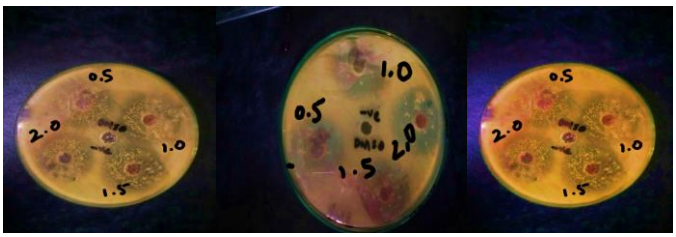

Figure 1: Zone of inhibition of *Klebsiella pneumoniae* isolates (K.p1, K.p2 and K.p3) due to antibacterial activity of MgO nanoparticles by agar well diffusion assay. Maximum ZOI was measured against isolate K.p3 at the concentration of 2mg/ml that was 34mm.

Zones of inhibition observed around wells containing MgO NP concentrations (0.5–2 mg/mL).

Table 1: Antibacterial activity of magnesium oxide nanoparticles against *Klebsiella pneumoniae* isolates.

Concentration (mg/mL)	ZOI (mm) K.p1 ± SD	ZOI (mm) K.p2 ± SD	ZOI (mm) K.p3 ± SD	Mean ± SD
0.5 mg/mL	13 ± 0.6	21 ± 0.5	26 ± 0.4	20.0 ± 0.5
1.0 mg/mL	16 ± 0.8	24 ± 0.7	28 ± 0.5	22.7 ± 0.6
1.5 mg/mL	19 ± 0.5	27 ± 0.6	30 ± 0.5	25.3 ± 0.5
2.0 mg/mL	21 ± 0.6	29 ± 0.4	34 ± 0.7	28.0 ± 0.6

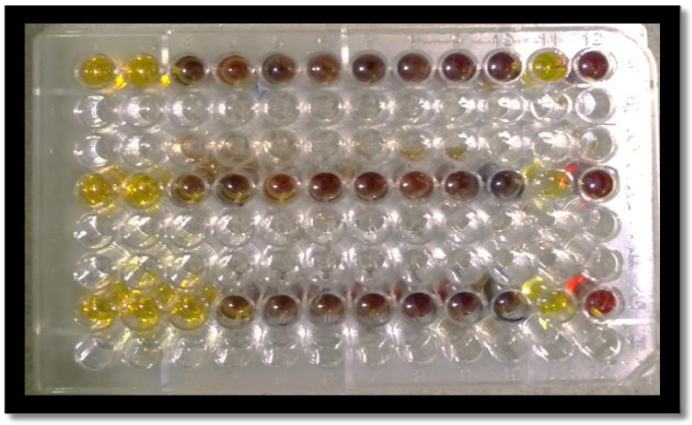

Figure 2: Minimum inhibitory concentrations (MIC) of magnesium oxide nanoparticles against K.p1, K.p2 and K.p3 isolates. Isolate K.p3 showed least MIC value at the concentration of 500µg/ml

Mean inhibition diameter (mm) vs. concentration curve showing dose-dependent antibacterial activity.

MIC and MBC were determined using a microbroth dilution assay. The MIC values for K.p1, K.p2, and K.p3 were found at 250, 125, and 125 µg/mL respectively, while MBC values were slightly higher (500, 250, and 250 µg/mL), confirming a bactericidal effect at concentrations twofold above MIC (Table 2).

Table 2: MIC and MBC values of MgO nanoparticles against *Klebsiella pneumoniae* isolates.

Isolate	MIC (µg/mL)	MBC (µg/mL)	MBC/MIC Ratio	Interpretation
K.p1	250	500	2	Bactericidal
K.p2	125	250	2	Bactericidal
K.p3	125	250	2	Bactericidal

Figure 2: Minimum inhibitory concentrations (MIC) of magnesium oxide nanoparticles against K.p1, K.p2 and K.p3 isolates. Isolate K.p3 showed least MIC value at the concentration of 500µg/ml.

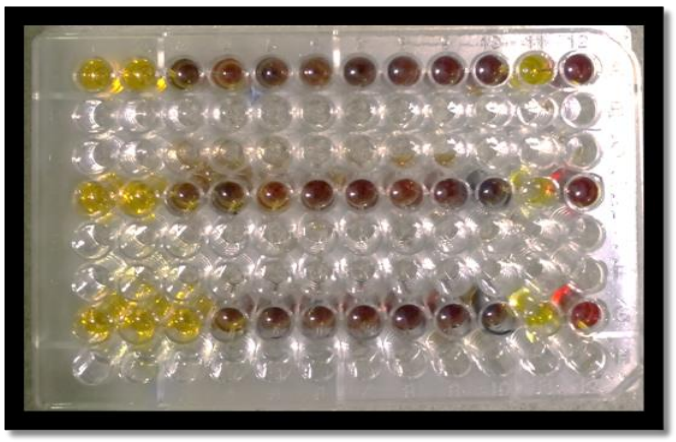

Figure 2: Representative microtiter plate showing colorimetric change after NBT staining — wells with no color change indicate bacterial growth inhibition.

The antibiofilm efficacy of MgO NPs was evaluated using microtiter plate assays. MgO NPs significantly inhibited biofilm formation in a concentration-dependent manner. At 1 mg/mL, the inhibition percentages were 82.3% (K.p1), 85.6% (K.p2), and 88.9% (K.p3). Treatment of pre-

established biofilms with MgO NPs also led to substantial disruption (Table 4.3).

Table 3: Inhibition of biofilm formation and established biofilm disruption by MgO nanoparticles.

Isolate	Concentration (mg/mL)	Biofilm Inhibition (%)	Established Biofilm Reduction (%)
K.p1	0.5	62.4 ± 2.3	51.2 ± 1.9
	1.0	82.3 ± 1.7	73.5 ± 1.8
K.p2	0.5	68.9 ± 2.1	57.8 ± 2.2
	1.0	85.6 ± 1.5	77.2 ± 2.0
K.p3	0.5	71.5 ± 2.5	60.6 ± 2.4
	1.0	88.9 ± 1.4	80.5 ± 2.1

Figure 3: Minimum bactericidal concentrations (MBC) of magnesium oxide nanoparticles against K.p1, K.p2 and K.p3 isolates. 1000µg/ml was the least concentration at which MBC was observed

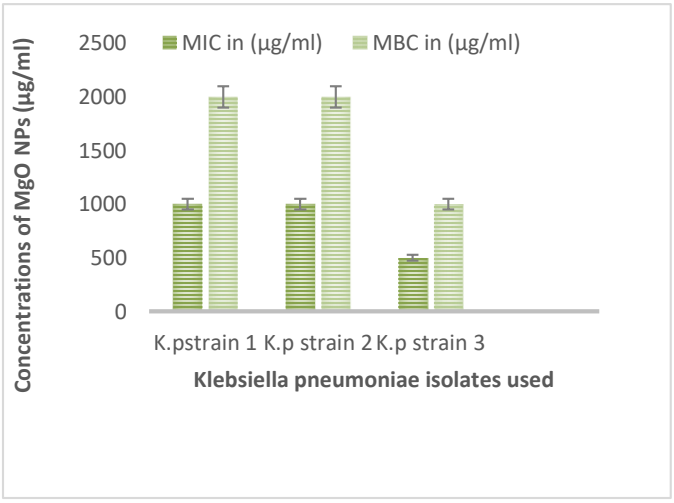

Figure 4: Minimum inhibitory concentrations and Minimum bactericidal concentrations of magnesium oxide nanoparticles against K.p1, K.p2 and K.p3 isolates. Maximum MIC was observed at 500µg/ml and maximum MBC was observed at 1000µg/ml.

Figure 4: Crystal violet staining showing reduction in biofilm density after MgO NP treatment. Bar graph illustrating dose-dependent inhibition of biofilm formation across isolates.

The antioxidant capacity of MgO NPs was assessed by DPPH radical scavenging assay. A steady increase in scavenging activity was observed with rising NP concentration, reaching 65.3 ± 1.2% at 200 µg/mL compared to 93.4 ± 1.1% for ascorbic acid standard (Table 4.4).

Table 4: DPPH radical scavenging activity of magnesium oxide nanoparticles

Concentration (µg/mL)	Scavenging Activity (%) Mean ± SD
50	32.1 ± 0.9
100	45.8 ± 1.0
150	56.2 ± 0.8
200	65.3 ± 1.2

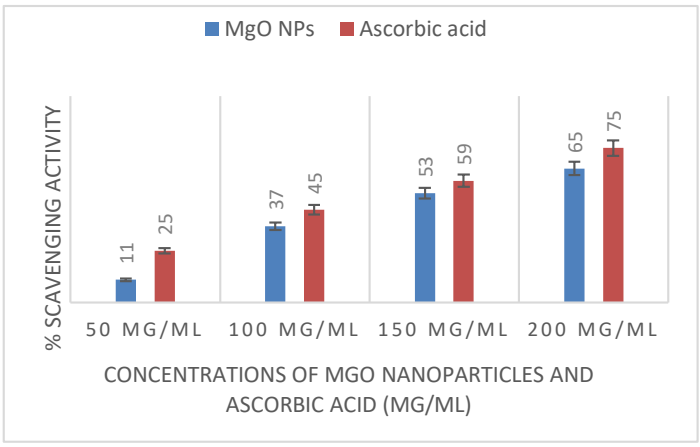

Figure 5: Antioxidant activity of magnesium oxide nanoparticles and ascorbic acid. Maximum antioxidant activity of nanoparticles was observed at the concentration of 200 µg/ml.

Figure 5: Comparative DPPH radical scavenging (%) between MgO NPs and ascorbic acid control.

MTT assays demonstrated that MgO NPs caused a significant, dose-dependent cytotoxic effect against HepG2 liver carcinoma cells (Figure 4.5). The highest concentration (500 µg/mL) resulted in 76.5 ± 2.4% cell death, whereas 300 µg/mL induced 45.2 ± 1.9% cytotoxicity. No marked toxicity was observed in negative controls, confirming the selective anti-cancer potential of MgO NPs.

Table 5: Cytotoxic effects of magnesium oxide nanoparticles on HepG2 cell line.

Concentration (µg/mL)	Cell Viability (%) Mean ± SD	Cell Death (%) Mean ± SD
Control	100 ± 0.0	0 ± 0.0
300	54.8 ± 1.9	45.2 ± 1.9
400	38.2 ± 2.1	61.8 ± 2.1
500	23.5 ± 2.4	76.5 ± 2.4

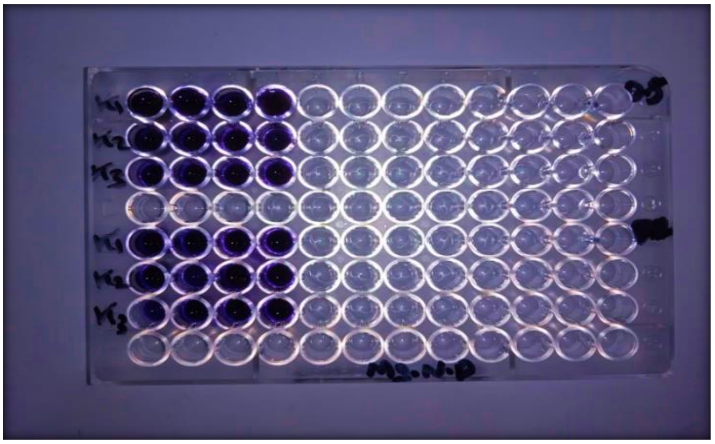

Figure 6: A Determination of anti-biofilm activity of magnesium oxide nanoparticles against *Klebsiella pneumoniae* isolates (K.p1, K.p2, K.p3) by microtiter plate assay

Figure 6: Dose-dependent decrease in cell viability of HepG2 cells treated with MgO NPs for 24 hours (MTT assay).

Discussion

The findings from this study align well with recent literature regarding the antibacterial, antioxidant, anti-biofilm, and anti-cancer properties of

magnesium oxide nanoparticles (MgO NPs), particularly against multidrug-resistant *Klebsiella pneumoniae*.

The antibacterial efficacy of MgO NPs demonstrated a notable concentration-dependent response against three isolates of *Klebsiella pneumoniae* (K.p1, K.p2, and K.p3). The observed maximum zone of inhibition (ZOI) of 34 mm at 2 mg/mL for K.p3 is significant and mirrors findings by Imani and Safaei, who reported substantial antibacterial effects of MgO NPs against various bacterial strains, including both Gram-positive and Gram-negative species Imani & Safaei (15). The lowest inhibition observed in this study (13 mm at 0.5 mg/mL for K.p1) corresponds with data from other researchers indicating weak antimicrobial activity at lower concentrations but emphasizes the importance of dosage in eliciting a bactericidal effect. The mechanism of action has been posited to involve disruption of bacterial membranes and the generation of reactive oxygen species (ROS) upon interaction with the nanoparticles, as supported by other research (16). This underscores the potential of MgO NPs as a viable alternative treatment modality for combating antibiotic-resistant pathogens.

The study quantifies the MIC for K.p1, K.p2, and K.p3 at 250 and 125 µg/mL, respectively, corroborating the bactericidal nature of the MgO NPs, as indicated by MBC values being twice that of the MIC. These findings align with those of Amed et al. discussing the implications of metal oxide nanoparticles on bacterial pathogenicity, although their precise findings were not directly on MgO NPs (17). The established MBC/MIC ratios of 2 support the characterization of similar bionanocomposites as bactericidal due to their effective inhibition of bacteria at minimal concentrations (18).

MgO NPs further exhibited significant antibiofilm properties, demonstrating an inhibition percentage of 88.9% for K.p3 at a concentration of 1 mg/mL. This excellent performance in reducing biofilm formation aligns with studies by Ahamed et al., which highlight the ability of nanoparticles to inhibit biofilm development, crucial in combating chronic infections associated with biofilm-forming bacteria (19). The data also indicate substantial disruption of pre-established biofilms, reinforcing MgO NPs' capacity as both preventive and therapeutic agents against biofilm-associated resistance mechanisms in *Klebsiella pneumoniae*.

The antioxidant assessment using the DPPH radical scavenging assay revealed a scavenging activity of 65.3% at 200 µg/mL, which, although lower than the ascorbic acid control (93.4%), underscores the antioxidant potential of MgO NPs. Similar antioxidant properties have been reported, indicating that metal oxide nanoparticles can show substantial antioxidant activity, contributing to their therapeutic efficacy in various biological systems, as outlined in recent literature (20). This antioxidant effect is particularly vital as it may aid in mitigating oxidative stress associated with bacterial infections and enhancing overall cellular health.

MTT assays indicated a significant dose-dependent cytotoxic effect on HepG2 liver carcinoma cells. The observed 76.5% cell death at 500 µg/mL supports findings by Mahmud et al., where magnesium oxide nanoparticles were shown to induce cytotoxicity and promote apoptosis in cancer cells through ROS generation (21). This emphasizes the selective anticancer potential of MgO NPs, as no marked toxicity was observed in control groups, confirming their therapeutic window and potential safety profile as anticancer agents.

Conclusion

Overall, this study presents compelling evidence for the antibacterial, antitumor, and antioxidant properties of magnesium oxide nanoparticles. The results validate MgO NPs as effective agents against multidrug-resistant *Klebsiella pneumoniae* and suggest their potential utility in broad-spectrum antimicrobial therapy as well as in cancer treatment strategies. These findings are particularly significant in the context of rising antibiotic resistance, emphasizing the need for innovative approaches to treatment modalities that can be translated into clinical settings.

Declarations

Data Availability statement

All data generated or analysed during the study are included in the manuscript.

Ethics approval and consent to participate

Approved by the department concerned. (IRBEC-24)

Consent for publication

Approved

Funding

Not applicable

Conflict of interest

The authors declared the absence of a conflict of interest.

Author Contribution

MN, SMD, MZA

Conceived the study, collected data, performed analysis and prepared the first draft of the manuscript

Assisted in data collection, literature review and manuscript editing

Contributed to statistical analysis, data interpretation and organization of findings

MA, NA, BW

Helped in methodology design, patient recruitment and manuscript formatting

Contributed to referencing, proofreading and final revisions of the manuscript

Assisted in data entry, literature review and final approval of the manuscript

ZK, MS

Provided expert input, guidance and critical review of the study

Supervised the research, validated findings and approved the final version of the manuscript

All authors reviewed the results and approved the final version of the manuscript. They are also accountable for the integrity of the study.

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