Effect of Magnesium Oxide Nanoparticles on Biofilm Formation Against Multidrug Resistant *Klebsiella pneumoniae* Isolates

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Abstract- ***The main purpose of this investigation was to access the impact on biofilm formation, antibacterial activity and anticancer potential of magnesium oxide nanoparticles (MgO NPs) against three multidrug resistant Klebsiella pneumoniae isolates. Agar well diffusion method was applied for the evaluation of antibacterial potential and maximum activity was reported at 2mg in all three isolates (21mm, 29mm, 34mm). MIC and MBC were calculated by broth micro-dilution method, two isolates showed MIC at 1000µg/ml and MBC at 2000µg/ml. While, one isolate showed MIC at 500µg/ml and MBC at 1000µg/ml. Effect of MgO nanoparticles was studied through microtiter plate assay using crystal violet stain. Magnesium oxide nanoparticles exhibited maximum reduction in biofilm formation against K.p3 isolate at the concentration of 1mg/ml that was 63.21% and on established biofilm it was 59.78%. Anticancer potential was accessed by MTT assay, research showed that anticancer activity of MgO NPs was dose-dependent manners so that the higher anticancer activity was correlated with high concentration of nanoparticles. Maximum anticancer activity was observed at the concentration of 500µg/ml that was 56.82-60.54%.***

I. INTRODUCTION

In this cutting-edge-era bacteria and other microorganisms are causing serious illness and infections in human body, antibiotics are being used to control these infections. However, antibiotic resistance has been emerged in bacteria due due to gene transfer, gene mutations, over-dosage and rapid production of antibiotics [1]. Several antibiotic resistant bacteria have been developed into multidrug resistant bacteria (show resistance against several drugs) which are not easy to treat and become the reason of numerous deaths [2]. *Klebsiella pneumoniae* isa gram negative and multidrug resistant bacteria that has ability to form biofilm (organized colonies of microorganisms). Biofilm is stress resistant and stable structure (not easy to destroy), which protects the microbes from unsuitable (biological, chemical and physical) conditions [3]. Microbial biofilms are dangerous for health, a wide variety of antibiotics have been developed which were considered as a final solution to fight against multidrug resistant (MDR) and biofilm associated infections but these antibiotics have failed to perform their functions against them [4]. So, there is an urgent need to develop an effective antibiotic to overcome these challenges.

Nanoparticle (alternative to the antibiotics) technology is an emerging and advance strategy in the field of medicine which provides an excellent platform to overcome these problems [5]. Different nanoparticles (MgO, ZnO, CuO, CaO, Au) exhibited antibacterial effect against a wide range of pathogenic bacteria and also help to eliminate biofilm [6]. Food and Drug Administration of United State have been declared the magnesium oxide nanoparticles as nontoxic and an antibacterial agent. Magnesium oxide nanoparticles exhibited their effect against both gram positive and gram negative bacteria, moreover nanotechnology inspires the use of magnesium as an antibiotic agent because it is also an important nutrient of human body [7]. So the aim of this study was to investigate the effect of magnesium oxide nanoparticles on biofilm formation against multidrug resistant *Klebsiella pneumoniae* isolates.

II. RELATED WORK

Nanotechnology offers a way to fight against biofilm associated multi-drug resistant bacteria. Various nanoparticles have been recognized as valuable

antimicrobial agent. So, the current study was designed to see the effect of magnesium oxide on biofilm associated multidrug resistance bacteria.

III. MATERIALS AND METHODS

A. Collection of bacterial isolates:

Three MDR isolates of *Klebsilla Pneumoniae* (K.p1, K.p 2, K.p 3) were provided by Department of Microbiology, Government College University Faisalabad.

B. Preparation of bacterial cultures:

Single colonies of three isolates were removed from agar plates and mixed in Luria-Bertani (LB) broth separately to achieve bacterial suspension. Turbidity of suspensions was adjusted according to the 0.5 McFarland standards (1.5×10^8 CFU/ml).

C. Evaluation of Antibacterial Activity of MgO NPs:

Antimicrobial potential of magnesium oxide NPs was assessed by agar well diffusion assay [8]. Muller Hinton agar plates were swabbed with bacterial after that, wells were made on swabbed agar plates and four different concentrations of MgO nanoparticles (2, 1.5, l and 0.5 mg/ml) were dispensed into the marked wells with the help of micropipette under aseptic conditions and DMSO was used as a negative control. Prepared plates were incubated at 37ºC for 24 hours and antibacterial activity was evaluated by measuring the ZOI (expressed in mm) against tested bacterial isolates.

D. Determination of MIC for MgO nanoparticles:

MIC of magnesium oxide nanoparticles was evaluated using broth dilution method [5]. 96 wells microtiter plate was used, LB broth was added in all 12 wells of microtiter plate. Twofold Serial dilutions of MgO nanoparticles was performed by adding 100 µl dissolved magnesium oxide nanoparticles into 1st well and then serially diluted up to 10th well of microtiter plate. Finally bacterial inoculum of 100 µl was dispensed up to 10th well and then into 12th well. Positive control presented by 12th well had bacterial inoculum and LB broth only, while 11th well was used as a negative control had only LB broth. After that the plates were properly covered with aluminium foil and incubated for 24h at 37 ºC. After incubation, viability of bacterial cells was determined using the redox nitro-blue tetrazolium chloride (NBT) dye by a color change from yellow to blue. Change in color from yellow to blue indicated the presence of viable cells.

E. Determination of MBC for MgO Nanoparticles:

MBC defined the lowest concentration of an antibacterial agent that needed to kill bacteria under a specific set of conditions. Minimum inhibitory concentration of Magnesium Oxide nanoparticles was evaluated [5] by sub-culturing the 100µl of broth dilutions (MIC and above) containing no visible growth on LB agar plates followed by incubation for 24 hours. Lowest concentration exhibiting no growth on LB agar plates was considered as minimum bactericidal concentration.

F. Biofilm formation assay:

Biofilm formation activity was quantified followed the method of 96 wells plate with some modifications [9]. Two different concentrations (0.5mg/ml, 1mg/ml) of magnesium oxide nanoparticles were used, wells were inoculated with 10 µl bacterial cultures, 10 µl nanoparticles of magnesium oxide and 180 µl of LB broth. After that the plate was sealed with aluminum foil and incubated at 37°C for 24 hours. The contents of the wells were discarded to remove non-adhered cells. Wells were washed with normal saline (0.85 % NaCl) and air dried for 30 minutes. The adhered cells were examined by fixing with sodium acetate and further staining with 200µl of 0.1 % (v/v) crystal violet (CV) for 10 minutes at room temperature. After staining, the excessive dye was removed and washed each well thrice with 200µl of deionized water. To quantify the biofilm, 200µl ethanol added into the well and absorbance was measured at 620 nm by the use of microplate reader, negative control and positive controls were also used in this assay.

The %age of biofilm inhibition was calculated by the following formula.

$$\%\text{age biofilm inhibition} = \frac{\text{OD620 Control} - \text{OD620 Treated}}{\text{OD620 Control}} \times 100$$

G. Effect of magnesium oxide nanoparticles on the established biofilm:

The effect of metallic magnesium oxide nanoparticles on established biofilm was monitored followed the procedure with minor modifications. Biofilms were established for 24 hours and then treated with magnesium oxide nanoparticles at sub-inhibitory concentrations (0.5mg/ml, 1mg/ml). Quantification of attached cells was done by washing the wells with

normal saline and staining with 200µl of 0.1 % crystal violet (CV) for 10 minutes. After staining, the excessive dye removed by washing each well thrice with 200µl deionized water, 200µl of 95% ethanol was added and absorbance measured at 620 nm.

H. Evaluation of anticancer activity of MgO nanoparticles:

Anticancer activity of Magnesium oxide nanoparticles evaluated using 3-(4, 5- dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) method [10]. HepG2 human liver cell lines were used, the cells were maintained in a suitable medium.

The cell density was 2×10^4 cell/mL for anticancer potential assays. 96 wells culture plate was used for experimentation, 100 µl cells were dispensed into each well and treated with different concentrations (500 µg/ml, 400 µg/ml and 300 µg/ml) of nanoparticles, negative control was also used in this assay (without nanoparticles treatment) and incubated for 24 hours under suitable conditions. After incubation period, the sample with the medium was removed from the wells and the cells were washed with PBS (pH 7.4). After that, 10 µl MTT dye (5mg/ml) was added into the wells and incubated for 4 hours then, 150 µl DMSO was added to each well to take optical density (OD) at 590 nm using reader plate. Each experiment was performed in triplet.

The percentage of cell death was calculated according to following equation

$$\%\text{age cell death} = \frac{\text{Control} - \text{Treated}}{\text{Control}} \times 100$$

IV. RESULTS AND DISCUSSION

Magnesium oxide nanoparticles exhibited antibacterial activity against three selected *Klebsiella pneumoniae* isolates. Zone of inhibition increased with increase in the concentration of MgO NPs. Higher antibacterial activity was observed at 2mg in all tested isolates (Fig.1) and (Fig.2). In the current study, maximum zone of inhibition was measured against isolate 3 (34mm) and slightly less activity was measured in isolates 1(21mm) and 2 (29mm) (Table.1). Similarly, Hayat et al [13] reported significant antibacterial activity of magnesium oxide nanoparticles. In another study, antibacterial potential of MgO nanoparticles was tested against pathogenic gram negative and gram positive bacteria [15].

The MIC is the lowest concentration of antimicrobial agents that completely inhibits growth of the microorganisms, (Fig.3) show the results of MIC values of magnesium oxide nanoparticles with different concentrations against *Klebsiella pneumoniae* isolates. Least MIC value of MgO nanoparticles was observed against *Klebsiella pneumoniae* isolate 3 (500 µg/ml) and other two isolates exhibited MIC value at the concentration of 1000 µg/ml (Table.2). Gokulakrishnan et al [16] referred the inhibitory action of different nanoparticles against pathogenic microbes, they observed that MgO and other nanoparticles are effective to fight against pathogenic bacteria. Minimum bactericidal concentration (MBC) values were evaluated on agar plates by the absence of growth of bacteria. Minimum inhibitory concentration values were observed at 2000µg/ml, 2000µg/ml and 1000µg/ml against isolates K.p1, K.p2 and K.p3 respectively (Table.2).

We analyzed the impact of magnesium oxide NPs at different concentrations on the formation of biofilm by *Klebsiella pneumoniae* isolates. It was noticed that when the concentration of nanoparticles was increased the inhibition of biofilm was also increased, which attributed directly to the dose-dependent manners. It was observed that the percentage of reduction in biofilm formation by magnesium oxide nanoparticles against *Klebsiella pneumoniae* isolates (K.p1, K.p2, K.p3) were 32.14%, 56.52 and 58.04% by using the concentration of 0.5 mg/ml. However, the % of biofilm inhibition was 42.85%, 59.23% and 63.21% at 1mg/ml concentration. The maximum biofilm inhibition was observed against K.p3 isolate (Fig.4). Biofilm inhibitory effect of MgO nanoparticles against Bacterial Leaf Blight pathogen was reported earlier [17].

Effects of MgO nanoparticles on already established biofilm was determined at different concentrations. Percentage of inhibition of established biofilm was 30%, 55.60% and 54.89% against *Klebsiella pneumoniae* isolate (K.p1, K.p2 and K.p3) respectively at the concentration of 0.5mg/ml. However, the percentage of inhibition of established biofilm was 40%, 56.18% and 59.78% at the concentration of 1mg/ml respectively against all K.p1,

K.p2 and K.p3 (Fig.5). Similarly, Iribarnegaray et al [18] described the anti-biofilm activity of magnesium oxide nanoparticles

Anticancer potential of MgO nanoparticles was investigated at different concentrations against used HepG2 human liver cell lines. Results showed that anti-cancer activity of MgO NPs was dose-dependent, percentage of cancer cells death were 44.48-51.56%, 51.67-60% and 56.82-60.54% at the concentrations of 300 µg/ml, 400 µg/ml and 500 µg/ml receptively. Maximum anticancer activity was observed at the concentration of 500µg/ml (Fig.6). In another study anticancer potential of MgO NPs was tested against (adipose and VERO) cell lines [14]. Similarly, Jebali et al [19] reported anticancer potential of different nanoparticles against mice cells and observed that anticancer activity of nanoparticles was in dose-dependent manners.

CONCLUSION

In the present study, magnesium oxide nanoparticles exhibited a valuable reduction in biofilm formation and also showed antibacterial and anticancer activity against three *Klebsiella pneumoniae* isolates. So, these nanoparticles can be used as an alternative of antibiotics to treat infections which are caused by biofilm associated multidrug resistant bacteria.

Table.1. Antimicrobial activity of MgO NPs against three *Klebsilla pneumoniae* isolates

Bacterial isolates	Zones of inhibition in mm			
	0.5 mg/ml	1 mg/ml	1.5 mg/ml	2 mg/ml
K.pneumoniae 1	13	16	19	21
K.pneumoniae 2	21	24	27	29
K.pneumoniae 3	26	28	30	34

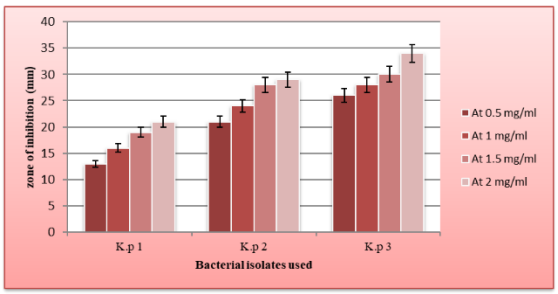
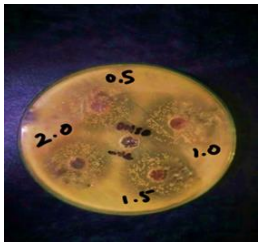


Fig.1. Zones of inhibition of *Klebsiella pneumoniae* isolates, Maximum ZOI was measured against isolate K.p3 at the concentration of 2mg/ml that was 34mm.

(1)



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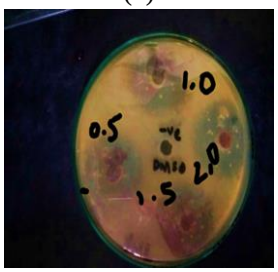

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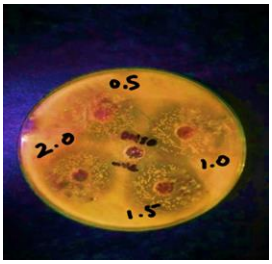

Fig.2. Zone of inhibition of *Klebsiella pneumoniae* isolates (K.p1, K.p2 and K.p3) due to antibacterial activity of MgO nanoparticles by agar well diffusion assay.

Table.2. Minimum Inhibitory Concentrations and Minimum Bactericidal Concentration of MgO NPs against selected *Klebsiella pneumoniae* isolates.

Bacterial isolates	MIC (µg/ml) of magnesium oxide nanoparticles	MBC (µg/ml) of magnesium oxide nanoparticles
Klebsiella pneumoniae 1	1000	2000
Klebsiella pneumoniae 2	1000	2000
Klebsiella pneumonia 3	500	1000

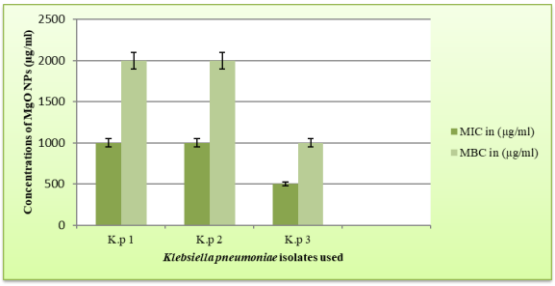

Fig.3. Least MIC was observed at 500µg/ml and least MBC was observed at 1000µg/ml.

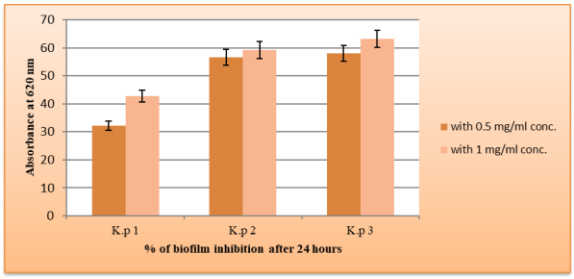

Fig.4. Magnesium oxide nanoparticles exhibited maximum reduction in biofilm formation against K.p3 isolate at the concentration of 1mg/ml that was 63.21%.

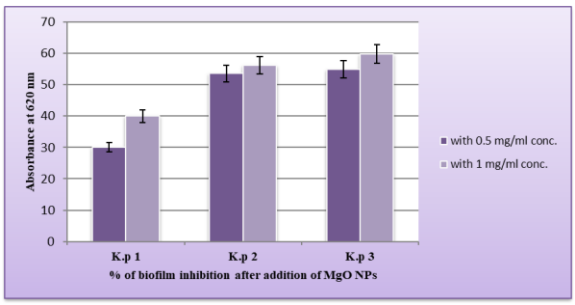

Fig.5. Percentage of reduction in established biofilm, by increasing the concentration of MgO nanoparticles established biofilm was decreased.

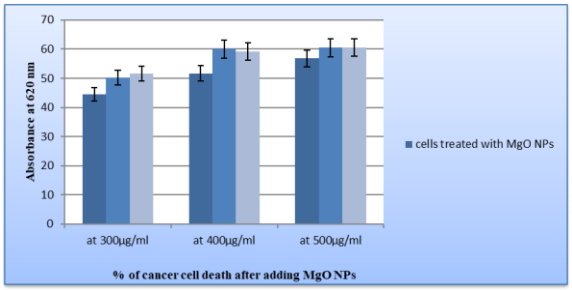

Fig.6. At the concentration of 500µg/ml maximum cell death was observed, so by increasing the concentration of nanoparticles anticancer activity increased.

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