Zinc oxide nanoparticles and antibiotics mediated combinatorial approach to enhance antibacterial potential

Article · March 2020

DOI: 10.31838/ijpr/2020.12.03.007

11 authors, including:

Hardeep Singh Tuli
Maharishi Markandeshwar University, Mullana
90 PUBLICATIONS **1,204** CITATIONS
SEE PROFILE

Ajinkya Nene
Chubu University
14 PUBLICATIONS **22** CITATIONS
SEE PROFILE

Zahoor Abbas
Maharishi Markandeshwar University
2 PUBLICATIONS **1** CITATION
SEE PROFILE

Vinit Prakash
Maharishi Markandeshwar University, Mullana
12 PUBLICATIONS **94** CITATIONS
SEE PROFILE

ISSN 0975-2366
DOI:https://doi.org/10.31838/ijpr/2020.12.03.007

Research Article

Zinc oxide nanoparticles and antibiotics mediated combinatorial approach to enhance antibacterial potential

HARDEEP SINGH TULI[1], GURPREET KAUR[2], SHIVANI SOOD[3], DIKSHA SHARMA[1], PRABHJOT KAUR[1], PARDEEP KUMAR[4], AJINKYA G. NENE[5], SANDEEP PUNIA[6], ZAHOOR ABBAS[7], VINIT PRAKASH[7], MANOJ KUMAR[7*]

[1]Department of Biotechnology, Maharishi Markandeshwar (Deemed to be)University, Mullana, Ambala.
[2]Department of Physics, Maharishi Markandeshwar (Deemed to be) University, Mullana, Ambala.
[3]Department of Biotechnology, Mukand Lal National College, Yamuna Nagar, India
[4]Department of Electronics Science, Kurukshetra University, Kurukshetra.
[5]Applied Science Innovations Private Limited, Pune,
[6]Department of Biotechnology, Multani Mal Modi College, Patiala, India.
[7]Department of Chemistry, Maharishi Markandeshwar University, Sadopur-Ambala
*Corresponding Author
Email ID: manojraju27@gmail.com
Received: 24.01.20, Revised: 09.02.20, Accepted: 21.03.20

ABSTRACT

Objectives: Bacterial diseases are increasing at an alarming rate. Therefore, the intention of this study was to examine the antibacterial action of Zinc oxide nanoparticles and their combined effect with different antibiotics including Erythromycin, Azithromycin, and Norfloxacin against various human pathogenic gram-positive (*Staphylococcus*) and gram-negative (*Escherichia coli, Klebsiella, Shigella, Pseudomonas*) bacterial strains.
Methods: Synthesized ZnO nanoparticles were analyzed by using UV spectroscopy. The antibacterial activity of ZnO nanoparticles at different concentrations was determined by the agar well diffusion method.
Results: The results showed that ZnO nanoparticles possessed antibacterial effects and synergistic activities even on drug resistance Staphylococcus sp. We get the results of MIC in the range of 35-50 μg/ml. The synergistic action of antibacterial agents can reduce the need for high dosages, minimize side effects and reduces multidrug resistance. We have observed the highest synergistic effects against *Escherichia coli* and *Staphylococcus* sp.
Conclusion: These results signify that this nanoparticle potentiates the antimicrobial action of possible utilization of nano compounds in combination effect against drug-resistant bacterial strains.

Keywords: Zinc Oxide, Antimicrobial, Characterization, Metal Nanoparticle

INTRODUCTION

Currently, nanoparticle synthesis is being explored exponentially due to their amazing application in medical field [1]. Nanoparticles have a high surface area which is utilized to load drugs and to enhance their distinct feature. Nanoparticles possess great antifungal and antibacterial activity [1-3]. Zinc oxides have promising uses in medical health [4]. Zinc oxide nanoparticles were reported as non-toxic to human cells in several studies [2]. Zinc oxide is regarded as safe for nutrient compounds by the U.S. These days antibiotics drug resistance represents a serious problem to the public health. According to a report, a large number of deaths are caused due to antibiotic-resistant bacterial infection. Nanotechnology could have potential to diminish drug resistance by growing the efficiency of drug synergistically [1]. Thereby, the synergism of drugs with nanoparticle may expose new paths and revolutions in existing therapeutic boundaries. Previous, reports has suggested the role of zinc-based nanoparticles to have synergistic effect with commonly used antibiotics towards infectious diseases [6] [7]. Study conducted by Bhande et.al.,2013 [8] investigated the synergistic effect of zinc oxide nanoparticles (ZnO NPs) with existing antibiotics to inhibit the growth of *E. coli, K. pneumoniae, S. paucimobilis, and P. aeruginosa* and suggested their implications in urinary tract infection treatment.

MATERIALS AND METHODS

A. Glassware and Apparatus

All glass wares and apparatus such as measuring cylinders, beakers, conical flasks, funnel, test tubes, petri plates, filter paper, Eppendorf tube, micropipette, Hot plate, Analytical balance, Autoclave, Incubator, Centrifuge, Furnace, Hot air oven, Magnetic stirrer, Water bath etc. were used from microbiology lab of biotechnology department MMDU, Mullana.

B. Procurement of bacterial culture

The antibacterial activity of the ZnO nanoparticles were tested individually on bacterial (gram-positive and gram-negative). All bacterial strains were obtained from microbiology laboratory MMDU, Mullana, Ambala. The gram-positive bacterial strains used was *Staphylococcus aureus*, and gram-negative bacterial strain used were *Escherichia coli*, *Klebsiellasp, Shigellasp,* and *Pseudomonas sp*. Bacterial strains were maintained on nutrient agar at 4°C and sub-cultured after every2-3 weeks in our laboratory.

C. Chemicals

For all experimental studies pure and analytical grade chemicals were used. For synthesis of ZnO nanoparticles zinc acetate and potassium hydroxide (KOH) and methanol of HIMEDIA were used. Nutrient agar media and nutrient broth of HIMEDIA were used for growth of microorganisms. All these chemicals were collected from the microbiology lab of Biotechnology department MMDU, Mullana (Ambala)

Experimental work

Synthesis of zinc oxide nanoparticles: Zinc oxide nanoparticles were synthesized by the low-temperature sol-gel method. Basically, 0.09 g $Zn(CH3COOH)_2$ and 0.12 g KOH were dissolved into 50 ml methanol and mixed rapidly while stirring at 1,000 rpm at 60ºC for 5 min and then cooled to room temperature so as to obtain a transparent solution of Zinc oxide nanoparticles. The resulting nanoparticles were then washed with distilled water by using a centrifugal method several times to remove the organic impurities present in it. The Zinc oxide nanoparticles thus prepared were then dried by placing them in hot air over for half an hour and the white color powder of Zinc oxide nanoparticles [8].

Characterization of the chemically synthesized nanoparticles:

D. UV-Vis spectroscopy

The formation of nanoparticles and their optical properties were analysed via UV-visible Spectrophotometer at wavelength200-800 nm with ZnO nanoparticles using Dual beam spectrophotometer operated at a resolution of 50nm in the Biotechnology Department, MMDU University, Mullana in the range of 200 to 800 nm.

E. Preparation of standard culture

A loop full culture of the different strains was inoculated in separate 25 ml of nutrient broth in a conical flask and incubated at room temperature (37ºC) on a rotary shaker for 24 hrs. to activate the test bacteria.

F. Maintenance and preservation of cultures of bacteria

To maintain the culture of bacterial strain, we periodically shift the old bacterial culture on new nutrient agar petri plates by quadrat striking method. Then placed these petri plates in an incubator for 24 hours at 37ºC.

G. Suspension formation of nanoparticles with antibiotics

Evaluation of synergistic anti-bacterial activity Norfloxacin, Azithromycin and Erythromycin were selected in the present study. All drugs was obtained from Pharmacy department MMDU, Mullana, Ambala. 1mg/ml of respective antibiotics was mixed with respective concentration of zinc oxide nanoparticle, the mixture was kept under stirring for three hours at room temperature. After stirring, the suspension was left overnight and used for further studies. Nanoparticle and drug synergism with different concentration (50,100,200 and 400μg/ml) were used for anti-bacterial activity against tested organisms as described earlier.

H. Antibacterial activity tested by agar well diffusion method

To determine the antibacterial activity of ZnO nanoparticles, nutrient agar (Hi media, India) was used as culture media. The antibacterial activity of ZnO nanoparticles at different concentrations were determined by agar well diffusion method as discussed previously (10, 11).

RESULTS

Chemical synthesis and characterization of nanoparticles

Zinc oxide nanoparticles (ZnO-NPs) were chemically synthesized and analysed by UV-Vis spectroscopy in order to insure the completion of the nano formation and detection of their characteristics.

A. Visual inspection

The synthesis of Zinc oxide nanoparticles was indicated by the formation of white precipitate[9]. After drying these precipitates, the white colour powder was obtained.

B. UV-Vis spectroscopy

For zinc oxide nanoparticles earlier reported broad absorption peak in the range of 340-380 nm which is a characteristic band for the pure ZnO nanoparticles[12]. In our study, we also found maximum absorption in the region of 360 to 380 nm as shown in the Figure 1, no other significant peaks were observed in the spectrum besides ZnO NPs.

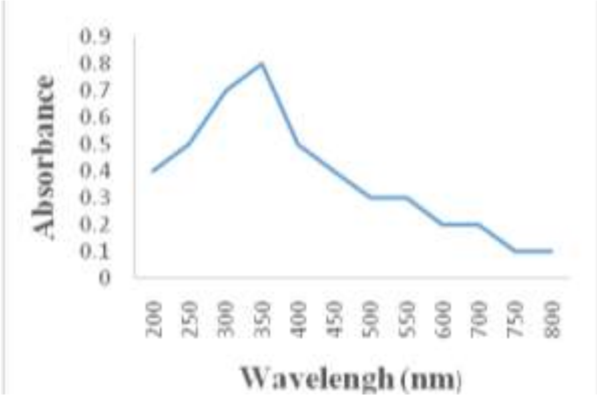

Fig.1: The graph showing the absorption at 360 nm by ZnO NPs.

Antibacterial activity of ZnO nanoparticles

Antibacterial activity of ZnO nanoparticles were determined by well diffusion method. Maximum anti-bacterial effect of NPs were at 400 μg/ml (*E.coli*; 22 mm, *Klebsiellasp;* 20mm, *Staphylococcus sp;* 27mm, *Pseudomonas sp;* 22mm, and *Shigella sp;* 23 mm). The minimum inhibitory zone formed by *Klebsiellasp* was observed at 50μg/ml concentration. Against *E.coli, Pseudomonas sp.* and *Shigella,* ZnO-NPs have shown minimum inhibitory concentration at 48μg/ml concentration. In recent years treatment of bacterial infection has become serious concern due to the increased resistance against antibiotics. It remains major challenge to treat intracellular infections because many antimicrobials are not able to diffuse easily through cell membrane, thus have low reactivity inside the cell. Therefore, it is important to develop an alternate therapeutical strategies including new class of antibiotics, which can kill drug resistant strains without harming the cell.

DISCUSSIONS

Our findings revealed that antibacterial activity of ZnO nanoparticles increases as the concentration of nanoparticles increases. We observed maximum inhibitory zone at 400 μg/ml concentration of ZnO nanoparticles for all pathogenic strains that we were tested i.e. *Klebsiellasp, Shigella, Pseudomonas sp, Staphylococcus sp* and *E. coli*. As Thangam et al.,2014 [13] have reported the zone of inhibition formed at concentration range 100-400 μg/ml of ZnO nanoparticles against pathogenic bacterial strains. We observed the MIC values for all pathogenic strains including *Klebsiellasp, Shigella, Pseudomonas sp, Staphylococcus sp* and *E. coli* at 50, 48, 48 35 and 48 μg/ml respectively. Fattah .et al.,2017 [14] have reported the MIC values for both *E. coli* and *Klebsiella sp*. in the range of 50-100 μg/ml. We have observed that *E. coli* and *Klebsiellasp* both were resistant to Azythromycin and Erythromycin drugs. The zone of inhibition formed by *E. coli* and *Klebsiellasp* by ZnO nanoparticles were observed 14mm and 12mm respectively at 100 μg/ml concentration of ZnO nanoparticles. The MIC value for *Staphylococcus aureus* was 625μg/ml reported by Aleaghil et.al.,2016[15]in their studies. We also observed a remarkable increase in inhibition zone by ZnO nanoparticles in all tested pathogenic bacterial strains. According to the obtained results it was clearly demonstrated that zinc oxide nanoparticles are able to enhance the efficiency of antibiotics. Among five tested bacterial strains with Azithromycin and Erythromycin,*E.coli, Staphylococcus sp*and*Shigella*had been show resistant against tested drugs . But on combining these drugs with ZnO nanoparticles all tested bacterial strains were shows zone of inhibition. In addition, for Norfloxin; one bacterial strain had been shows resistance i.e. *E.coli*. After combining with ZnOnanaoparticles we are able to overcome the multi-drug resistance in tested pathogenic bacterial strain. However, the combination between ZnO nanoparticles and other antibiotics such as Azithromycin resulted in increasing in inhibition zone diameter indicating that the effect of ZnO nanoparticles could be varied according to antibiotic class and mode of action. These findings were in good agreement with Fattah et.al.,2017 [14] who have been studied the mechanism of synergistic activity of ZnO nanoparticles and antibiotics (Penicillin and Ampicillin) on gram negative bacterial strains. They have reported that antibiotics shows synergistic results along with silver and zinc nanoparticles. Abdulrahman and Nssaif ,2016 [16] have worked on the multidrug-resistant to most using antibiotics (ampicillin, amoxicillin, gentamicin, tetracycline clindamycin and erythromycin) *Staphylococcus aureus*. These drugs have used along with nanoparticles that confirmed the synergism activity towards the tested bacterial strains.

CONCLUSION

Pathogens are showing drug-resistant against existing antibiotics. In the present study chemically synthesized ZnO nanoparticles were initially confirmed by UV-Vis spectroscopy at 365 nm. Toxicity studies of zinc oxide nanoparticles on pathogenic bacteria species have opened a new array of antimicrobial agents. The antimicrobial activity test performed by the well diffusion method showed that at 50μg/ml concentration, zinc oxide nanoparticles have better antimicrobial properties against human pathogenic bacterial strains (Staphylococcus, Escherichia coli, Klebsiella, Shigella, and Pseudomonas). We have also observed the synergistic effect of ZnO nanoparticles with tested drugs against multidrug-resistant human pathogenic bacterial strains. NPs can help in absorbance of the drug on its surface and enhance the antibacterial activity of drugs show a synergistic effect. Therefore, this study may give an insight into new area of research.

Author contribution

HST, MK, PK, ZA, DS: Designed& preformed the experiment; PK, FT, SP, AGN: contributed in experiment designing and result analysis; VP & GK: Proof reading and result analysis

Conflicts of Interest: Authors declare no conflict of interests.

REFERENCES

1. Ehsan S, Sajjad M., Bioinspired synthesis of zinc oxide nanoparticle and its combined efficacy with different antibiotics against multidrug resistant bacteria, Journal of Biomaterials and Nanobiotechnology, 2017;8(2):159-175.
2. Geetha A, Sakthivel R, Mallika J, Kannusamy R, Rajendran R., Green Synthesis of antibacterial Zinc oxide Nanoparticles using biopolymer Azadirachtaindica gum,Oriental Journal of Chemistry, 2016;32(2):955-963.
3. Joel C, Badhusha MSM., Green synthesis of ZnO nanoparticles using phyllanthus embilica stem extract and their antibacterial activity, Der Pharmacia Lettre, 2016;8(11):218-223.
4. Raut SB, Thorat P., A review on preparation, characterization and application of zinc oxide (ZnO) nanoparticles by green synthesis method, international journal of emerging technology and engineering, 2015;5(3):521-524.
5. Palanikumar L, Ramasamy S, Hariharan G, Balachandran C., Influence of particle size of nano zinc oxide on the controlled delivery of Amoxicillin, Applied nanoscience, 2013;3(5):441-451.
6. Mirhosseini M., Synergistic antibacterial effect of metal oxid nanoparticles and ultrasound stimulation, Journal of Biology Today's World, 2015;4(6):138-144.
7. Bayroodi E, Jalal R., Modulation of antibiotic resistance in Pseudomonas aeruginosa by ZnO nanoparticles, Iranian journal of microbiology, 2016; 8(2):85-92.
8. Bhande RM, Khobragade CN, Mane RS, Bhande S., Enhanced synergism of antibiotics with zinc oxide nanoparticles against extended spectrum β-lactamase producers implicated in urinary tract infections, Journal of Nanoparticle Research, 2013;15(1):1413.
9. Chauhan R, ReddyA, Abraham J., Biosynthesis of silver and zinc oxide nanoparticles using Pichia fermentans JA2 and their antimicrobial property, Applied nanoscience, 2015; 5(1): 63-71.
10. Kumar P, Nene AG, Sood S, Kaur G, Punia S, Kumar M, Thakral F, Tuli HS., Synthesis and evaluation of antibacterial activity of Zinc Oxide nanoparticles. International Journal of Pharmaceutical Research, 2020 ;12 (1):878-881.
11. Kumar P, Nene AG, Punia S, Kumar M, Abbas Z, Thakral F, Tuli HS., Synthesis, Characterization and Antibacterial activity of CuO nanoparticles, International Journal of Applied Pharmceutics, 2020;12 (1): 17-20.
12. Kołodziejczak-Radzimska A, Jesionowski T., Zinc oxide—from synthesis to application, A review Materials , 2014;7(4):2833-2881.
13. Thangam A, Pritam Ramalakshmi S. Effect Of ZnO nanoparticles against strains of *Escherichia coli* , Asian Journal of Pharmaceutical and clinical Research 2014;7(7):202-206.
14. Fattah K, Gamal A, Ibrahim Z, Mohamed E, Saleh A. Investigation of the Efficacy of Synthesized Silver and Zinc Oxide Nanoparticles against Multi-Drug Resistant Gram Negative Bacterial Clinical Isolates, Archives of Clinical Microbiology 2017;8(5):67.
15. Aleaghil SA, Fattahy E, Baei B, Saghali M, Bagheri H, Javid N, et-al. Antibacterial activity of Zinc oxide nanoparticles on Staphylococcus aureus, International Journal of Advanced Biotechnology and Research 2016; 7(3):1569-1575.
16. Abdulrahman NBA, Nssaif ZM. Antimicrobial Activity of Zinc Oxide, titanium Dioxide and Silver Nanoparticles Against Mithicillin-Resistant *Staphylococcus aureus* Isolates, Tikrit Journal of Pure Science 2018;21(3):49-53.
17. Ruba kello (2016) oral vial with plunger and tear off cap (vptc) is an alternative dosage form for dietary supplements. Journal of Critical Reviews, 3 (4), 6-10.