Int. J. Bio-Inorg. Hybr. Nanomater., 10(2): 103-108, Summer 2021

Antibacterial activity of Chitosan & ZnO nanoparticles on gram positive and gram-negative bacteria

S. Salemi-Najaf abadi[1], M. Doudi[1], A. Tahmourespour[2], Gh. R. Amiri[3] and Z. Rezayatmand[4]*

[1] *Department of Microbiology, Falavarjan Branch, Islamic Azad University, Isfahan, Iran*
[2] *Department of Basic Medical Sciences, Isfahan (Khorasgan) Branch, Islamic Azad University, Isfahan, Iran*
[3] *Department of Basic Science, Falavarjan Branch, Islamic Azad University, Isfahan, Iran*
[4] *Department of Plant Biology, Falavarjan Branch, Islamic Azad University, Isfahan, Iran*

Received: 6 December 2020; Accepted: 8 February 2021

ABSTRACT: Nanotechnology deals with the manufacture and application of materials with size of up to 100 nm. They are widely used in a number of processes that include material science, agriculture, and food industry, cosmetic, medical, and diagnostic applications. Nanotechnology science can create products with enormous potential to treat all the diseases. Antibacterial substances in nanoparticles are able to fight bacteria and inhibit and slow the growth of bacteria. The aim of this study was to investigate the antibacterial properties of chitosan & ZnO nanoparticles against *Escherichia coli* & *Staphylococcus aureus*. Investigation of the antibacterial properties of chitosan& ZnO nanoparticles on *Escherichia coli* & *Staphylococcus aureus* by qualitative agar well diffusion test and microdilution was performed to the concentration of 0.032 g/ml to 0.512 g/ml. The size of chitosan & ZnO nanoparticles in this study was 100, 100 nm, and spherical in shape. The sizes of the inhibition zone were different according to the type of bacteria and the concentrations of chitosan& ZnO nanoparticles, the maximum zone diameter 24 and 22 mm in concentration 0.512 g/ml observed.

Keywords: *Antibacterial effect, Chitosan, Escherichia coli, Staphylococcus areuse, ZnO nanoparticles*

INTRODUCTION

Nanotechnology includes applied knowledge in various fields. The main field of nanotechnology in the production of materials or tools in dimensions less than one hundred nanometers [1]. New applications of nanoparticles and nanomaterial depend on their improved and new property, which is related to their size morphology, and distribution [2]. A large number of materials which were considered to be safe develop toxicity at nano size ranges. A larger surface area (as in case of nanoparticles) ensures an increased range of probable interaction with bio-organics present on the viable cell surface [3]. Currently, various methods are used to produce nanoparticles [4]. Zinc oxide nanoparticles (ZnO NPs) have a wide range of applications in electrical, optical industries and cosmetics. The wide range of applications of ZnO NPs, especially in medical care products, Water treatment, photo catalytic [5]. ZnO

(*) Corresponding Author - e-mail: monirdoudi@yahoo.com

nanoparticles as one of the multifunctional inorganic nanoparticles have many significant features such as chemical and physical stability, high catalysis activity, effective antibacterial activity as well as intensive ultraviolet and infrared adsorption with a broad range of applications as semiconductors, sensors, transparent electrodes, and solar cells [6]. Antibacterial activity properties ZnO NPs are their greater effectiveness on resistant strains of microbial pathogens, less toxicity and heat resistance. In addition, they provide mineral elements essential to human cells and even small amounts of them exhibit strong activity [7]. ZnO NPs with caused oxidative stress which caused eventual cell growth inhibition and cell death, generation of reactive oxygen species (ROS), Zn^{2+} release and causes death bacteria. Chitosan has been investigated as an antimicrobial material against a wide range of target organisms like algae, bacteria, yeasts and fungi in experiments involving in vivo and in vitro interactions with chitosan in different forms (solutions, films and composites) [8]. Chitosan nanoparticles (CNs) have antibacterial activity due to the presence of positively charged amine groups. These amino groups react with the cell membrane of microorganism, which has a negative charge. This is followed by the deposition and oxidation of the intracellular protein components of the microorganisms. These proteins also have the function of transporting nutrients in bacteria and waste products out, causing cell death [9]. Factors affecting antibacterial properties of chitosan such as pH, concentration, temperature, molecular weight, type of microorganisms [10]. The present investigation was aimed to determine the antibacterial activity of Chitosan & ZnO nanoparticles toward *E. coli* as Gram-negative bacteria and *S. aureus* as Gram-positive bacteria in laboratory.

MATERIALS AND METHODS

Preparation of the materials and bacterial cultures

ZnO & Chitosan nanoparticles powder which were prepared by Salemi, et al. 2020 by wet chemical & sol gel method were used in this experiment [11]. The size of prepared ZnO & Chitosan nanoparticles was 100,100 nm. *Staphylococcus auerus* ATCC 25923 and *Escherichia coli* ATCC 25922 were bought from ideal company. All these strains were grown aerobically in nutrient broth for 24 h at 37°C before using as target organisms.

Antibacterial activity assay

For this purpose, qualitative diffusion method was used in agar wells and the quantitative microdilution method. The standard bacteria were cultured from 1.5×108 cfu/ml (0.5 MacFarland) suspension on Muller-Hinton agar medium while maintaining standard conditions for susceptibility testing Different concentrations of 0.032 g/ml to 0.512 g/ml were prepared from ZnO & Chitosan nanoparticles. 80µl of each concentration was poured into the well. Gentamicin (10µg/disc) antibiotic was used as positive control and sterile distilled water was used as a negative control. To determine the minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), used microdilution method. For this purpose, 96-well polystyrene round-bottomed sterilized microtiter plates were used. 80µl of nanoparticle concentrations, Added in columns 1 to 4, respectively. Then it was transferred to each of the wells 80 µl of microbial suspension with a turbidity equivalent of 1.5×10^8 cfu/ml (0.5 MacFarland) to the wells. Also, 50 µl of Muller -Hinton broth was added to each well. Row 5 wells were considered as a negative control containing distilled water and Row 6 wells were considered as a positive control containing culture medium and bacteria as a positive control. 96- Well plates were incubated at 37 °C for 24 hours. And then light absorption at 620 wavelengths was read by Eliza Reader, model 800 Bio Tec Elx made in the USA. The results were reported as average after 3 repetitions [12].

Statistical analysis of data

To statistically analyze the data obtained from this study, SPSS version 21 was used. Descriptive statistics were obtained using standard deviation and corresponding values of P and $P< 0.05$.

RESULTS AND DISCUSSION

The antibacterial activity of ZnO & Chitosan nanopar-

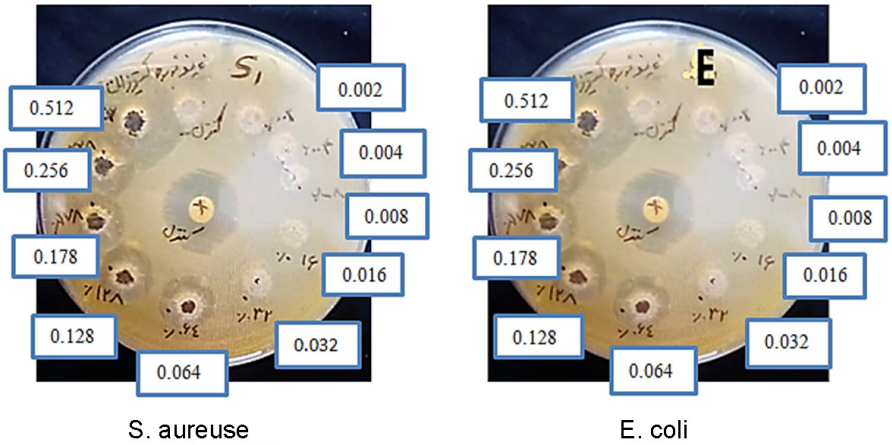

Fig. 1. Zone of inhibition for different concentration (g/ml) in standard E.coli & S.aureus against chitosan nanoparticle

ticles were tested by microdailution and well diffusion agar methods (Tables 1). The presence of an inhibition zone clearly indicated the antibacterial effect of ZnO & Chitosan nanoparticles. As it was also shown in the study of Rizwan, et al., it has been seen in this study that by increasing the concentration of ZnO nanoparticles in wells and discs, the growth inhibition has also been increased. The size of inhibition zone was different according to the type of bacteria, the size and the concentrations of ZnO & Chitosan nanoparticles. Particle concentration seems to be more effective on the inhibition of bacterial growth than particle size under the condition of this work (Lingling, et al., 2006). In Fig. 1 showed zone of inhibition for different concentration in standard *E.coli* & *S.aureus* against chitosan nanoparticle.

The result of the antibacterial effect of chitosan & ZnO nanoparticles on standard *E.coli* & *S.aureus* bacteria, according to the evidence and results of the maximum inhibition zone diameters related to concentration of 0.512 g/ml chitosan nanoparticle with 24 mm in *E.coli* and the same concentration of 0.512 g/ml ZnO in *S.aureus* with 17 mm. The Bacteria were more sensitive to nanoparticles than antibiotics. Standard *E.coli* & *S.aureus* had the largest diameter of growth inhibition zone compared to chitosan nanoparticles, so that, compared to the positive control of antibiotics, it has the greatest effect in increasing the diameter of growth inhibition zone in bacteria). In Figs 1 and 2 showed zone of inhibition for different concentration in standard *E.coli* & *S. aureus* against chitosan nanoparticle.

The concentration of chitosan & ZnO nanoparticle seems to be more effective on the inhibition of bacterial growth than particle size under the condition of this work (Figs 2 and 3). The enhanced bioactivity of smaller particle Chitosan & ZnO nanoparticle probably are attributed to the higher surface area to volume ratio and increase antibacterial activity. According to the results in this study, Chitosan nanoparticle and ZnO nanoparticle are antibacterial activity agents both gram - negative & positive bacteria. Based on the results obtained from MIC and MBC, disc and well agar diffusion methods, it can be suggested that in comparison with gram-positive bacteria, the growth of gram-negative bacteria is inhibited at higher concentrations of ZnO nanoparticles. Reddy, et al. (2007), have reported the same results, emphasizing on the higher susceptibility of gram-negative bacteria in comparison with gram-positive bacteria. In the study

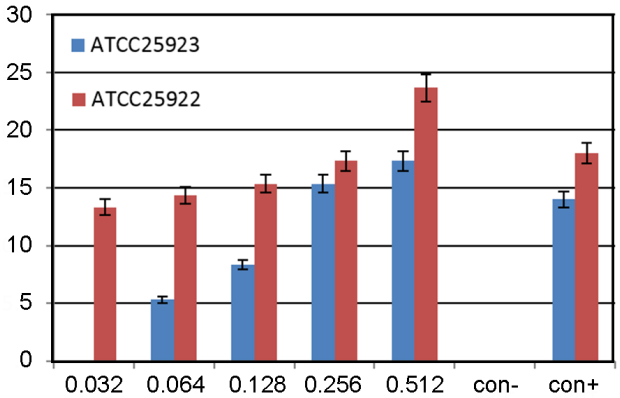

Fig. 2. Comparison of zone of inhibition for different concentration in standard E.coli & S.aureus against ZnO nanoparticle

Table 1. MIC and MBC Chitosan nanoparticles in standard E.coli & S.aureus against chitosan & ZnO nanoparticle

Bacteria	Chitosan nanoparticle		ZnO nanoparticle	
	E. coli ATCC25922	*S.aureus* ATCC25923	*E. coli* ATCC25922	*S.aureus* ATCC25923
MIC	0.032	0.064	0.064	0.128
MBC	0.064	0.128	0.128	0.256

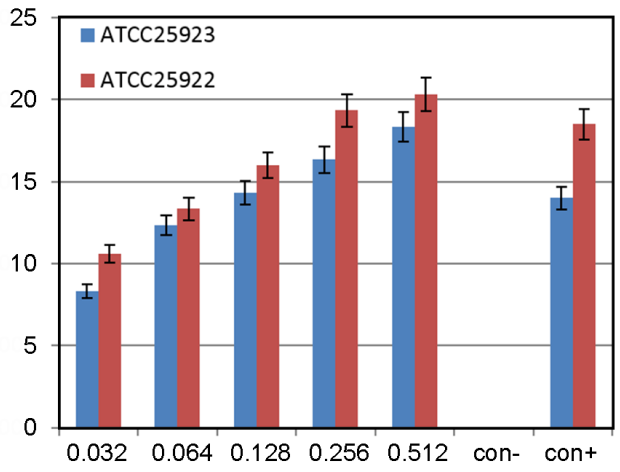

Fig. 3. Comparison of zone of inhibition for different concentration in standard E.coli & S.aureus against chitosan nanoparticle

done by Selahattin, et al. (1998), it has been proposed that the higher susceptibility of gram-negative bacteria could be related to differences in cell wall structure, cell physiology, metabolism or degree of contact. This study showed that nanoparticles of ZnO and Chitosan have a greater effect on *E. coli*. This is probably due to the gram-negative cell wall structure. Chitosan nanoparticles have a positive charge due to having amine acid groups and tend to bind to the surface of bacteria. *S.aureus* bacteria compared to both nanoparticles did not show much sensitivity to positive control. Emami karvani, et al. (2011), have reported the same result that ZnO nanoparticle increase on *E.coli* than *S.aureus*. Ghsemzade, et al. (2009), have reported the different result about effect Chitosan nanoparticles in *S.aureus* bacteria, It can be explained that the production of chitosan nanoparticles in the results of studies. They have different effects and chitosan nanoparticles did not have an acceptable effect on *Staphylococcus aureus* in this study.

ACKNOWLEDGEMENT

The authors would like to thank the esteemed officials of the research laboratory of the Flavarjan Branch, Islamic Azad University for their executive support.

CONCLUSIONS

In this study, the antibacterial activity of Chitosan & ZnO nanoparticles was assessed by the well and microdilution methods in standard *E.coli* & *S.aureus*. By increasing the nanoparticle concentration in wells and discs, the growth inhibition and diameter of inhibition zone have also been increased. The sizes of the inhibition zone were different concentration Chitosan & ZnO nanoparticles, the maximum diameter being observed for *E.coli* concentration of 0.512 g/ml than *S.aureus* in the same concentration. Chitosan nanoparticles by damaging the cell membrane and entering the cell cause destroyed DNA and proteins and cause bacterial dysfunction, and ZnO nanoparticles with generation ROS, oxidative stress, and Zn^{2+} releases, damage with important biomolecules such as DNA, RNA and, proteins caused death bacteria.

REFERENCES

[1] Sheydaei, P., Bayrami, A., Azizian, Y., Parvinroo, S. (2017). Study on the toxicity effects of Zinc Oxide nanoparticles on hematological and serum parameters in mice. J. Arak. Uni. Med. Sci., 19(115), 39-47.

[2] Khalil, K., Fouad, H., Elsarnagawy, T., Almajhdi, F. (2013). Preparation and characterization

of electrospun PLGA/silver composite nanofibers for biomedical applications. Int. J. Electrochem Sci., 8, 3483-93.

[3] Rizwan, W., Young-Soon, K., Amrita, M., Soon, Y., Hyung-Shik, Sh. (2010c). Formation of ZnO micro-flowers prepared via solution process and their antibacterial activity. J. Nanoscale Res. Lett., 5(10), 1675–1681.

[4] Reddy, K.M., Kevin, F., Jason, B., Denise, G.W., Cory, H., Alex, P. (2007). Selective toxicity of zinc oxide nanoparticles to prokaryotic and eukaryotic systems. J. Appl. Phys. Lett., 90(21), 1-3.

[5] Jansen, J., Karges, W., Rink, L. (2009). Zinc and diabetes clinical links and molecular mechanisms. J. Nutr. Biochem., 20, 399-417.

[6] Kim, M.H., Seo, J.H., Kim, H.M., Jeong, H.J. (2014). Zinc oxide nanoparticles, a novel candidate for the treatment of allergic inflammatory diseases. Eur. J. Pharmacol., 738, 31–39.

[7] Sawai, J., Shoji, S., Igarashi, H., Hashimoto, A., Kokugan, T., Shimizu, M., Kojima, H. (1998). Hydrogen peroxide as an antibacterial factor in zinc oxide powder slurry. J. Ferment Bioeng., 86, 521–522.

[8] Sudarshan, N.R., Hoover, D.G., Knorr, D. (1992). Antimicrobial action of chitosan. Food Biotechnol., 6, 257-272.

[9] No, H.K., Park, N.Y., Lee, SH., Meyers, S.P. (2002). Antimicrobial activity of chitosan and chitosan oligomers with different molecular weights. Int. J. Food Microbiol., 65-74.

[10] Baron, E.J., Rinegold, M.S. (1990). Diagnosis Microbiology. The C.V Mosby Company, New York, USA, 171-174.

[11] Salemi Najafabadi, S., Doudi, M., Tahmourespour, A., Rezayatmand, Z., Amiri, Gh.R. (2021). Evaluation of antibacterial properties of chitosan nanoparticles against Esherichi coli isolated from diabetic ulcers. Int. J. Bio-Inorg. Hybr. Nanomater., 10(1), 11-15.

[12] Salemi Najafabadi, S., Doudi, M., Tahmourespour, A., Rezayatmand, Z., Amiri, Gh.R. (2020). Synthesis of ZnO/Urtica Dioica Nanoparticles by Wet Chemical Procedure and Investigation of Its Antibacterial Activity Against Multiple Drug Resistant (MDR) *Staphylococcus aureus* Isolates of Diabetic Ulcers. J. JAMT, 10(3), 79-88.

[13] Lingling, Z., Yunhong, J., Yulong, D., Malcolm, P., David, Y. (2006). Investigation into the antibacterial behavior of suspensions of ZnO nanoparticles (ZnO nanofluids). J. Nanoparticle Res., 9(3), 479-489.

[14] Selahattin, A., Kadri, G., Ramazan, A. (1998). The effect of zinc on microbial growth. Tr. J. Med. Sci., 28, 595-597.

[15] Emami karvani, Z., Chehrazi, P. (2011). Antibacterial activity of ZnO nanoparticles on Gram positive and Gram negative bacteria. Africa J. Microbiology Res., 5(12), 1368-1373.

[16] Ghasemzadeh Daghigh, N., Rezazadeh Bari, M., Razavi Rohani, S.M. (2009). Study on antibacterial effects of artemia urmiana cyst shell chitosan on listeria monocytogenes, *Staphylococcus aureus*, Salmonella typhimurium and Escherichia coli. J. V. Res., 64(1), 79-83.

[17] Shantikumar, N., Abhilash, S., Divya, R., Deepthy, M., Seema, N., Manzoor, K., Satish, R., (2009). Role of size scale of ZnO nanoparticles and microparticles on toxicity toward bacteria and osteoblast cancer cells. J. Mater. Sci. Mater Med., 20, 235–241.

AUTHOR (S) BIOSKETCHES

Samaneh Salemi-Najaf abadi, PhD. Student, Department of Microbiology, Falavarjan Branch, Islamic Azad University, Isfahan, Iran

Monir Doudi, Associate Professor, Department of Microbiology, Falavarjan Branch, Islamic Azad University, Isfahan, Iran, *Email: monirdoudi@yahoo.com*

Arezoo Tahmourespour, Associat Professor, Department of Basic Medical Sciences, Isfahan (Khorasgan) Branch, Islamic Azad University, Isfahan, Iran

Gholam Reza Amiri, Associat Professor, Department of Basic Science, Falavarjan Branch, Islamic Azad University, Isfahan, Iran

Zahra Rezayatmand, Assistant Professor, Department of Plant Biology, Falavarjan Branch, Islamic Azad University, Isfahan, Iran