Int. J. Bio-Inorg. Hybr. Nanomater., 10(1): 11-15, Spring 2021

Evaluation of antibacterial properties of chitosan nanoparticles against Esherichi coli isolated from diabetic ulcers

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Received: 30 September 2020; Accepted: 2 December 2020

ABSTRACT: Nanotechnology is the science to create products with enormous potential to treat diseases. Diabetic ulcers and their infections have affected many people around the world, and many people die each year from infections caused by microorganisms in these ulcers. The aim of this study was to investigate the antibacterial properties of chitosan nanoparticles against *Escherichia coli* isolated from diabetic wounds. Morphological evaluation and substructure of nanoparticles synthesized by ion gelling method were performed by XRD and TEM. Investigation of the antibacterial properties of chitosan nanoparticles on *Esherichia coli* by qualitative agar well diffusion test and microdilution was performed to the concentration of 0.064 g/l to 0.256 g/l. The size of chitosan nanoparticles in this study was 100 nm and spherical shape. The sizes of the inhibition zone were different according to the type of bacteria and the concentrations of chitosan, the maximum zone diameter 20 mm in concentration 0.256 g.l being observed.

Keywords: *Antibacterial effect, Chitosan nanoparticles, Diabetic ulcers, Escherichia coli , Nanotechnology*

INTRODUCTION

Due to the increasing prevalence of diseases diabetes and diabetic ulcers in people, whom are suffering from jaundice, it is known as a health threat in the 21st century [1]. Diabetes is a disease that increases the susceptibility of infections in people. In this systemic disease, many organs of the body are involved, including the skin [2]. According to the predictions made, the prevalence of diabetic wounds in human society has increased. Ischemia, neuropathy, and infection are three important pathological factors that lead to complications of diabetic ulcers [3]. *Escherichia coli* (*E.coli*) is the most successfully pathogenic and human life-threatening bacterium. This Gram-negative bacillus is the cause of many nosocomial infections. This bacterium plays an important role in causing diseases such as food poisoning, urinary tract infections, diarrhea, and diabetic wound infections. Deep and chronic

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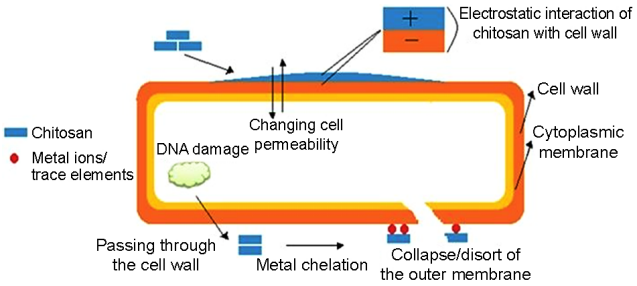

Fig. 1. The antibacterial effect chitosan nanoparticles in bacteria [5].

diabetic wounds often contain Gram- negative and forced anaerobic or polymicrobial bacteria [4]. One of the cases that researchers are studying is to heal diabetic wounds. Use a combination of intelligent polymers such as chitosan. Chitosan is made from a combination of glonoric acid and N-acetyl glucose amine. It is rapidly degraded in the body and mostly injected as a gel. Chitosan nanoparticles 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 [5,6].

MATERIALS AND METHODS

Preparation of chitosan nanoparticles

The preparation of chitosan nanoparticles in this study is through ionic interactions between the positive charge of the chitosan amine group and the negative charge of the polyanion sodium triphosphate groups. Preparing chitosan nanoparticles, the first 2 mg of chitosan powder was added 1cc of 0.1 M acetic acid, then the pH of the solution was raised to 4.4 with NaOH hydroxide. By adding 1 mg of sodium tripolyphosphate (TPP) to 1 ml of sterile distilled water, sodium tripolyphosphate solution was obtained. From this solution was added dropwise to the chitosan solution. It was then placed on the magnetic stirrer at room temperature for 1 hour. After this period, the supernatant containing chitosan nanoparticles was collected and used [7].

Sensitivity test for bacterial isolates

In this study, the first from 30 diabetic wound patients were sampled, and bacteria were identified using direct slides and biochemical tests. To determine the susceptibility of the isolates to the antibiotics, suspension of 1.5×108 CFU/ml (0.5 MacFarland) was prepared from the bacteria, and it was cultured Muller Hinton agar. The antibiogram test was done by the agar disc diffusion method. Then, antibiotics discs including Cefotaxim (30µg/disc), Tetracycline (30µg/disc), Gentamicin (10µg/disc), Imipenem (10µg/disc), and Cefoxetine (30 µg/disc) were placed at appropriate intervals. After inoculation at 37°C for 24 hours, the inhibition zone diameter was measured with a millimeter ruler after 3 replications [8].

The Antibacterial effect of chitosan nanoparticles on bacteria isolated from diabetic ulcers

For this purpose, were used qualitative diffusion method in agar wells and the quantitative microdilution method. The antibiotic-resistance bacterium was 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 0.002 g/l to 0.256 g/l were prepared from chitosan nanoparticles in (methyl sulfoxide) DMSO. 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 1.5×108 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.

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 DISCUSION

Results of TEM transposition electron microscopy of chitosan nanoparticles, it nanoparticles in this study was spherical and 100 nm dimensions as shown in Fig. 1 and XRD diagram shows, chitosan nanoparticles have with peaks at 19.5 ° and 20.5° levels shown in Fig. 2.

Among 30 of the bacterium isolated from diabetic wounds, 12 samples were *E.coli* Gram- negative bacilli, after the biochemical examination. The results show the percentage of bacterial resistance to common antibiotics including Cefotaxim (30µg/disc), Tetracycline (30µg/disc), Gentamicin (10µg/disc), Imipenem (10µg/disc), and Cefoxetine (30 µg/disc) in Fig. 4. Four antibiotic- resistance *E.coli* bacteria were selected and treated with different concentrations of chitosan nanoparticles.

The result of the antibacterial effect of chitosan nanoparticles on bacteria, according to the evidence and results of the maximum inhibition zone diameters related to concentration 0.256 with 25 mm shown in

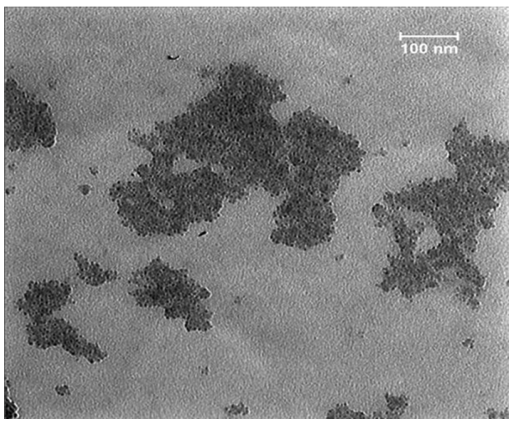

Fig. 2. Transmission Electron Microscope of chitosan.

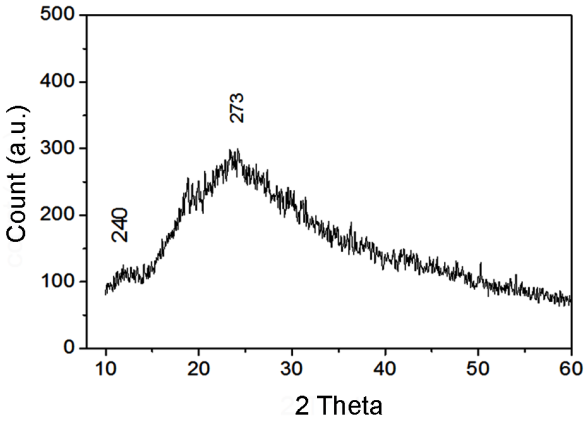

Fig. 3. XRD patterns of the chitosan.

Fig. 4.

Diabetic wound infection is one of the most important chronic complications of diabetes. The infection can progress rapidly and reduce the sense of touch at the site of infection and damage to the affected limb by reducing blood flow to the limb [9]. The most important cause of these infections in people is the Gram-negative bacillus of E. coli. Alexander, *et al.* studied, diabetic infection wound isolated 7.5% bacillus of E. coli [10]. In our research results, E. coli was isolated from 40% of the diabetic wounds. Pınar, *et al.* studied, E. coli which was reported to be the cause of 16.8% of peripheral wound infections in diabetics and 11.8% of infection ulcers. Zahedi yegane, *et al.* reported chitosan nanoparticles antibacterial activity against E. coli. In the study, Mahmudi, *et al.* the size of chitosan nanoparticles was 100 nm, which result in the size of chitosan nanoparticles being the same in our study. Eslami, *et al.* studied, the antibacterial

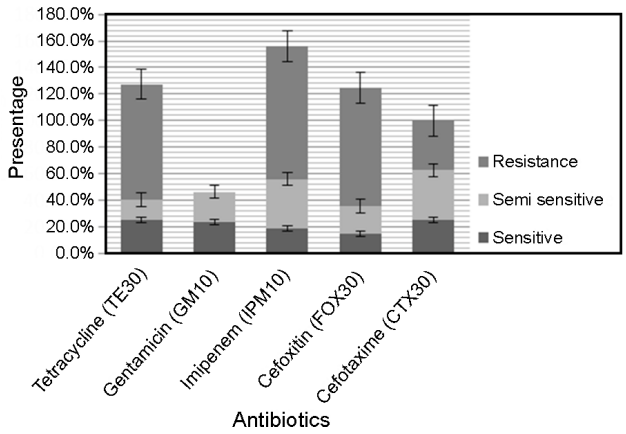

Fig. 4. The results from antibiotic resistant pattern of the isolated E. coli strains by antibiogram testing.

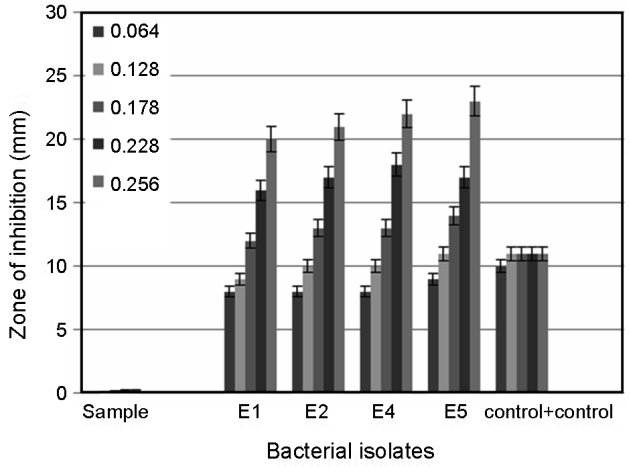

Fig. 5. Comparison of zone of inhibition for different concentration in resistant antibiotic E.coli, E: *E.coli*

Table 1. MIC and MBC chitosan nanoparticles in *E.coli*

Sample	MIC (g/l)	MBC (g/l)
E. coli	0.128	0.178

effect of chitosan nanoparticles was investigated in *E.coli*, and this result is the same in our study [11]. In this study, there was a significant relationship between concentrations of chitosan nanoparticles to kill *E.coli*. The antibacterial properties of chitosan nanoparticles were also higher than antibiotics. Chitosan is a natural, renewable, and environmentally friendly biopolymer with suitable antibacterial properties against resistance to several antibiotics E. coli. In this study, it can be said that chitosan nanoparticles can be used to kill and inhibit the growth of E. coli isolated from diabetic ulcers. Chitosan nanoparticles are capable of killing many species of bacteria, so mass production may be an available alternative to antibiotics in the treatment of infections in the future.

ACKNOWLEDGEMENTS

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, Chitosan nanoparticles were synthesized by the ionic gel method. The antibacterial activity of Chitosan nanoparticles were assessed by the well diffusion agar and microdillution methods. By increasing the nanoparticle concentration in wells and discs, the growth inhibition and diameter of inhibition zone have also been increased. The sizes of inhibition zone were different concentration Chitosan nanoparticles, the maximum diameter being observed for resistance – antibiotics *E.coli* concentration of 0.256 g/l. Chitosan nanoparticles by damaging the cell membrane and entering the cell cause destroyed DNA and proteins and cause bacterial dysfunction.

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