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Research Article

The Effects of Silver and Iron Nanoparticles on *Bacillus cereus* and *Pseudomonas aeruginosa* by spectrophotometry

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ABSTRACT

Introduction: Nanoscience, new ways to deal with diseases caused by bacteria and it has brought . The use of metals such as silver and iron nanoparticles in the nano against microbes has led to strong chemicals into the treated area. This study aimed to determine the antibacterial activity of silver and iron nanoparticles on reference bacteria such as *Bacillus cereus* and *Pseudomonas aeruginosa* were performed.

Materials and Methods: In this study , silver and iron nanoparticles approximately 20 nm and the *Bacillus cereus* and *Pseudomonas aeruginosa* was used. The concentrations of 1 , 2 and 5 µg/ml of nanoparticles were prepared. Using a spectrophotometer at a wavelength of 600 nm and measured bacterial growth curves were plotted.

Results: The results showed that the inhibitory power of the silver and iron nanoparticles bacteria are selected. In the meantime, more power than the iron nanoparticles, silver nanoparticles inhibit the growth of bacteria.

Conclusion: The results could reveal way more effective use of these compounds at the appropriate dose. Clinical research to clinical application of these extracts is necessary.

Keywords: Silver Nanoparticles, Spectrophotometry, Antibacterial activity, *Pseudomonas aeruginosa*

INTRODUCTION

Using nanoparticles and their oxides to fight against bacterial infections can be effective as an alternative method for antibiotics. Studies have shown that as nanoparticle size is smaller it will show new and different properties and activities. Nowadays, the rate of using of nanomaterials is increasing so that it is used at all aspects of life, including the fight against disease-causing microbes, diagnosis and treatment of diseases. Nanomaterials have shown the lowest level of toxicity in life cycle and ecosystem, and using these materials can be considered as appropriate option to fighting against germs. In the conducted studies, microbial and bactericidal properties of nanoparticles such as Ag, Zn, Fe, Ti, Cr, and their oxide have been proven (Kumar and Jakhmola, 2007). At the nanoscale, Silver has impact on metabolism, respiration and reproduction of microorganisms. Silver nanoparticles, without increasing drug resistance, inhibit the bacterial respiratory system. This element has a specific effect on microbial

enumeration, but its preparation is difficult and expensive. Iron nanoparticles, especially their oxides, have inhibitory properties n growth of bacteria. A few studies have been conducted on anti-bacterial property of nanoiron, but extensive studies have been conducted on nanosilver. In the case of nanoiron, it seems that its oxide to be more effective (Sun Yg. Et al, 2003). Pseudomonas and gram negative, aerobic and mobilebacilli. Pseudomonas aeruginosa often constitute normal intestinal flora in small numbers and human skin, and it is considered as opportunistic pathogen in patients with impaired immune system. Pseudomonas aeruginosa producing Metallo beta lactamases (MBL) was reported for the first time in Japan in 1991. Then, it was identified in different parts of the world, including Asia, Europe, Australia, North and South America.

Pseudomonas aeruginosa strains carrying MBL gens are a serious clinical threat (Aoki et al, 2004). Bacillus cereus is spore-containing Gram-positive basil belonging to Bacillaceae family. Spore of the bacteria is widely found in nature, water and dust so that it can be isolated from various food products.

Bacillus cereus is able to produce extracellular material such as beta-hemolysin that is important in identifying it. This enterotoxin-producing bacteria causes diarrhea and nausea, and it can create vomiting syndrome and diarrhea. *B. cereus* was recognized as food poisoning agent in 1950 (Rajkowski and Bennett, 2003).

MATERIALS AND METHOD

Preparation of nanoparticles: silver nanoparticles and iron nanoparticles were purchased from NanoSany Engineers Company at the dimensions of approximately 20 nm. Preparation of bacterial strains: In this study, the standard strains (*Bacillus cereus* (ATCC / 1052 and *Pseudomonas aeruginosa* (ATCC / 27853) were prepared in lyophilized form from Microbial Collection of Biotechnology Research Institute of Tehran University.

Preparation of dilutions: to do spectroscopy tests, nanoparticles of dilutions of 1, 2 and 5 micrograms per ml were prepared. For the negative control, a dilution containing nanoparticle and medium was selected, and for positive control, a dilution containing bacteria and medium was selected.

Spectrophotometry:

bacterial growth curve was drawn by spectrophotometry according to reliable sources (Smith et al, 2003). One day before start of Spectrophotometry stages about considered bacteria, at least 14 flasks each containing 100 mlMueller Hinton Broth medium were prepared, that were placed in the in a mixer with 37 ° C. Usually, 18 hours before, one of these two flasks was inoculated with two loops of the sample. At the initial stage of Spectrophotometric stage, inoculated flask at a rate of 1 ml was inoculated to each of the flasks that their absorption should be measured.

Registration of absorption of each of flasks after zero time immediately after inoculation of 1 mg microbe to each of them (except the control flasks) and adding the nanoparticles are required flasks. In addition, all of these stages were sterilized under hood with UV radiation at the beginning and they performed with appropriate ventilation beside flame. Flasks were placed in the mixer with same temperature of 37 ° C and approximately 120 rpm rounds.

In each step, with related control samples, device absorption became zero (control of nanoparticles to read, flasks containing nanoparticles and control flask to read absorption of control flasks). Then, required data from spectrophotometer that is rate of absorption, optical density and percentage of passage were recorded approximately at least 11 hours after the zero time.

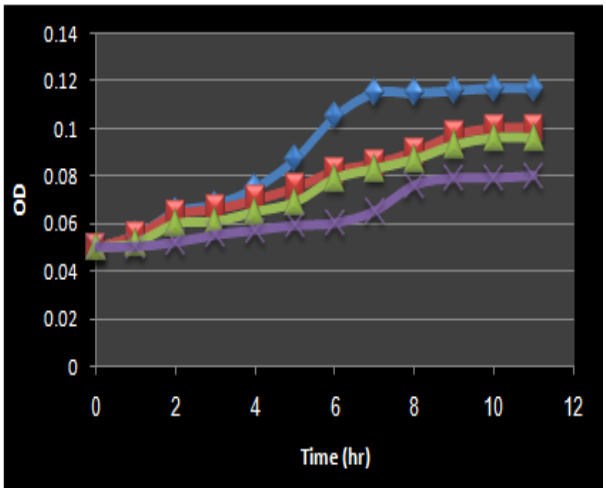
This time depends on absorption rate or bacteria growth. All spectrophotometric stages were performed in the optical absorption of 600 nm

RESULTS

Changes for the growth of *Bacillus cereus* in the absence or presence of silver nanoparticles:

The results indicate that silver nanoparticles have significant inhibitory effects on the growth of *Bacillus cereus*. Comparison of different concentrations of silver nanoparticles shows that nanosilver has had a significant impact on all hours of the growth curve. This effect reached to it maximum value at late hours of growth. By increasing the concentration of the nanoparticle, this effect also increases. Details of growth curve can be seen in following diagrams:

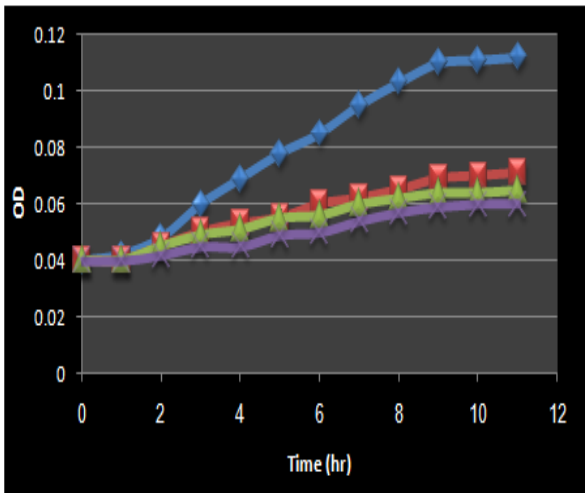
Figure 1: a Growth curve of *B. cereus* bacteria at different silver nanoparticles concentrations



Changes for *Pseudomonas aeruginosa* bacteria growth in the absence or presence of silver nanoparticles:

Results showed that nanosilver inhibited the growth of the bacteria with more power. This effect was higher than Bacillus bacteria.

Figure 2: a Growth curve of *P.aeruginosa*bacteria at different silver nanoparticles concentrations



Changes for the growth of *Bacillus cereus* bacteria in the absence or presence of iron nanoparticles:

The results show that iron nanoparticles against this bacterium is almost ineffective or less effective in high concentrations, so that at concentrations of 1 and 2 micrograms of this nanoparticle, no effect was observed in bacterial growth curve, and only slight inhibition of bacterial growth by this nanoparticle was performed at the concentration of 5 mg.

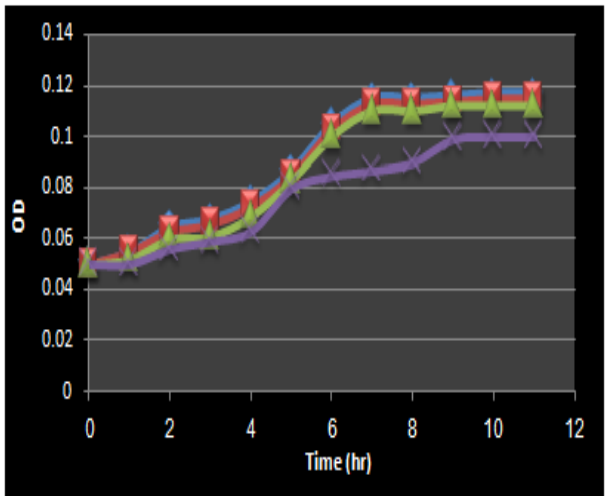


Figure 3: a Growth curve of *B. cereus* bacteria at different Iron nanoparticles concentrations

Changes for growth of *Pseudomonas aeruginosa* bacterium in the absence or presence of silver nanoparticles:

The results showed that iron nanoparticles had no effect on the bacteria. In other words, this bacterium showed resistance to nanoiron.

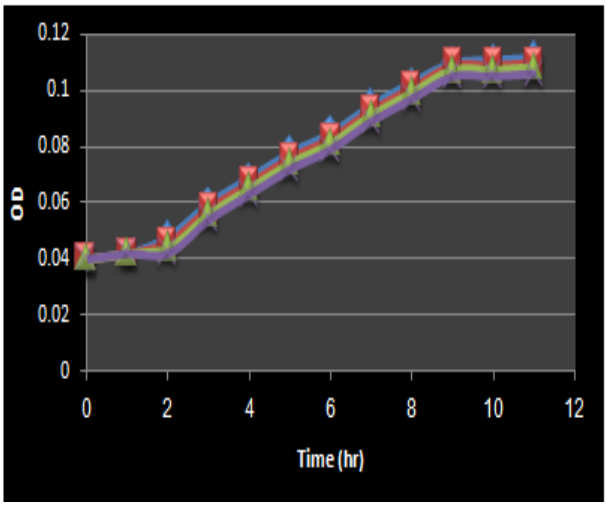


Figure 4: a Growth curve of *P.aeruginosa*bacteria at different Iron nanoparticles concentrations

DISCUSSION

Nanoparticles have been discussed in two metallic and nonmetallic parts since old days. Metal nanoparticles are used in bactericides and pesticides for many years. Some of nanoparticles are considered as modern method in the development of modern pharmaceutical science, that it is used widely in biology and pharmaceutical studies due to having high potential to do specialized treatment activities (Hardman RA, 2005). Silver nanoparticles used in this study showed different antibacterial properties based on surface to volume ratio. Gram-positive bacterium showed higher resistance compared to gram-negative bacterium. It can be due to the structure of their cell walls. Numerous studies have been conducted based on the possible reactions between nanoparticles and living organisms macromolecules. The difference between the positive charge of microorganism and positive charge of nanoparticle acts as electromagnetic absorbent between microbe and nanoparticle, and it can cause connection of nanoparticle to cell surface. As a result, it can cause cell death. Many of these contacts finally lead to oxidation of the surface molecules of microbes and their quick death (Amanda et al, 2010). In this study, it was found that silver nanoparticles have more power to inhibit selected bacteria. Meanwhile, nanosilver showed the greatest impact on the Pseudomonas aeruginosa bacterium. Interpreting of data shows that nanoiron had lower impact on the growth of Bacillus cereus and this impact was only at concentration of 5 micrograms per ml, but it had no impact on Pseudomonas aeruginosa bacterium. In other words, this bacterium was resistant against this nanoparticle. Numerous studies have been conducted on the antibacterial properties of silver so far. In an article entitled "the effect of silver nanoparticles on the growth of E. coli," Naghsh et al (2012) stated that at concentration of 400 ppm, the mean of zones diameter in the bacterium was 2.20 ± 0.43 mm that it showed significant increase compared to control groups (Naghsh et al., 2012). In a study entitled "the laboratory antimicrobial effect of silver nanoparticles solutions and chlorhexidine on Streptococcus sanguinis solutions and Actinomycesviscosus", Sadeghi et al (2012) concluded that the MIC value of silver nanoparticle solution and chlorhexidine against Streptococcus sanguinis was respectively 16 and 256 mg per ml and 4 and 64 mg per ml for Actinomycesviscosus. MBC value of silver nanoparticles and chlorhexidine against Streptococcus sanguinis was 64 and 512 mg per ml respectively, and 16 and 102 micrograms per ml for Actinomycesviscosus. In general, silver nanoparticles have good antimicrobial activity against the bacteria that this impact is achieved with lower concentrations of silver nanoparticles compared to chlorhexidine (Sadeghi et al., 1390). In an article entitled "investigating the antimicrobial effect of nylon carpets containing nano-silver ", Haj Mirza Baba et al (2011) showed that in a sample in which nylon carpet was covered with 25 ppm nano-silver solution, reduction of bacteria was 73.3%. However, in the samples in which nylon carpets were covered with 50 ppm nanosilver, reduction of bacteria

was 99.99% (Haj Mirza Baba et al., 2011). In an article entitled "examining the antimicrobial activity of silver and copper nanoparticles and comparing with Sodium hypochlorite on vegetative cells and spore of Bacillus subtilis and Bacillus cereus", Khosravi Iqbal et al (2010) concluded that Bacillus subtilis had the most sensitivity to both nanoparticles compared with Bacillus cereus. Its MIC was 7 ppm in the nanosilver, 50 ppm in nanocopper, and 700 ppm in sodium hypochlorite. Bacillus subtilis spores were at lower concentrations than antimicrobial substances, and they were eliminated in zero time. As a result, it was observed that spore and vegetative cell of Bacillus subtilis were more sensitive to silver nanoparticles and these results indicate that silver nanoparticles are more effective than other antimicrobial substances (Khosravi Iqbal et al, 2010).

CONCLUSION

It can be concluded that the nanoparticles especially silver nanoparticles can be used to fight against pathogenic bacteria without risk of increasing bacterial resistance. We hope to fight against them in disease-causing microbes in especial cases such as reemerging and epidemic cases using these nanoparticles in pure or combined form.

Recommendations

It is recommended that other researchers to examine the combined effect of these nanoparticles on bacteria. In addition, iron oxide nanoparticles to be evaluated to fight against microbes. Before making medicines of these compounds, clinical experiments should be done.

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