Short Communication

J Babol Univ Med Sci
21; 2019. P:237-241

Evaluating Antibacterial Effect of Green Synthesis Oxide Iron Nanoparticles Using Cytoplasmic Extract of Lactobacillus casei

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J Babol Univ Med Sci; 21; 2019; PP: 237-41
Received: May 1st 2018, Revised: Nov 24th 2018, Accepted: Feb 3rd 2019.

ABSTRACT

BACKGROUND AND OBJECTIVE: With regard to the drug resistance, special attention has been placed on researches for discovering new antimicrobial substances such as nanoparticles. In green synthesis biological resources such as bacteria and yeast are used for producing metal nanoparticles. In this research, we used the Lactobacillus casei extract as a biological source for producing iron oxide nanoparticles and antibacterial effects of these nanoparticles against the standard strains of Escherichia coli and Staphylococcus aureus were investigated.

METHODS: for synthesis of green iron oxide nanoparticles, cytoplasmic extract of Lactobacillus casei and iron sulfate solution 10-3M were mixed and incubated for 3 weeks at 37 ° C in the presence of 5% carbon dioxide. Synthesis of iron oxide nanoparticles were evaluated using X-ray diffraction pattern and Transmission electron microscopy. Antibacterial effects of nanoparticles with dilution of 10, 100 and 1000 micro-grams per milliliter on two standard strains of Escherichia coli and Staphylococcus aureus were evaluated.

FINDINGS: Synthesis of iron oxide crystals was confirmed by XRD analysis. Based on transmission electron microscopy the average of nanoparticles was about 15 nm with a spherical shape. Antimicrobial effect of nanoparticles showed that at concentration of 100 and 1000 µg/ml has an inhibitory effect on the growth on Staphylococcus aureus equal to 12.03±0.32 and 16±0.5, respectively. The only concentration that showed significant effect was 1000µg/ml ($p \leq 0.001$). While are ineffective against Escherichia coli.

CONCLUSION: The results of this study indicated that producing iron oxide nanoparticles using cytoplasmic extract of Lactobacillus casei is a biologically safe method and very noteworthy in medicine and pharmacology and may be considered as a good candidate for the treatment of bacterial infections.

KEY WORDS: *Iron Oxide Nanoparticles, Lactobacillus Casei, Staphylococcus Aureus.*

Please cite this article as follows:

Torabian P, Ghandehari F, Fatemi M. Evaluating Antibacterial Effect of Green Synthesis Oxide Iron Nanoparticles Using Cytoplasmic Extract of Lactobacillus casei. J Babol Univ Med Sci. 2019;21:237-41.

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[DOR: 20.1001.1.15614107.1398.21.1.37.2]
[DOI: 10.22088/jbums.21.1.237]

Introduction

Nowadays researchers use biological systems to produce nanoparticles, which have minimal environmental hazards and have simple and environmentally friendly production methods (1). In this science, researchers use biological resources, including microorganisms, plant extracts and their metabolites (2,3). In recent years, following the widespread use of popular drugs to treat a variety of diseases, particularly microbial infections and cancers, numerous problems, including drug resistance and side effects from chemotherapy drugs are expanding. Antibiotics have the property that frequent use eliminates them, as well as the fact that challenges such as finding new antibiotic drugs have led to the low priority of discovery of new antibiotics for the pharmaceutical industry despite the clinical needs. Today, microbial resistance and the side effects of chemotherapy have created a major concern among researchers and have led to the need for alternative medicines.

Therefore, the pharmaceutical industry has focused its efforts on finding safe, non-toxic and effective drugs for the treatment of bacterial infections and reducing the side effects of chemotherapy (4-6). The aim of this study was to produce iron oxide nanoparticles using Lactobacillus casei extract as a probiotic bacterium and its effect on two standard strains of Staphylococcus aureus and Escherichia coli.

Methods

Lactobacillus casei with ID (PTCC 1608) was purchased from Microbial Bank of Iran Scientific and Industrial Research Organization. Cytoplasmic extract was prepared by freeze-thaw method and was added to aqueous solution of 3-10 mM iron oxide and incubated at pH 5.6 for 3 weeks at 37 ° C and incubated in the dark (7,8). At the end, the reaction solution was powdered by drying apparatus and X-ray diffraction (Philips11800) was used to study the production of nanoparticles. The specimen was placed on a glass substrate and the scan was performed at an angle of θ2 in the range of 20 to 80 degrees. The voltage used was KV40 and the power flow was 30 mA. The particle size was calculated by using the following formula:

$$D= 0.9\lambda/\beta\cos\theta$$

Transmission electron microscopy (Philips 208S 100Kv, Netherlands) was used to study the shape and size of the nanoparticles (10, 9). Standard strains of Staphylococcus aureus (ATCC 25923) and Escherichia coli (ATCC 25922) purchased from Daroush Tehran were cultured on Blood Agar and MacConkey agar medium and incubated for 24 h at 37 °C. 100 microliters of microbial suspension at half-McFarland concentration was bubbly cultured on Müller Hinton agar medium and drilled on 6 mm wells and 100 µl for dilutions of 10, 100, 1000 µg/ml. The nanoparticles were inoculated. 100 µl of cefalexin antibiotic (100 mg/ml) was used as positive control. After 24 hours' incubation at 37 °C, the diameter of non-growth zone was measured. All data were analyzed by one-way ANOVA and Measure Repeat test using SPSS 2016 software and $p<0.05$ was considered significant.

Results

The color of ferrous sulfate solution was changed from colorless to black after reduction of the iron ion by the cytoplasmic extract. The color intensity is due to vibrations of the active surface plasmon in iron oxide nanoparticles. The X-ray diffraction pattern confirmed the existence of iron oxide nanoparticles (Fig 1). Findings obtained from X-ray diffraction at scanning angle of 2θ and at degrees of 20 to 80 indicated four peaks at 311,220, 400 and 440, respectively that they correspond to the iron oxide sample pattern and the size of the nanoparticles after calculating with the 15 nm Debrec formula, it was found to be consistent with the size of the analysis obtained from XRD (Fig 2). Figure 2 shows the spherical shape of nanoparticles and the size of nanoparticles are on average between 10-15 nm, which is consistent with the size obtained by XRD analysis.

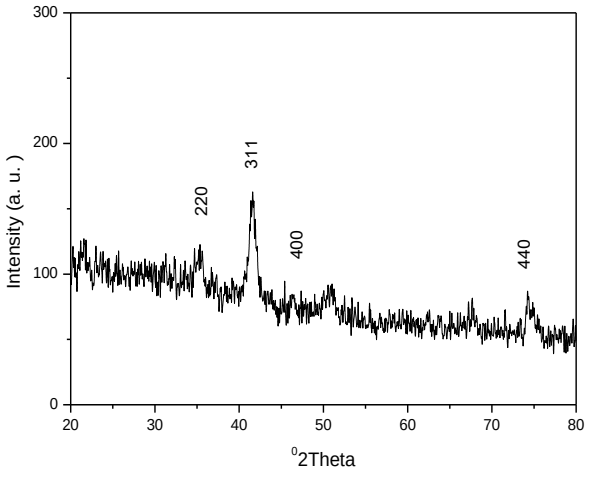

Fig. 1. The X-ray diffraction curve shows the size of the nanoparticles at 15 nm

[DOR: 20.1001.1.15614107.1398.21.1.37.2]
[DOI: 10.22088/jbums.21.1.237]

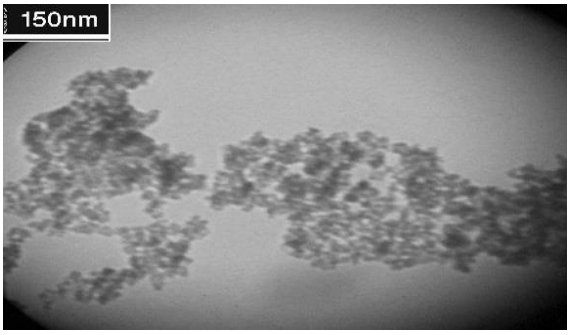

Figure 2. Electron microscope image of the nanoparticles showed a spherical shape with an average size of 10-15 nm. Bar = 150 nm

The results of antimicrobial effects by well plate method showed that iron oxide nanoparticles had inhibitory effect on the growth of Staphylococcus aureus while it had no inhibitory effect against Escherichia coli. The results showed that the nanoparticles at 10 µg/ml had no antimicrobial effect on Staphylococcus aureus while the concentrations of 100 and 1000 µg/ml had an inhibitory effect of 12.03±0.32 and 16±0.5, respectively. Results of statistical analysis showed a significant difference ($p<0.001$) between two concentrations of 100 and 1000 µg/ml of iron oxide nanoparticles with positive control group. Comparison of 100 and 1000 µg/ml concentrations of iron oxide nanoparticles also indicated that there was a significant difference between these two concentrations ($p\leq0.001$). These results indicate that the antimicrobial effects of the nanoparticles are dose-dependent and that as the concentration of nanoparticles increases, the antimicrobial effect increases (Fig 3).

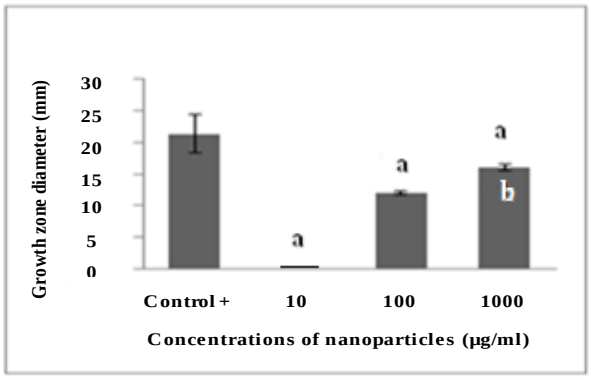

Figure 3. Comparison of the antimicrobial effect of different concentrations of nanoparticles against Staphylococcus aureus.

a letter indicates the comparison of concentrations of 10, 100, and 1000 green synthesis compared to positive control, and the letter b below the chart indicates the comparison of 1000 to 100 concentrations. The significance level is p<0.001.

Discussion

Based on the results of this study, iron oxide nanoparticles were synthesized from the extract of Lactobacillus casei. The iron oxide nanoparticles synthesized in this method are very small, 10-15 nm in size and spherical. It seems that the presence of reductive compounds and specific enzymes in the cytoplasmic extract reduces metal ions and produces iron oxide nanoparticles. In a study by Hilger et al., 15 nm silver nanoparticles were synthesized, spherical, stable, small and highly affected by the Lactobacillus fermentum supernatant (11).

One of the biggest medical problems is the elimination of microorganisms that have become resistant to chemical drugs over time. Increasing the dosage of the drug used to counteract resistant bacteria will increase the risk of accumulation of chemicals in the body, causing side effects and increasing their detrimental effect on the environment. Therefore, research into the discovery of new antimicrobial agents has attracted interest. The results of our study indicate that the synthesized iron oxide nanoparticles showed a good antimicrobial effect on Staphylococcus aureus. As the concentration of iron oxide nanoparticles increased, the growth zone diameter increased and the results of statistical analysis showed that after comparing two concentrations 100 and 1000 µg/ml of nanoparticles with positive control group, there was a significant difference between the two concentrations with the positive control group and with increasing the concentration, inhibitory effect on Staphylococcus aureus will increase. However, these nanoparticles had no inhibitory effect on Escherichia coli.

The mechanism of the inhibitory effect of iron oxide nanoparticles on Staphylococcus aureus as a Gram-positive bacterium appears to be different from that of Escherichia coli. The level of cytotoxicity of iron oxide nanoparticles is highly dependent on the type of organism and the concentration of the nanoparticles. At low concentrations, the nanoparticles can act as sources of iron to provide the organism with the iron needed to promote growth (12,13).

The results of the present study, together with the results of Nhiem et al., indicate that the gram-positive Staphylococcus aureus is more sensitive to the synthesized iron oxide nanoparticles than the gram-negative bacterium Escherichia coli (14). Gram-positive bacteria have mucopeptides in their cell wall, whereas gram-negative bacteria only have a thin layer of mucopeptide and most of the wall structure contains

[DOR: 20.1001.1.15614107.1398.21.1.37.2]
[DOI: 10.22088/jbums.21.1.237]

lipoproteins and lipopolysaccharides. They have an outer membrane around their cell wall and on the other hand they contain enzymes in the periplasmic space that are able to break down the outer molecules, thereby making them more resistant to antibacterial substances. However, in the study of Khatami et al., unlike the present study, iron nanoparticles showed more effect on Escherichia coli and this difference may be due to differences in the shape, size and concentration of nanoparticles and the test method (15). In general, regarding the possible mechanisms of interactions of nanomaterials with biological macromolecules, the difference between the negative charge of the microorganism and the positive charge of the nanoparticles acts as an adsorbent electromagnetism between the microbes and the nanoparticles and causes the nanoparticles to bind to the cell surface. Eventually, many of these contacts lead to the oxidation of the surface molecules of the microbes and their rapid death (16). Green chemistry-based nanoparticles may be used to treat microbial infections as a viable alternative to antibiotics in medicine.

Acknowledgment

Hereby, we would like to thank the Islamic Azad University of Falavarjan for providing the necessary facilities to conduct this research.

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[DOI: 10.22088/jbums.21.1.237]

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