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Evaluation of Antibacterial Activity of Iron Oxide Nanoparticles Against *Escherichia coli*

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Abstract

Introduction: Considering the usefulness of metal oxide nanoparticles in biology and biomedicine, iron oxide nanoparticles were biosynthesized using bioresource engineering to evaluate its antibacterial activity against *Escherichia coli*.

Methods: Macrodilution method was used for calculating the lowest concentration which prevented the growth of bacteria (minimum inhibitory concentration [MIC]), and the lowest concentration that destroyed all bacterial cells (minimum bactericidal concentration [MBC]).

Results: The lowest concentration of iron oxide nanoparticles that inhibited the growth of *E. coli* (MIC) was recorded at 250 µg/mL. On the other hand, the MBC of iron oxide nanoparticles was calculated at 500 µg/mL.

Conclusion: Iron oxide nanoparticles were produced by a green and eco-friendly, simple and inexpensive method. The results showed the inhibitory effect of iron oxide nanoparticles on *E. coli* at 250 µg/mL. This may suggest using these nanoparticles as potential antibacterial agents.

Keywords: Minimum inhibitory concentration, Minimum bactericidal concentration, Iron oxide nanoparticles, Antibacterial, Macrodilution, *Escherichia coli*

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Introduction

Antimicrobial agents considerably help to prevent and treat infectious diseases in humans and animals; however, the increase of resistant microbial strains has turned to a serious challenge in medicine.[1-4] Nanomaterials have attracted the researchers' interest due to their significant antibacterial activity.[5,6] In recent years, antimicrobial nanoparticles have paved the way for medicine and biotechnology.[7,8] Nanoparticles with high antimicrobial activity are a new class of biomedical materials.[9-13]

Iron oxide nanoparticles have a high specific surface area, so they are able to interact with bacterial surface structures. Furthermore, because of their relatively small size, they can facilitate the particle uptake by bacterial cells. The iron oxide nanoparticles are produced through various physicochemical methods.[14-17] The chemical approaches may be harmful for human and impose environmental and health risks. In another approach, magnetic nanoparticles are produced by biological resources (biosynthesis or green synthesis). Here, we aimed to evaluate the antibacterial impacts of iron oxide nanoparticles on a common bacterial strain in clinical practice.[18,19]

Methods

The iron oxide nanoparticles were synthesized using bioresource engineering, as previously described.[3] Briefly, *Rosmarinus officinalis* leaves (20 g) were washed, dried and powdered. The obtained powder was added to the glass flask containing 2000 mL sterile water. The final mixture was heated

at 80°C for 30 minutes. The sample was centrifuged. All steps were carried out under sterile condition (laminar air-flow). The $FeCl_3 \cdot 6H_2O$, 98% (ferric (III) chloride hexahydrate) stock solution (0. 1 M) was prepared. Twenty mililiters of obtained extract was mixed with 1 mM of $FeCl_3$ solution under constant stirring. The color change from light yellow to black indicated the production of iron oxide nanoparticles.[20]

Antibacterial Test

Bacterial strain used in this study (*Escherichia coli,* 1330) was supplied from Microbial Collection of Iranian Science and Technology Organization, with the Persian Type Culture Collection (PTCC). First, for the bacterial strain, the cell suspension with 0.5 McFarland turbidity ($10^8 \times 1.5$) was prepared and each well of the ELISA plate was filled with 100 mL of Muller-Hinton broth (MHB) and then 100 mL of iron oxide nanoparticles was poured in the first cast well; Afterward, 100 µL of this mixture was removed and transferred to the next well. This process continued to the last well. Finally, the concentrations of iron oxide nanoparticles (2000, 1000, 500, 250 and 125 µg/mL) were obtained.[21]

Minimum Inhibitory Concentration and Minimum Bactericidal Concentration Determination

The control sample (positive control) only contained 100 µL of bacteria with cell culture medium that reflected the growth of the bacteria in the absence of iron oxide nanoparticles. After dilution of bacterial strains, the final suspension was incubated for 24 hours at 37°C. Afterward, the growth of bacterial strains was determined by visual observation. The concentration of nanoparticles that prevented the growth of bacteria was recognized as minimum inhibitory concentration (MIC) and the lowest concentration that destroyed all bacterial cells was considered as minimum bactericidal concentration (MBC).[22]

Results

Transmission electron microscopy (TEM) images of bio-synthesized iron oxide nanoparticles are shown in Figure 1; the particle size range was from 1 to 12 nm. The iron oxide nanoparticles were mainly spherical and approximately monodisperse.

The study of antimicrobial effects of iron oxide nanoparticles using macrodilution method and the calculation of MIC and MBC against *E. coli* were performed. The antibacterial activity of iron oxide nanoparticles was investigated at concentrations of 2000, 1000, 500, 250 and 125 µg/mL. The lowest concentration of iron oxide nanoparticles that inhibited the growth of *E. coli* (MIC) was observed at 250 µg/mL, while the MBC of iron oxide nanoparticles against *E. coli* was calculated at 500 µg/mL.

Discussion

Initially, we eco-friendly synthesized iron oxide nanoparticles. These nanoparticles have small size and good stability when natural resources are used. In biosynthetic production of iron oxide nanoparticles, in comparison with physicochemical methods, less time is needed.

The findings showed that biogenic iron oxide nanoparticles represent antibacterial activity against *E. coli* in a dose-dependent manner. Thukkaram et al[23] reported that the highest inhibition of iron oxide nanoparticles (29 mm) against *Staphylococcus aureus* was observed at 150 ug/mL. Masadeh et al[24] reported MIC value of iron oxide nanoparticles against *Enterobacter aerogenes, Proteus mirabilis,* and *Klebsiella pneumoniae* in the range of 10-320 µg/mL of iron oxide nanoparticles. In the present study, MIC and MBC of iron oxide nanoparticles against *E. coli* was obtained at 250 µg/mL and 500 µg/mL, respectively. The mechanisms of antibacterial activity of these agents are not well known; however, there may be a role for these agents to act as membrane permeability enhancers, or as disruptors of the cell wall by generating reactive oxygen species.[25]

These results were obtained in vitro, but it should be mentioned that the high antibacterial effects of the studied iron oxide nanoparticles are not selective, and it can be generally effective on all kinds of human cells.

The application of iron oxide nanoparticles in pharmaceutical fields dates back to the beginning of the 1970s. Iron oxide nanoparticles have also been used for drug targeting, magnetic resonance imaging,[3] as spoilers for magnetic spectroscopy, and more recently as sensors for biomolecules.

The use of iron oxide nanoparticles as antibacterial agents has also been reported.[26] Engineering designs, physiochemical characteristics, biomedical applications, toxicity and magnetic nanotoxicology of iron oxide nanoparticles have been discussed.[27] In this study, we explored the antibacterial activity of iron oxide nanoparticles against a common bacterial strain.

Conclusion

Iron oxide nanoparticles were synthesized by a green

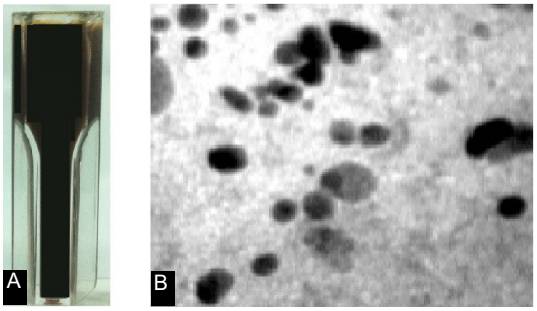

Figure 1. (A) Iron Oxide Nanoparticles Solution. (B) TEM Images of Iron Oxide Nanoparticles

and eco-friendly, simple and inexpensive method. The nanoparticles showed dose-dependent toxicity against *E. coli* growth. The iron oxide nanoparticles can be used as alternative antibacterial agents.

Ethical Approval

The study protocol was approved by Ethics Committee of Bam University of Medical Sciences, Bam, Iran.

Competing Interests

Authors declare that they have no potential conflict of interests.

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