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IN VITRO ANTIBACTERIAL ACTIVITY OF IRON OXIDE NANOPARTICLES AGAINST *ESCHERICHIA COLI* ISOLATED FROM UTI

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ABSTRACT

In this study , the suitability of commercial dry–powder iron oxide nanoparticles against Escherichia coli isolated from patients with urinary tract infection was evaluated . Antibacterial activity of iron oxide nanoparticles were investigated on E.coli by Qualitative and Quantitative screening methods . IONPs were characterized by Atomic Force Microscope (AFM) , Scanning Electron Microscope (SEM) , X–Ray Diffraction (XRD) and zeta potential , consequently , found nanoparticles with average particles size equal to 75nm . The result provide evidence that IONPs had excellent bactericidal effect on the concentration of 0.5mg/ml and bacteriostatic effect on (0.1 , 0.25 , 1 , 2)mg/ml concentration .

Key words : IONPs , *E.coli* , UTI .

Introduction

Nanotechnology is at the leading edge at the rapidly developing new therapeutic and diagnostic concepts in all areas of medicine (1) .

Nanotechnology offers tools that maybe able to detect disease in a very small volume of cells or tissue. In general , nanotechnology may offer a faster and more efficient means for scientists (2) .

Fe_3O_4 are superior to other metal oxide nanoparticles for their biocompatibility and stability . Recently , considerable research has been focused on iron oxide due to their potential uses such as Magnetic Drug Targeting , Magnetic Resonance Imaging (MRI) for clinical diagnosis (3) , detection of biological entities (cell , protein , nucleic acid , enzyme , bacteria , virus , ect.) (4) , gene therapy , stem cell tracking , magnetic separation of bacteria (5) , detection of infection disease pathogens

(6) and isolation of genomic DNA from *Escherichia coli* k12 (7) .

Fe_3O_4 nanoparticles has long been of great interest because of their immense usefulness in different domains , especially in water purification for the production of safe drinking water (8) .

Smaller particle size of IONPs have higher specific surface area , and so are better able to interact with bacterial surface structure because IONPs have a strong antibacterial power , also more easily facilitate particle uptake by microorganisms , and so possibly contribute to the fatality mechanism for microbe–nanoparticle interaction (9) .

Due to the increased resistance to existing antibiotics of various microorganisms , many researchers have turned towards engineered nanoparticles for finding a solution.

The ability of pathogenic bacteria to resist antibacterial agent , by genetic mechanisms and by phenotypic resistance due to the biofilm growing state , has emerged in the recent years and became a major health problem .

The studies reported various metal nanoparticles to exhibit antibacterial effect (10) .

The purpose of this study was to evaluate the in vitro antibacterial activity of iron oxide nanoparticles against *E.coli* .

Collection of the Urine specimens and cultured

Hundred and sixty (160) samples of urine specimens were collected with aseptic technique in sterile tubes . Mid–stream urine was taken from the patients and inoculated on both MacConkey agar and blood agar plates by direct streaking method (11).

Isolation and identification of bacteria

Bacteria isolated as pure colonies on MacConkey agar and Eosin methylene blue agar , then bacterial isolates were examined and identified by microscopic , cultural and biochemical test (12) and by aid Vitek2 automatic system . Vitek2 cards for identification and susceptibility testing were inoculated and incubated according to the manufacture's recommendations .

Iron oxide nanoparticle (Fe_3O_4)

Iron oxide nanoparticle is obtained from EPRUI nanoparticles & Microspheres Co. Ltd from China as powder black color with radius size 20nm MW 536.39 . The concentration was prepared by dissolving of iron oxide nanoparticle in sterilized distilled water.

Identification of nanoparticle

Identification of iron oxide nanoparticle in Ministry of science and technology in Nanotechnology Laboratories by using Atomic Force Microscopy (AFM) , Scanning Electron Microscopy (SEM) X-Ray Diffraction (XRD) and zeta potential . These techniques are used to measure the size of iron oxide nanoparticle as well as illustrate the shape and dimension (surface and three dimensional view) . XRD used to understanding of material and molecular structure . Zeta potential explain electrokinetic potential in colloidal dispersion in particle .

Antibacterial activity of iron oxide nanoparticles

Antibacterial activity of iron oxide nanoparticles was determined by preparation of inoculum 1.5×10^8 CFU/ml of *E.coli* isolates

after adjusted with 0.5 Mcfarland turbidity standard and also prepared various concentration of IONPs (0.1 , 0.25 , 0.5 , 1 , 2)mg/ml respectively .

Qualitative screening of antibacterial activity

Qualitative screening of antibacterial activity was carried out by an adapted agar diffusion technique using disk diffusion method saturated with various concentration of IONPs (0.1 , 0.25 , 0.5 , 1 , 2)mg/ml bacterial inoculum 1.5×10^8 CFU/ml spread on Muller–Hinton agar medium by sterile cotton swab , the saturated disks with IONPs were placed on the agar and incubated at 37°C for 24hrs (13) .

Quantitative screening of antibacterial activity

Quantitative screening of antibacterial activity achieved by added 0.5ml of inoculum 1.5×10^8 CFU/ml to test tubes containing 5ml of tryptic soy broth , then added 0.5ml of various concentration of IONPs (0.1 , 0.25 , 0.5 , 1 , 2)mg/ml , incubated in shaking incubator at 37°C for 24hrs . Later pulled 0.1ml of each test tubes spread on brain heart infusion agar plates , incubated at 37°C for 24hrs .

Tested these tubes with spectrophotometer the lowest concentration of IONPs that inhibited the growth of *E.coli* isolate considered as minimum inhibitory concentration (MIC) but the lowest concentration of IONPs that killed the bacterial isolates considered as minimum bactericidal concentration (MBC) (8) .

Results and discussion

The result showed that hundred and forty three bacterial isolates (89.38%) were identified 66 isolates 46.15% belonged to *E.coli* which was the most predominant in UTI and 23 isolates 16.08% belonged to *Kebsiella pneumoniae* , 19 isolates 13.29% belonged to *Proteus mirabilis* , 8 isolates 5.59% to *Pseudomonas aeruginosa* , these results showed when using the card for Gram–negative bacteria and others bacteria obtained from the card for Gram–positive bacteria including 27 isolates 18.88% belonged to *Staphylococcus aureus* .

The prevalence of UTI among females subject was found to be 54 isolates 81.8% against 12 isolates 18.2% in males .

The sensitivity of *E.coli* isolates 66 causing UTI was done against 16 type
of antibiotics from different classes by Vitek2 . The results shows in figure (1) that all isolates of *E.coli* were resistant to (Ampicillin , Cefazolin , Ceftazidime , Ceftriaxone , Cefepime) with percentage 100% while totally sensitive towards (Imipenem 0% , Ertapenem 5% , Piperacillin /Tazobactam 10% , Tobramycin 20% , Gentamycin 30% , Nitrofurantoin 30%) and sensitivity to other antibiotics at different proportions varied .

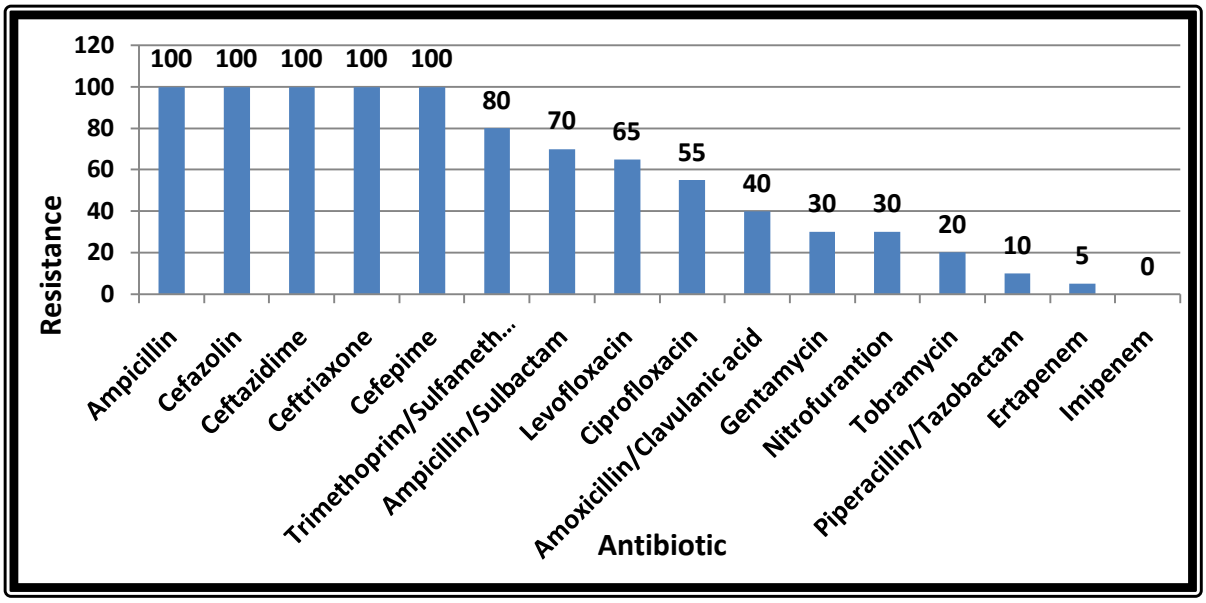


Figure (1) Percentage of antibiotics resistance of *E.coli* isolates from UTI .

The *E.coli* 1 and *E.coli* 2 isolates developed the highest multidrug resistance ; hence it was chosen for further experiments .

Characterization of iron oxide nanoparticles

Scanning electron microscopy (SEM)

SEM identified physical properties of nanoparticles such as morphology (shape , size) . The obtained results using scanning electron microscopy analysis clearly show that the iron oxide nanoparticles have cubic shape and the optimum size of magnetic nanoparticles to promote an effective bio–distribution is ranging from 10 to 100nm figure (2) .

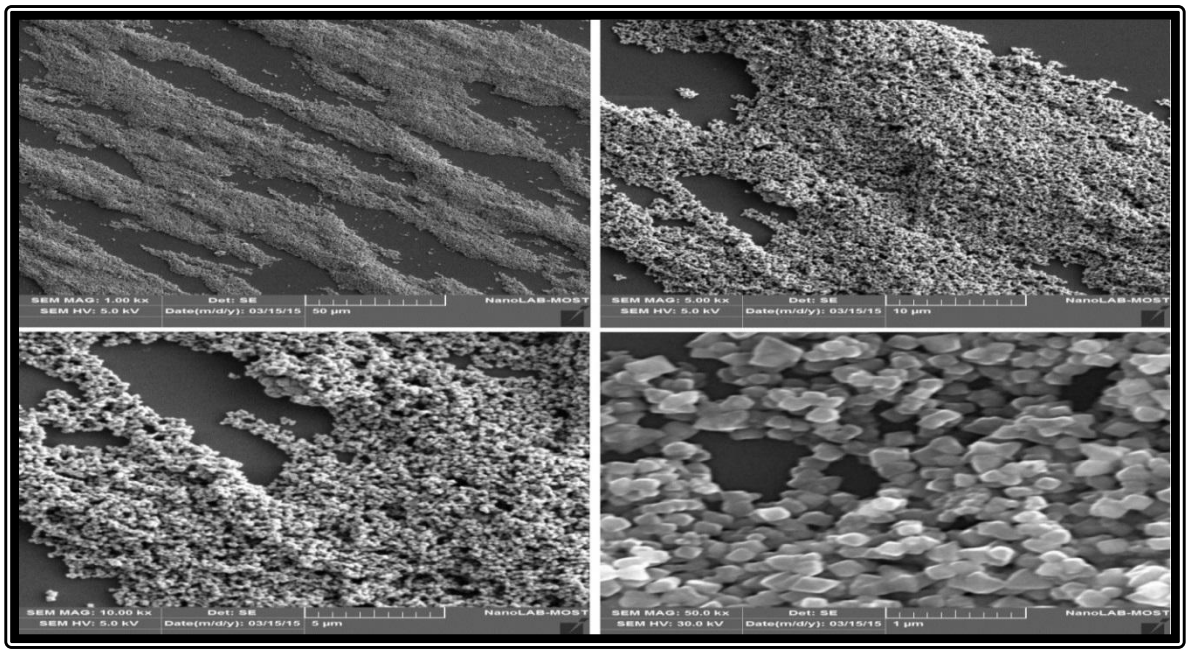


Figure (2) Scanning electron microscopic (SEM) images of Fe_3O_4 nanoparticles .

XRD pattern

Figure (3) shows the x–ray diffraction patterns for iron oxide (Fe_3O_4) nanoparticles powder . The peaks of the Fe_3O_4 are compared with those of standard . A series of characteristic peaks at 2Θ = 30.09° , 35.42° , 43.05° , 53.39° , 56.94° and 62.51° which corresponds to 220 , 311 , 400 , 422 , 511
and 440 Bragg reflection , respectively . This figure agree with standard magnetite (Fe_3O_4) XRD patterns , identify that the Fe_3O_4 nanoparticles are cubic structure .

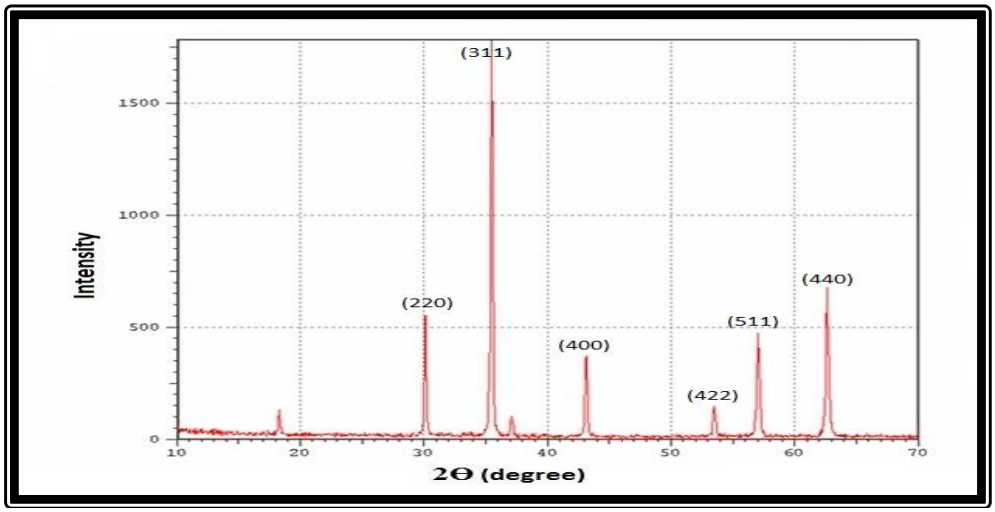
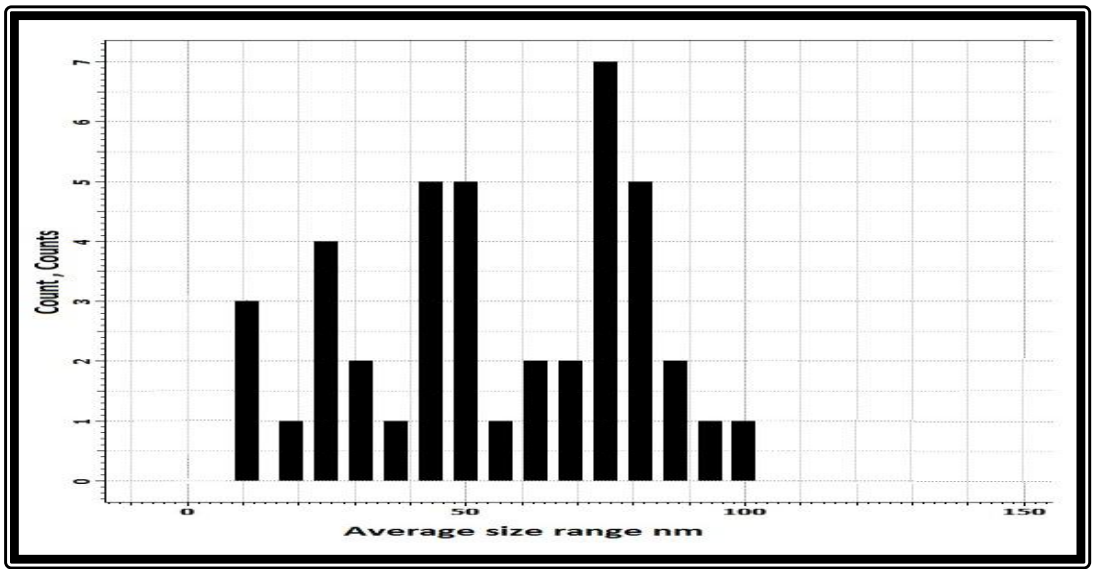


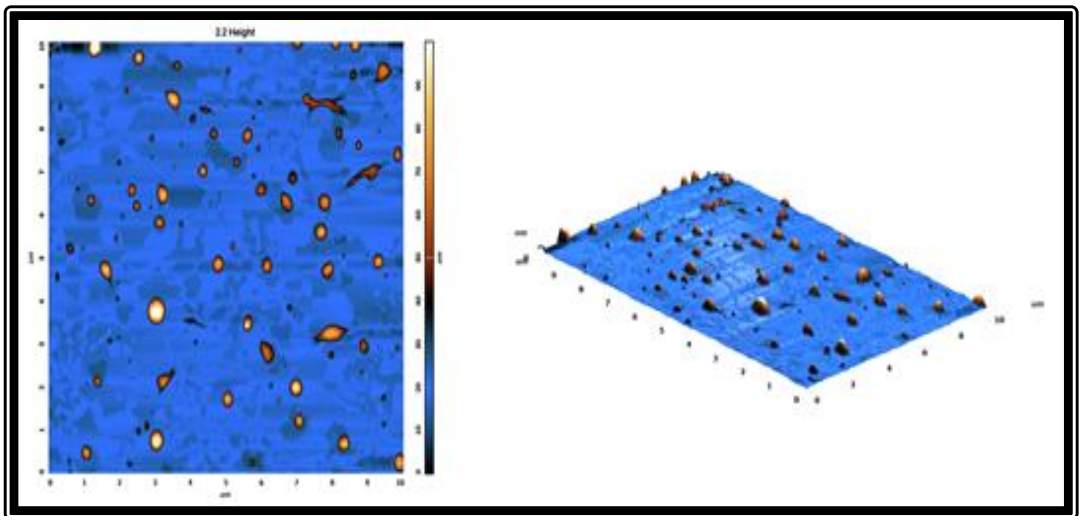
Figure (3) X–Ray Diffraction (XRD) patterns of Fe_3O_4 nanoparticles .

Atomic Force Microscopy (AFM)

AFM is a suited tool to determine the average size diameter of nanoparticles as well as surface texture and roughness in three dimensions , the results shows that the average size of iron oxide nanoparticles 75nm figure (4) .



A



B

Figure (4) Atomic force microscopy (AFM) image of Fe_3O_4 nanoparticles. (A) Average size range (nm) ., (B) Surface and three Dimensional view .

Zeta potential

Zeta potential is a measure of the magnitude of the electrostatic or charge repulsion/attraction between particles , it is a scientific term for electrokinetic potential in colloidal dispersion , it is a key indicator of stability of colloidal dispersions . The results showed the surface of iron oxide nanoparticles was found to be negatively charged with average zeta potential (-22.27mV) as evident in figure (5) .

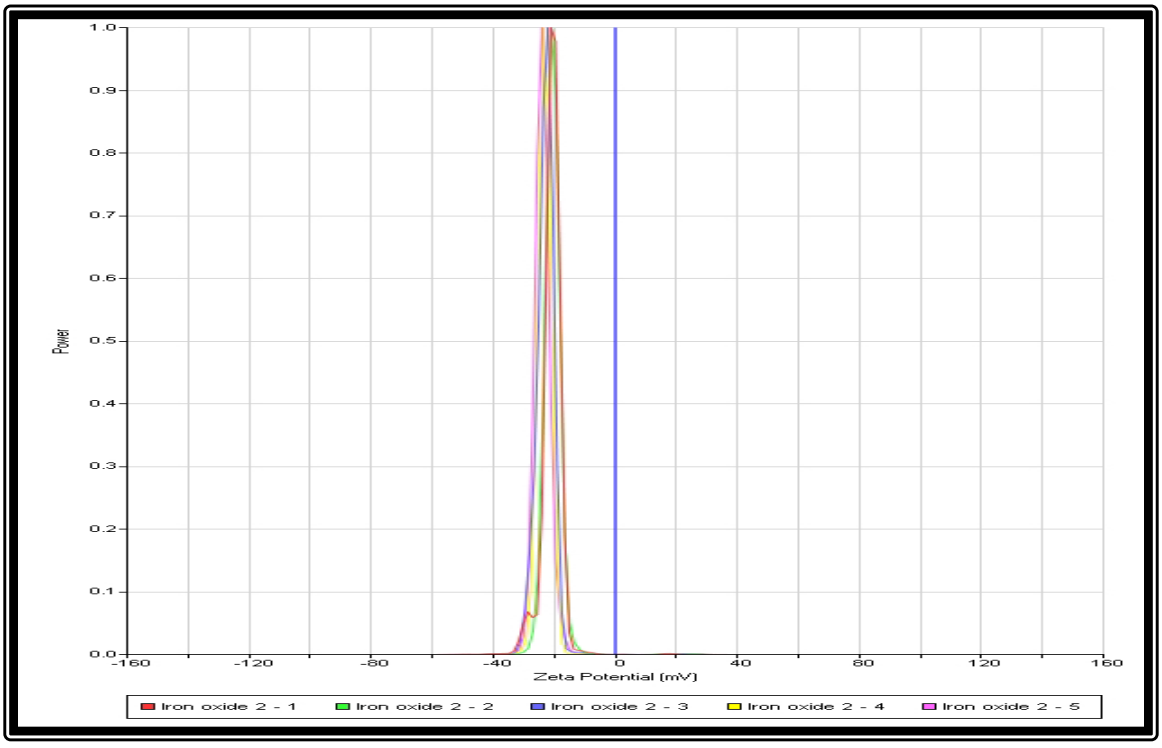


Figure (5) Zeta potential of Fe_3O_4 nanoparticles was measured by zeta plus with average zeta potential was -22.27 mV .

Antibacterial activity of iron oxide nanoparticles

Antibacterial activity of iron oxide nanoparticles was determined by using qualitative and quantitative screening method . In qualitative screening methods antibacterial activity of iron oxide nanoparticles was investigated at concentration (0.1 , 0.25 , 0.5 , 1 , 2)mg/ml the results showed that no effect of iron oxide nanoparticles at a concentration (0.1 , 0.25 , 1 , 2)mg/ ml when the plates were examined after 24hrs of incubation while contained inhibition zone at concentration 0.5mg/ ml (10 , 12)mm of iron oxide nanoparticles from two isolates (*E.coli* 1 , *E.coli* 2) .

In quantitative screening method iron oxide nanoparticles observed despite increasing interest in the antibacterial activity it shows a strong bactericidal activity at concentration 0.5mg/ml toward two isolates (*E. coli* 1 , *E. coli* 2) and bacteriostatic in the other concentration table (1) and table (2) .

Table (1) Effect of iron oxide nanoparticles on *E.coli* 1 growth by quantitative screening method .

Iron oxide nanoparticles Concentration mg/ml	Absorbance before incubation (nm)	Absorbance after incubation (nm)	Effect
0.1	0.280	0.21	Bacteriostatic
0.25	0.301	0.28	Bacteriostatic
0.5	0.353	0.004	Bactericidal
1	0.407	0.085	Bacteriostatic
2	0.41	0.088	Bacteriostatic

Table (2) Effect of iron oxide nanoparticles on *E.coli* 2 growth by quantitative screening method .

Iron oxide nanoparticles Concentration mg/ml	Absorbance before incubation (nm)	Absorbance after incubation (nm)	Effect
0.1	0.299	0.245	Bacteriostatic
0.25	0.302	0.237	Bacteriostatic

0.5	0.353	0.004	Bactericidal
1	0.407	0.048	Bacteriostatic
2	0.411	0.058	Bacteriostatic

At concentration 1mg/ml and 2mg/ml iron oxide nanoparticles shows higher activity in *E.coli* 1 and *E.coli* 2 but shows slightly higher activity at concentration 0.1mg/ml and 0.25mg/ml . The lowest concentration of iron oxide nanoparticles that inhibited the growth of *E.coli* 1 and *E.coli* 2 isolates considered as a minimum inhibitory concentration (MIC) was recorded at concentration 0.1mg/ ml but minimum bactericidal concentration (MBC) of iron oxide nanoparticles was recorded at concentration 0.5mg/ml from two isolates .

(13) reported that after the qualitative screening , the bacterial isolates which proved to be susceptible to the tested nanoparticles have been investigated in the quantitative assay for establishing the MIC value , IONPs generally stimulated the growth of *E.coli* at highest concentration 5mg/ml and bactericidal effect at low concentration 0.01mg/ml.

The bactericidal effect at currently study iron oxide nanoparticles could due to several mechanisms . The main mechanisms suggested is related to oxidative stress generated by ROS . ROS includes superoxide radicals , hydroxyl radicals , hydrogen peroxide , and single oxygen that may cause chemical damage to proteins and DNA in bacteria secondly , electrostatic interaction between nanoparticles and bacterial cell membranes or cell membranes proteins can results in physical damage , which ultimately leads to bacterial cell death (14) . Other studies demonstrated that the small size of nanoparticles could contribute to their antibacterial effects (15,16) . (17) reported that IONPs was inactivation of *E.coli* could be due to the penetration of nanoparticles with size ringing from 10 to 80nm in to *E.coli* membranes .

(18) reported that IONPs synthesized by *lactobacillus fermentum* isolate had inhibition activity against pathogenic bacteria (*E. coli*) with reduction of growth .

Conclusion

Recent development in the field of nanoparticles and promising results from in vitro studies provide for a new and urgently needed strategy for the treatment of biomaterial implant associated infections using nanoparticles .

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