Microbicidal Effect of Fe_2O_3 Nanoparticles in Antimicrobial Agent System

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ABSTRACT

Microbial antibiotics resistance is considered a serious health issue in the Middle East and developing countries. In this study, the Fe_2O_3 nanoparticles was prepared chemically, and the particles size and shape were analyzed by using Scan electron microscope (SEM) and X-Ray diffraction (XRD). Different concentration of Fe_2O_3 nanoparticles were used and examined on *E.coli* and *S. aureus*. Using liquid dilution and in vitro cytotoxicity assay by microplate toxicity test (MTT). The microbial cell metabolic activity was measured on gram-negative, gram-positive bacteria and fungi after treating with different concentrations of Fe_2O_3 nanoparticles. The results of liquid dilution method showed that the MIC of Fe_2O_3 nanoparticles are 30 µg/ml and 40 µg/ml on *E.coli* and *S. aureus* respectively. The results of MTT assay exhibited the ability of Fe_2O_3 nanoparticles to eliminate the gram negative bacteria (*E.coli* and *K. pneumonia*e) at 20 µg/ml, while *S. aureus*, *M. luteus*, *Candida albicans* and *Candida parapsilosis* were totally eliminated at 30 µg/ml.

Keywords: nanotechnology, Fe_2O_3, bactericide effect, gram-negative and gram-positive bacteria, fungi.

التاثير القاتل للمايكروبات لجسيمات أوكسيد الحديد النانوية في نظام المركبات المضادة للنمو المايكروبي

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الخلاصة

مقاومة المضادات الحياتية هي مشكلة صحية عامة رئيسية في بلدان الشر ق الأوسط. في هذه الدراسة تم تحضير مركب Fe_2O_3 النانوي كميائيا و تم التحري عن حجم الجزيئات وشكلها باستخدام تقنية مجهر المسح ألألكتروني (SEM) و الحيود أشعة X (XRD). تمت دراسة ادنى فعالية مضادة للنشاط المايكروبي ضد بكتريا *E.coli* و *St. aureus* بوجود تراكيز مختلفة من جسيمات Fe_2O_3 النانوي بطريقة الانتشار في الوسط السائل وأختبار قياس السمية خارج الخلايا الحية بطريقة الاطباق متعددة الحفر (MTT). تمت دراسة الفعالية المثبطة للنمو المايكروبي ضد البكتريا السالبة لصبغة غرام ،البكتريا الموجبة لصبغة غرام والفطريات بعد معاملتها بتراكيز مختلفة لاوكسيد الحديديك النانوي. اظهرت نتائج تجربة الانتشار في الوسط السائل أن ادنى فعالية مثبطة للنمو المايكروبي لاوكسيد الحديديك النانوي ضد بكتريا *St. aureus* ,*E.coli* كانت 30 µg/ml و 40 µg/ml على التوالي كما اظهرت نتائج فحص MTT قدرة جسيمات Fe_2O_3 النانوية على تثبيط واضح لبكتريا الاختبار كانت النتائج ضد البكتريا السالبة لصبغة غرام *E.coli* و *K. pneumonia* كانت 20 µg/ml كما حصل تثبيط تام بتركيز 30 µg/ml للبكتريا الموجبة لصبغة غرام والفطريات :

S. aureus, .*Candida albicans* and *Candida parapsilosis*, *M. luteus*

الكلمات الرئيسية : المواد النانوية.Fe_2O_3, , المضادات الحياتية, البكتريا سلبية لصبغة غرام ، البكتريا إيجابية لصبغةغرام والفطريات.

1. INTRODUCTION

According to world health organization, the hospitals in Middle East countries are facing a serious challenge represented by the failure of the used antibiotic system to treat neonatal infection because of the antibiotics resistance bacteria. To address this issue, many methods were adopted such as the logical usage of antimicrobial agents, guidance in the hospitals, and the community spreading microbial resistance. Thus, there was an urgent need to develop a new technique to increase the effectiveness of antibacterial against infectious agents to develop a public health free of resistance with less cost و **Park et al., 2009.**

Mineral aggregates might contain metals such as Fe, Cu and Ag with different oxidation states, and this increase their germicidal effect. These metals are responsible for inhibition of the cellular respiration process, DNA, RNA disruption due to its ability to inactivate of –SH radicals groups in respiration enzymes, **Chaloupka et al., 2010.** Natural mineral aggregates (NMAs) have shown germicidal activity, as it may cause defect in targeting cells losing homogeneous composition and containing undesirable metals **Miranda-Ríos and Luna Pabello, 2002-2003.** Highlights the importance of the current nanotechnology revolution in health care, medication sectors, pharmaceutical industries and the prediction of nanotechnology has the impact to change the ways of produce, process, package, transport, and consume **Chan et al., 1993**.

Nanoparticles were expected to open up some issues to fight and prevent diseases by atomic scale effect of materials, **Saba et al., 2012.** On the other hand, **Joo et al., 2005** focused on the relation between the iron oxide nanoparticles size (less than 100 nm) and its inhibitor effects on living cells. **Keenan et al., 2008** was used iron oxide nanoparticles for treating several sorts of cancer as targeted medication delivery. **Zhang et al., 2007** utilized iron oxide nanoparticles as external magnetic field on implant infection and could be used as an effective bactericidal agent. Recent investigation on iron oxide nanoparticles and its role in antimicrobial field are too limited; therefore, this study had made a trial to detect the potential influence of Fe_2O_3 nanoparticles as antimicrobial agent.

2. MATERIALS AND METHODS

2.1.Preparation of Fe_2O_3:

1. 32.31 g of Fe $(NO_3)_3.9H_2O$ was totally dissolved in 400 ml of deionized water to form a 0.2 M solution.
2. 28.77 g of NaOH was completely dissolved in 1200 ml of deionized water to form a 0.6 M solution, 1: 3 ratio.
3. NaOH solution was added to $Fe(NO_3)_3.9H_2O$ solution with continuous stirring at room temperature to increase the pH up to 14.
4. A brown color precipitate was rapidly formed. This precipitate was washed many times using deionized water, filtered, and dried by lyophilization.

2.2. Microorganisms and Culture Media

The organisms *K. pneumonia* (MTCC109), *E .coli* (MTCC118), *S. aureus* (MTCC96), *M. luteus*, (ATCC4698) *Candida albicans* (MTCC183) and *Candida parapsilosis* (MTCC2509) were used and inoculated with 50 ml of Mullar Hinton Broth (Difco Co., Detroit, Mich).

2.3 Determination the Cytotoxicity Effect of Fe_2o_3 Nanoparticles by MTT Test.

The living microbial cell activity was measured by the efficiency of dehydrogenase enzymes in the cell. 95µl of Muller Hinton Broth mixed with different concentration of Fe_2O_3 nanoparticles (10µg, 20µg, 30µg…. 100µg), poured in microplate and incubated at 37ºC for 24 hours. 10 µl of (5mg of MTT (3- (4, 5 –Dimethylthiazol-2-yl)-2, 5-Diphenyltetrazolium Bromide) was dissolved in 1 ml of deionized water), and added to each well of microplate and incubated for 4 hours. The contents of each well collected and centrifuged at 8000 rpm for 15 minutes and dissolved in 100µl of Dimethyl sulphoxide. Using ELISA reader, the microplate was read at 570 nm and the percentage of viable cells were calculated as followed formula:

$$\% \text{ Dead cells} = ((\text{Control O.D} - \text{Test O.D}) / \text{Control O.D}) * 100 \quad (1)$$

2.4. Minimum Inhibitor Activity Fe_2o_3 Nanoparticles Tested by Liquid Dilution Method

The influence of Fe_2O_3 nanoparticles against (*S. aureus* and *E .coli*) strains were studied by using liquid dilution method, **Shrivastava et al., 2007.** The bacterial cultures inoculated in 100 ml Mullar Hinton culture media until the broth density become 0.2 at 600 nm (OD of 0.2 were corresponded to a concentration of 10^8 CFU. mL^{-1} of medium).

Subsequently, 100 ml of Mullar Hinton medium mixed with different concentrations of Fe_2O_3 nanoparticles and inoculated with (*S. aureus* and *E .coli*). The growth rates of the organisms were studied by measuring the optical density at 600 nm in regular intervals.

3. RESULTS AND DISCUSSION

3.1. Synthesis and Characterization of Fe_2O_3 Nanoparticles

The shape and size of Fe_2O_3 nanoparticles were proved by (SEM) image**, Fig. 1(a)** which showed that synthesized particles were spherical and approximately 30 µm in size. Threads-like or tube-like structures are clearly visible between spherical particles **Fig. 1(b)** which are responsible for increasing the reaction surface area.

The X-ray diffraction presented in **Fig. 2** clarified the peaks of samples which refer to particle size. The formation of stoichiometric single maghemite phase and the main peaks of maghemite nanoparticles were at $2\theta \approx 24.2, 33.2, 35.6, 40.8, 49.2, 54.1, 62.5, 63.9$ correspond to (211), (302), (313), (219), (422), (426), (524), and (4112) respectively. The calculated cell parameters were (a = 8.4329; c = 24.9587; c/a = 2.9597) and the particle size was calculated according to Debye-Scherrer formula:

$$D = (0.9 * \lambda) / (\beta * \cos\theta) \quad (2)$$

The (313) reflection peak at $2\theta = 35.6$ indicated the formation of maghemite nanoparticles with approximately 24.7 nm diameter **Qusay et al., 2011**.

3.2. Inhibitor Activity of Fe_2O_3 Nanoparticles

The results of minimum inhibitor characterization of Fe_2O_3 nanoparticles on. *E.coli* and *S .aureus* detected by liquid dilution methods showed in **Fig. 3**. The MIC of Fe_2O_3

nanoparticles against *E.coli* and *S. aureus* were 30 mg/ml and 40 mg/ml respectively, while at 60 mg/ml the growth of *E.coli* and *S .aureus* were completely inhibited. **Fig.4** and **Table 1** showed the antimicrobial effectiveness of Fe_2O_3 nanoparticles on pathogenic strains which determined by MTT assay and indicated the complete inhibition of growth of gram negative bacteria (*E.coli* and *K. pneumonia*) at 20 µg/ml and 30 µg/ml for *(S. aureus* and *M. luteus*), in addition to completely blocking of the activity of (*Candida albicans* and *Candida parapsilosis*) at 30 µg/ml.

The less toxicity to mammalian cell, heat resistance and germicidal effect of Fe_2O_3 nanoparticles are related to several mechanisms. The main mechanism is the ability of ferrous oxide nanoparticles to cause oxidative stress created by ROS (the effect of hydroxyl radicals (-OH), superoxide radicals (O_2^-), singlet oxygen ($1O_2$) and hydrogen peroxide (H_2O_2)), **Sies, 1997**. The ROS effects cause proteins and DNA damage in the cell **Lee et al., 2000**. In this study, the metal oxide Fe_2O_3 nanoparticles might be acted as a source of hydroxyl radicals. **Keenan et al., 2008** noted similar approach, when H_2O_2 reacted with ferrous irons to produce hydroxyl radicals via the Fenton reaction. The created radicals caused damage to most biological macromolecules.

The other mechanism is the size of particles and surface morphology which might enhance the activity of metal nanoparticles to reduce the killing time. Entering the living cell and reacting with –SH thiol group of protein, the nanoparticles might deactivate cell membrane and cell organelles **Touati, 2000**. **Lee et al., 2000** reported that the use of zero-valent iron nanoparticles with sizes ranging from 10–80 nm lead to inhibit the growth of *E. coli* after interact with intracellular oxygen and cause disruption to cell membrane, proteins and DNA. The size of Fe_2O_3 nanoparticles (24.7 nm) used in this study may increase the bactericidal effects by zero-valent iron nanoparticles.

Also, the nanoparticles concentration were considered as a main cause of elevating the rate killing of *E.coli, K. pneumonia, S. aureus, M. luteus* and *Candida albicans, Candida parapsilosis as well* when incubated with different concentration. Increasing the density of nanoparticles might raise the rate of hydroxyl radicals (-OH), superoxide radicals (O_2^-), singlet oxygen ($1O_2$) and hydrogen peroxide (H_2O_2) around the microbial cell. **Tran et al., 2010** demonstrated the relation between the concentration of Fe_2O_3 nanoparticles and the bacterial inhibition time and showed the toxic effects of Fe_2O_3 nanoparticles in the cell wall might increase the concentration of lactate dehydrogenase enzyme which is considered as indicator for cell membrane damage.

Finally, this study supported the ability of Fe_2O_3 nanoparticles to eliminate the bacteria and fungi, and the possibility to use it, as solution, as antimicrobial agents.

4. CONCLUSIONS

Fe_2O_3 nanoparticles were prepared successfully and characterized by XRD and SEM. Fe_2O_3 showed microbicidal ability measured by liquid dilution method and MTT assay. Fe_2O_3 nanoparticles cause inhibition to all tested microbial strains in this study. Fe_2O_3 nanoparticles are easy to prepare with low cost and high reactivity. Thus, they could be considered as promising killing agents in antimicrobials system.

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NOMENOCLATURE

β = Beta, the line broadening at half the maximum intensity.
θ = Theta, the Bragg angle.
λ = Lambda, X-ray wavelength.
μm = micrometre

Ag = silver

C. albicans = Candida albicans

C. parapsilosis = Candida parapsilosis

CFU = cell forming unit

Cu = copper

DNA = Deoxyribonucleic acid

ELISA = enzyme-linked immunosorbent assay

E.coli = Escherichia coli

Fe = Iron

Fe (NO3)3.9H2O = Iron(III) Nitrate Nonahydrate

g = gram

H_2O_2 = hydrogen peroxide

K. pneumoniae = Klebsiella pneumoniae

M. luteus = Micrococcus luteus

MTT = microplate toxicity test; Cytotoxicity test.

MTCC = Microbial Type Culture Collection

MIC = minimum inhibition concentration

ml = milliliter

M = molarity

nm = nanometer,10^9 m

NMAs. Natural mineral aggregates

NaOH = sodium hydroxide

pH = the decimal logarithm of the reciprocal of the hydrogen ion activity in a solution.

Pb = lead

O.D = optical density

$^{\cdot}OH$ = hydroxyl radicals

$O_2^{\cdot}$ = superoxide radicals

$1O_2$ = singlet oxygen

ROS = Reactive Oxygen Species

RNA = Ribonucleic acid

SEM = Scan electron microscope

S. aureus = Staphylococcus aureus

XRD = X-ray diffraction

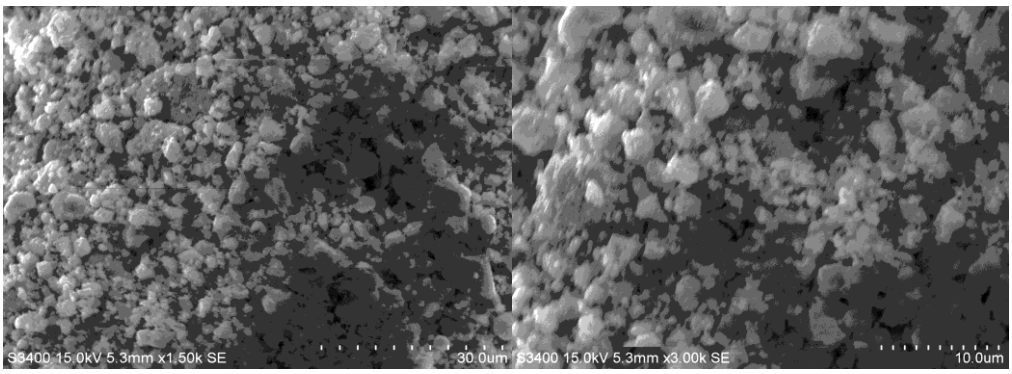

Figure 1. SEM Image of heated and non heated Fe_2O_3.

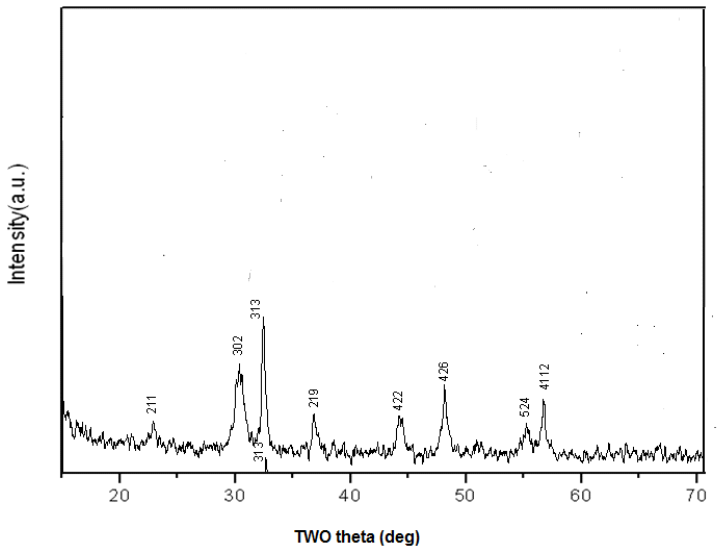

Figure 2. X-ray diffraction analysis of Fe_2O_3 nanoparticles samples.

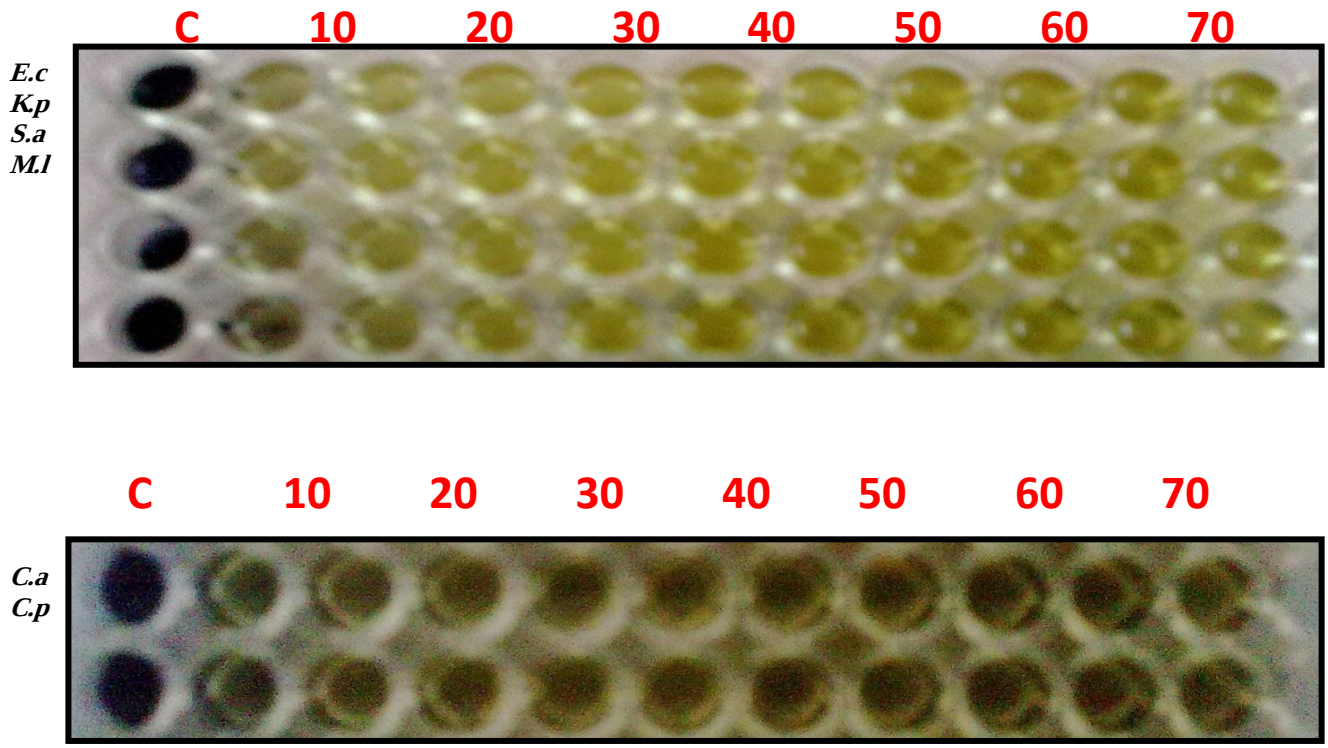

Figure 3. Antimicrobial activity of Fe_2O_3 nanoparticles by MTT - (3- (4, 5 – Dimethylthiazol-2-yl) -2, 5-Diphenyltetrazolium Bromide) assay

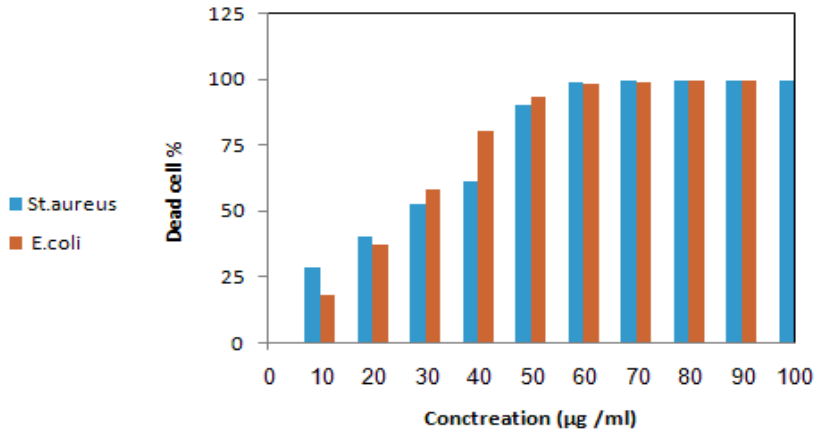

Figure 4. Antimicrobial activity of Fe_2O_3 nanoparticles

Table 1. Antimicrobial activity of Fe_2O_3 nanoparticles.

Concentrations	**O.D for *E.c***	**O.D for *S.a***
Control	0	0
10	20	35
20	35	45
30	55	50
40	82	65
50	92	80
60	100	100
70	100	100
80	100	100
90	100	100
100	100	100