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Antimicrobial Activity of Copper Oxide Nanoparticles on Multidrug Resistant Bacteria MDR and *C. albicans*

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Abstract

The increase of antimicrobial drug resistance of microorganisms and their harmful effect on human beings necessitate finding solutions and alternative methods to reduce the risk of this phenomenon. Nanoparticles have been focused on as an alternative method to prevent the spread of drug resistant microorganisms. Copper oxide nanoparticles (CuO-NP) were synthesized by Direct-Current (DC) plasma method. Polycrystalline monoclinic structures for CuO lattice consistent with the standard card No. 96-101-1195. The diffraction lines appeared at Bragg's angles of (35.5565º, 38.7921º, 48.8611º, and 58.4383º) matching the planes (11-1), (111), (20-2), and (202) respectively. The SEM image showed homogeneous Small rods in size and shape in the sample of diameter about 20 nm and a length of about 78 nm. MDR bacteria and fungi were isolated from Baqubah teaching hospital in Diyala governorate. CuO-NPs showed antimicrobial activity of these pathogenic by visualizing the inhibition zone around the CuO-NPs disks.

Keywords: Copper Oxide Nanoparticles CuO-NPs, Antimicrobial Agent, Multidrug Resistance Bacteria, Fungi. direct plasma technique.

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الفعالية ضد الميكروبية لجسيمات أوكسيد النحاس النانوية على بكتيريا متعددة المقاومة للأدوية و C. albicans

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الخلاصة

إن الزيادة الملحوظة في مقاومة الكائنات الحية الدقيقة للأدوية المضادة للميكروبات وتأثيرها الضار على البشر يستلزمان إيجاد حلول وطرق بديلة لتقليل مخاطر هذه الظاهرة. تم التركيز على الجسيمات النانوية كطريقة بديلة لمنع انتشار الكائنات الحية الدقيقة المقاومة للأدوية. تم تصنيع جزيئات أكسيد النحاس النانوية (CuO-NP) بطريقة البلازما ذات التيار المباشر (DC). الهياكل أحادية الميل متعددة البلورات لشبكة CuO المتوافقة مع البطاقة القياسية رقم 96-101-1195. ظهرت خطوط الحيود بزوايا (35.5565° و 38.7921° و 48.8611° و 58.4383°) مطابقة للمستويات (11-1) و (111) و (20-2) و (202) على التوالي. أظهرت صورة المجهر الالكتروني الماسح SEM ظهور عصيات صغيرة متجانسة في الحجم والشكل مطمورة في البوليمر في عينة قطرها حوالي 20 نانومتر وطولها حوالي 78 نانومتر. تم عزل االبكتيريا المقاومة للمضادات الحياتية MDR و فطريات المبيضات *Candida albicans* في مستشفى بعقوبة التعليمي في محافظة ديالى. أظهرت جزيئات اوكسيد النحاس النانوية CuO-NPs فعالية مثبطة لهذه الاحياء المجهرية الممرضة من خلال ملاحظة منطقة تثبيط النمو حول أقراص جزيئات اوكسيد النحاس النانوية CuO-NPs.

كلمات مفتاحية: البكتيريا المقاومة للمضادات، فطريات، جسيمات اوكسيد النحاس النانوية، تقنية البلازما المباشرة.

Introduction

Antibiotic resistance is a serious problem. The main cause of antimicrobial agent ineffectiveness is antibiotic- resistant bacteria. Bacterial resistance is a results of changes in microorganisms' capacity to resist antibacterial treatments, either by rendering them inactive or reducing their therapeutic efficiency. Due to genetic alterations, these resistances arise spontaneously in microorganisms throughout time. The misuse and abuse of antibiotics greatly encourage such changes [1]. Infectious disorders and the development of drug resistance in harmful bacteria and fungi are on the rise at an alarming rate, which is a cause for concern [2]. However, microbial infections continue to cause a significant proportion of morbidity and mortality rates [3]. As a result, there is a necessity to develop new antimicrobial compounds using simple, quick, and environmentally acceptable methods that have broad-spectrum antimicrobial action. The new generation of antimicrobial agents, which will control a wide spectrum of microbial illnesses will be such agents [4]. Metal nanoparticles are one of the antibacterial agents that have lately gained a lot of attention [5]. Due to its prospective uses in optoelectronics, nanoelectronics, nanodevices, nanosensors, catalysis, and, information storage; metal oxide nanoparticles (CuO-NPs) have gotten a lot of interest.

CuO has gained special attention among metal oxide NPs because it is one of the most basic member of the copper compound family and exhibits a variety of relevant physical features including electron correlation effects, high temperature superconductivity, and spin dynamics[6]. Metal nanoparticles' unique antibacterial characteristics are related to their small size and high surface area to volume ratio [7]. Other than releasing metal ions, these physiochemical features of nanoparticles allow for significant contact with bacterial membranes, resulting in wide effects antimicrobial agents [8]. Bacteria that are in contact with metal nanoparticles have specific characteristics that explain their behaviour [9] since it has a negatively charged surface [10]. Negatively charged cell walls of bacteria are attracted to the positively charged surface of nanoparticles due to electrostatic interactions. On the other hand, these nanoparticles showed a strong bond with membranes, resulting in distrupting of cell walls and, increases in their permeability. Also, nanoparticles can release metal ions which are, capable of entering the cell and disrupt its biological processes [11]. Within the cell, nanoparticles or metal ions can induce production of Reactive Oxygen Species (ROS). This oxidative stress causes the oxidation of glutathione, thus suppressing the antioxidant-defense mechanism of bacteria against ROS. These metal ions can interact with cellular structures (like, proteins, membranes, DNA), thus disrupting cell functions [11], besides, they can form strong coordination bonds with N, O, or S atoms which are abundant in organic compounds and biomolecules. Since this bond is generally non-specific, metal nanoparticles can exhibit a broad spectrum of activity on microorganisms [12]. The aim of this work is to synthesize Copper oxide nanoparticles (CuO-NP) by Direct-Current (DC) plasma method, and determine antibacterial activity of CuO NPs against MDR bacteria and fungi.

Material and methods

Synthesis of copper oxide nanoparticles

A copper oxide nanoparticle was synthesized by the plasma method. Figure 1 shows the atmospheric DC current argon plasma set-up for the preparation of CuO-NPs using direct-current (DC) plasma at room temperature between plasma discharge from capillary glass (0.5 mm inner diameter) with the surface of the distilled water as cathode and copper electrode as anode with 3 cm electrodes separation. The argon flow through was 50 (*sccm*-standard cubic *centimeters*). The distance of the plasma-liquid interface was 1 cm. as shown in Fig. 1. The process was applied for 10 min at a constant current of 10mA at 2.3 kV applied voltage [13].

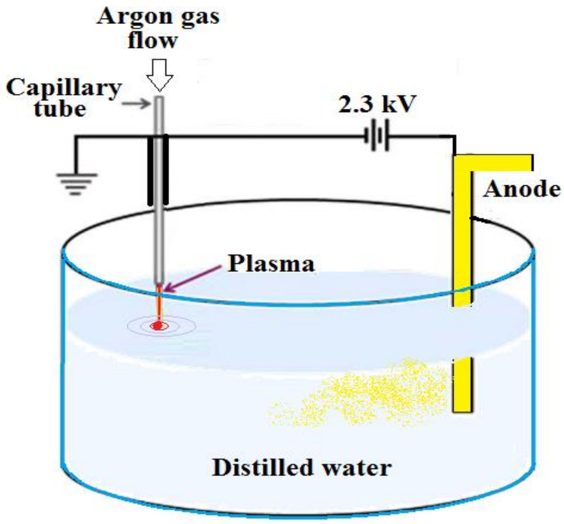

Figure 1: Schematic of the atmospheric argon plasma system for generation nanoparticles in liquid

Isolation of pathogenic bacteria and fungi

Pathogenic bacteria (*Staphylococcus aureus, E. coli, and Pseudomonas aeruginosa*) and *Candida albicans* were isolated and identified at Baqubah teaching hospital in Diyala governorate. *Staph. aureus*, and *P. aeruginosa* were isolated from patients while *E.coli* and *C. albicans* from Urinary Tract Infection (UTI) patients. All of these bacteria were MDR (multidrug resistant bacteria) and *C. albicans* was resisted to Fluconazole.

Antimicrobial activity of coper oxide nanoparticles

Whatman No.1filter paper was used for copper oxide nanoparticles disks. Disks were prepared with 5 mm in diameter, then placed on Petri dish for sterilization by autoclave. Prepared disks were immersed in the copper oxide nanoparticles solution, then left to dry in sterile conditions at room temperature. Isolated bacteria and *C. albicans* were inoculated on previously prepared Muller Hinton agar and left to dry. Previously prepared disks and the control disk were placed on inoculated media then incubated for 24 hours at 37°C. Antimicrobial activity of

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nanoparticles was observed by visualizing inhibition zone of these pathogens around these disks.

Results

Fig. 1 shows the XRD pattern for the prepared CuO NPs. Polycrystalline monoclinic structures for CuO lattice consistent with the standard card No. 96-101-1195. The diffraction lines appeared at Bragg's angles of (35.5565°, 38.7921°, 48.8611°, and 58.4383°) matching the planes (11-1), (111), (20-2), and (202) respectively. The lines broaden as a result of the nanostructure of the prepared particles. The intermolecular distances (dhkl) were measured using the Bragg formula while the crystallite size (C.S) was measured by applying the width at half maxima in Sheerer formulas shown in Table 1.

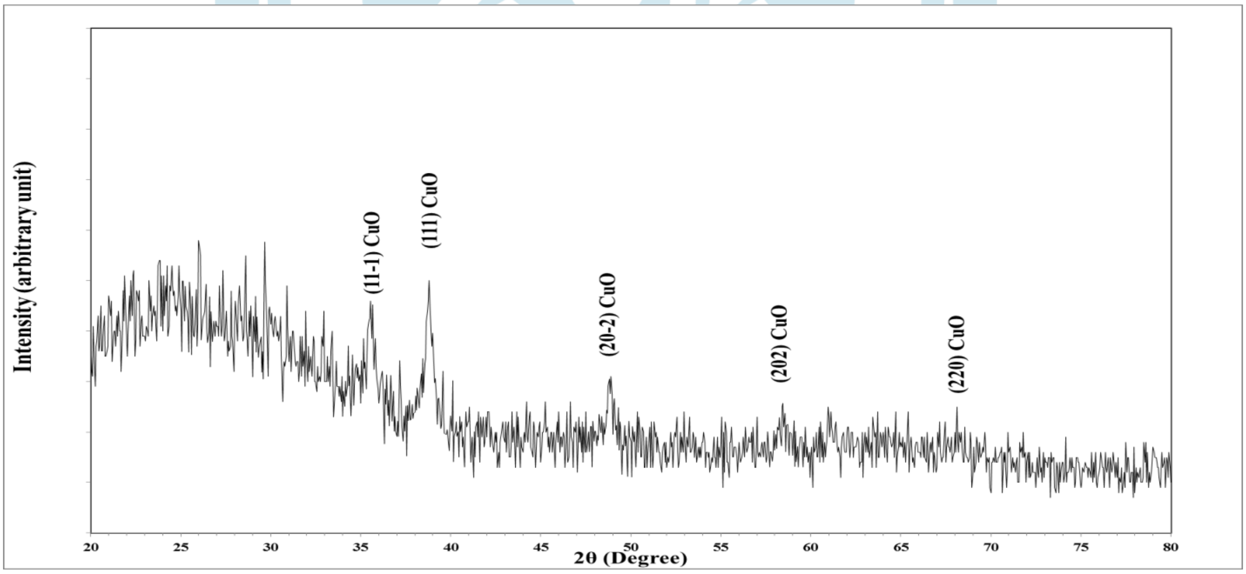

Figure 1: XRD pattern for the prepared CuO-NPs

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Table 1: X-ray diffraction parameters for the CuO-NPs

2θ (Deg.)	FWHM (Deg.)	dhkl Exp.(Å)	C.S (nm)	dhkl Std.(Å)	hkl
35.5565	0.4918	2.5228	17.0	2.5228	(11-1)
38.7921	0.4400	2.3195	19.2	2.3212	(111)
48.8611	0.4918	1.8625	17.7	1.8617	(20-2)
58.4383	0.4401	1.5780	20.7	1.5764	(202)

Fig. 3 Shows the SEM image at 100 kX magnification power for the prepared CuO-NPs. homogeneous small rods of in size and shape imbedded in the polymer appeared in the sample of diameter about 20 nm - 78 nm.

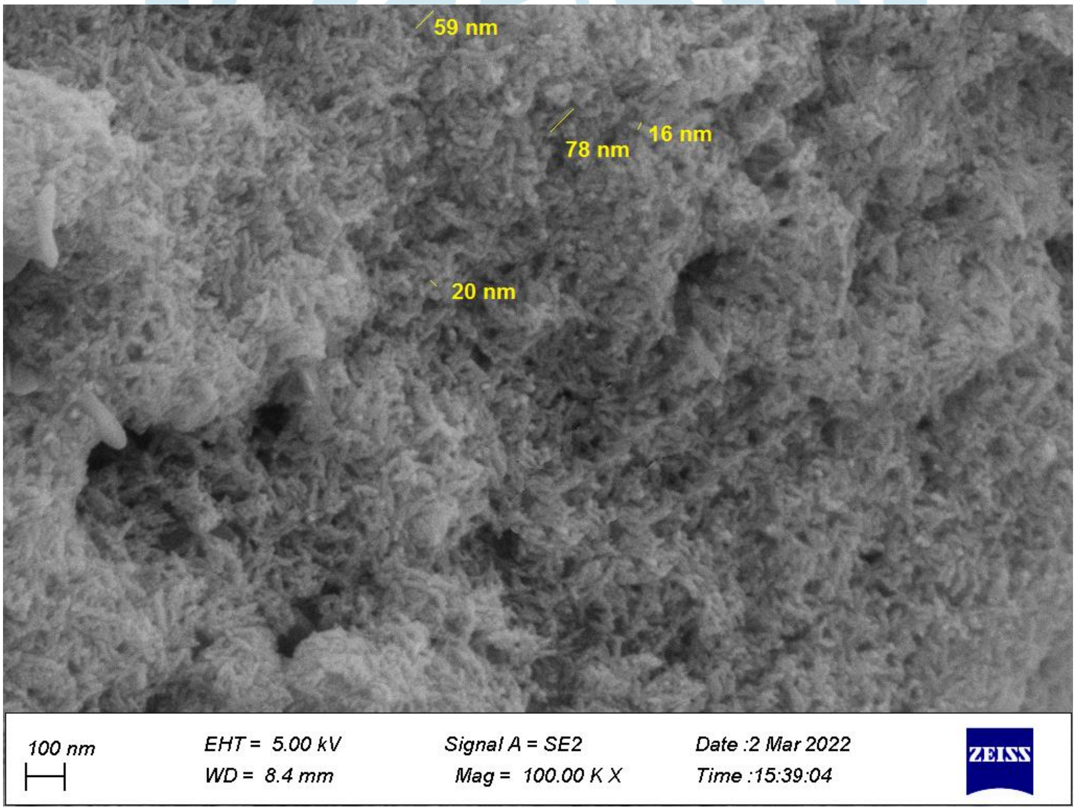

Figure 3: SEM image for the CuO-NPs

CuO-NPs show an antimicrobial effect on pathogenic bacteria and *Candida albicans* by observing the inhibition zone of growth around CuO-NPs disks as shown in Figure 4. A study showed a complete inhibition (100%) of *Candida* growth on TSB media by using a high concentration of CuO NPs (100-1000 µg/ml) [14]. Another study demonstrated that exposing the soft denture liners infected by *Candida albicans* and *Streptococcus spp*. to CuO NPs at a concentration of 500 µg mL−1 showed significant activity (75%) in inhibition of these oral pathogens [15].

The inhibition activity of CuO-NPs on these pathogenic microorganisms by causing damage to the cell envelope as well as, forming free radicals and copper(I)–peptide complex which they identified is the source of toxicity towards cells [16].

Table 2: Coper Oxid NP effect on inhibition diameter.

MICROORGANISMS	INHIBITION ZONE (MM)	
	Mean	SD
Staph. aureus	20.67	1.70
Pseudomonas aerogenosa	10.33	1.25
E. coli	12.00	0.82
Candida albicans	10.67	1.25

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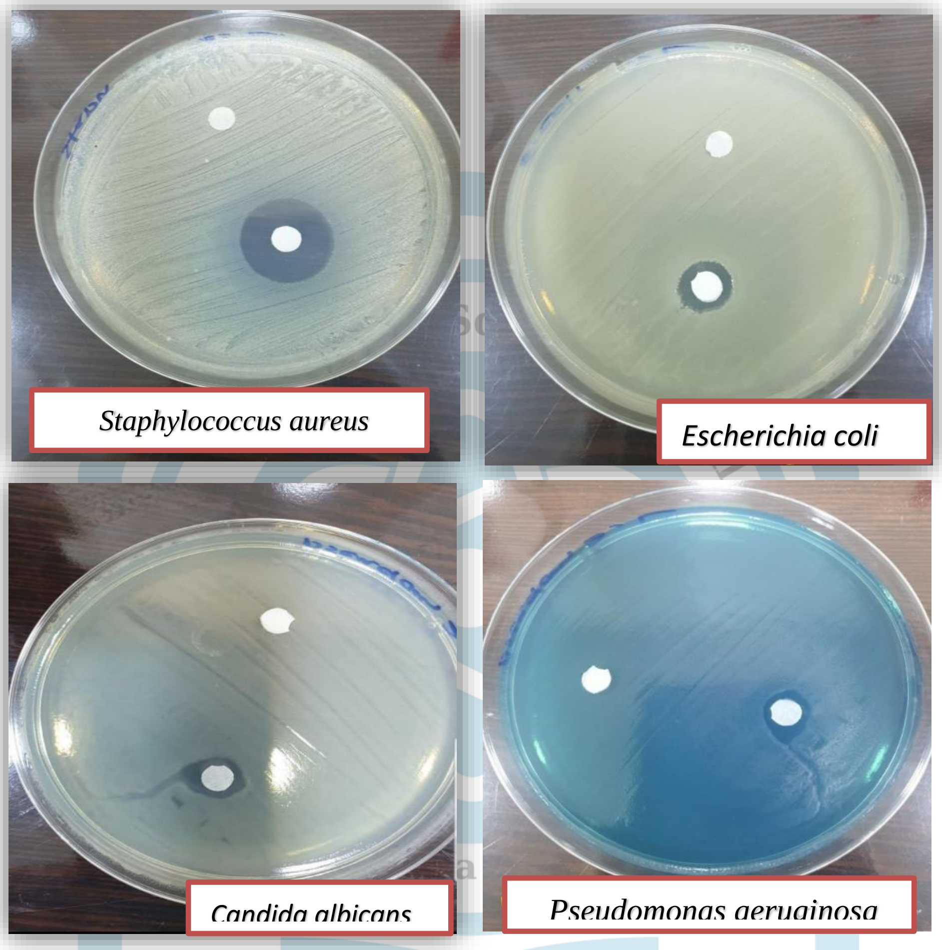

Figure 4: Inhibitory effect of CuO-NPs on some pathogenic bacteria and fungi.

Conclusion

According to the results of the current study, the plasma method was a good technique used for synthesis of CuO NPs, and our study confirmed the role of CuO NPs in inhibition growth of most local clinical isolates such as *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas spp.*, *Candida*.

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