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Article

Biologically synthesized Copper Nanoparticles from *S. epidermidis* on resistant *S. aureus* and cytotoxic assay

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ABSTRACT: The risk of significant concern is resistance to antibiotics for public health. The alternative treatment of metallic nanoparticles (NPs), such as heavy metals, effects on antibiotic resistance bacteria with different types of antibiotics of - impossible to treat using noval eco-friendly synthesis technique nanoparticles copper oxide (CuO NPs) preparation from *S. epidermidis* showed remarkable antimicrobial activity against *S.aureus* Minimum inhibitory concentra range (16,32,64,256,512) µg/ml via well diffusion method in vitro, discover those concentrations effected in those bacteria and the best concentration is 64 µg/ml, characterization CuO NPs to prove this included atomic force microscope, UV, X-ray Diffraction and TEM, and anticancer activity was tested against cell membrane A375. The cell viability was decreased with increasing the CuNPs. It displayed a dose-dependent sequence of progressive cytotoxicity beginning at a lower concentration to its maximum inhibition (22)% inhibition of HdFn cells and (66)% inhibition of A375 cells.
Keywords: CuO NPs, Green Synthesis, A375cells.

Introduction

Nanoscale demonstrates behavior materials frequently between that intermediate of microscopic solid and that of molecular system [1]. Among other inorganic nanoparticles, metal oxide Copper oxide (CuO), safe and simple, has been used excessively and is an effective antimicrobial agent [2]; it is less toxic, naturally eco-friendly and supports the Environmental Protection Agency for humans [3]. Different nanomaterials shaped Cu/ CuO green synthesis Cost-effective and practical agent against pathogens microorganism and complications environmental such toxicology field and potential risk [4] Refers Nanotoxicology to the assay of the interactions of biological systems with nanostructures such relationship between the physical and chemical properties with responses induction of toxic biological [5].

Natural processing agents biology nanoparticle completely eco-friendly the present that is less toxic and good antimicrobial agent [6] widely explored CuNPs have been for their effectiveness against a wide range of bacterial and fungal strains assay antimicrobial potentials in a demonstrating that they are a[7]

Materials and methods

Isolation and Identification of S. epidermidis and S.aureus :

Confirmation of Bacteria Thirty swap wounds were collected from (Al-Yarmouk Teaching Hospital/ Iraq) cultured on mannitol salt agar and biochemical characteristics and confirmed by Vitek 2 system [8].

Antibiotic Sensitivity Test:

The disk diffusion method against (Gentamicin, Azithromycin, Cefoxitin, Trimethoprim and vancomycin) According to the Clinical and Laboratory Standards Institute [9] determination antibiotic suitability test zone of inhibition (in mm).

Suspension of S. epidermidis:

Bacteria were cultured in Mueller Hinton Broth to prepare a suspension incubated for 18 h at 37° C. The bacteria were centrifuged at 10000 g for 10 min at 4° C. Remove bacteria cells and preservative suspension to synthesize copper nanoparticles [10].

Synthesis of CuONPs:

Synthesis of copper oxide nanoparticles

Green Synthesis of CuONPs was 0.1 M dissolved in 100ml of deionized water. A 15 ml of 1mM copper acetate was added to 15 ml of the isolated supernatant S. epidermidis in 100 ml Erlenmeyer flasks. The flasks were incubated at 30°C–32°C and observed for color change—copper acetate only, which depended on negative control. No color change was observed over time Figure 1.

Figure 1. Synthesis of CuONPs by biological method.

MIC determination

Doubling dilutions serial of CuONPs were prepared using MHB, added to each test culture bacteria was tube incubator for 24 hours at 37° C different concentrations between (16,32,64,256,512) µg/ml—figure 2.

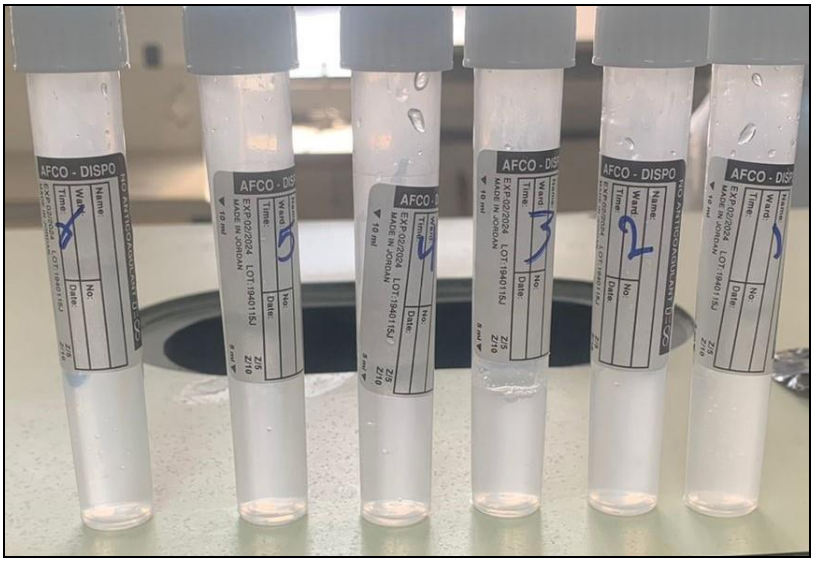

Figure 2. MIC CuONPs by biological method.

Characterization techniques included:

UV-vis Spectroscopy.

(XRD).

Transmission Electron Microscopy and Scanning Electron Microscopy.

(AFM)

Activity of CuONPs in vitro:

CuONPs synthesis by biological method assay Antimicrobial by Agar Diffusion Method. Against multidrug resistance (MRSA), MHA was used for bacteria. A 5mm diameter agar well was made using a sterilized cork borer. The several prepared concentrations of CuONPs were taken in different concentrations between (16,32,64,256,512) µg/ml, the minimum inhibitory concentration (MIC) that dissolved with sterile deionized water, in addition to on Muller Hinton agar incubator for 48 hrs at a 37 ° C and then the growth inhibitory zone was examined [12].

Cytotoxicity of Assay

Cell Line Maintenance

When the cells in the vessel formed a confluent monolayer, The A375 cells and HdFn cells from the human colon were using microtiter plates at a concentration of range (0.5 _ 2.5) mg mL [13].

MTT Protocol

The cytotoxic effect of CuONP synthesis from S. epidermis was performed using MTT ready-to-use Kit contents.

Result

Bacterial Isolates:

Isolates and Culture media:

The isolate was the source of isolation (wounds). It was diagnosed and identified; the isolates on Blood agar showed yellow-gray colonies (4-3) mm in diameter on the zones of β-hemolysis as shown in Figure 3.

Figure 3. A) *S. epidermi*s B) MRSA on MSA agar at 37c for 24 hrs.

Disk diffusion method Figure 4 The isolates of S. aureus susceptibility towards Azithromycin and cefoxitin. There was less resistance to vancomycin than other antibiotics.

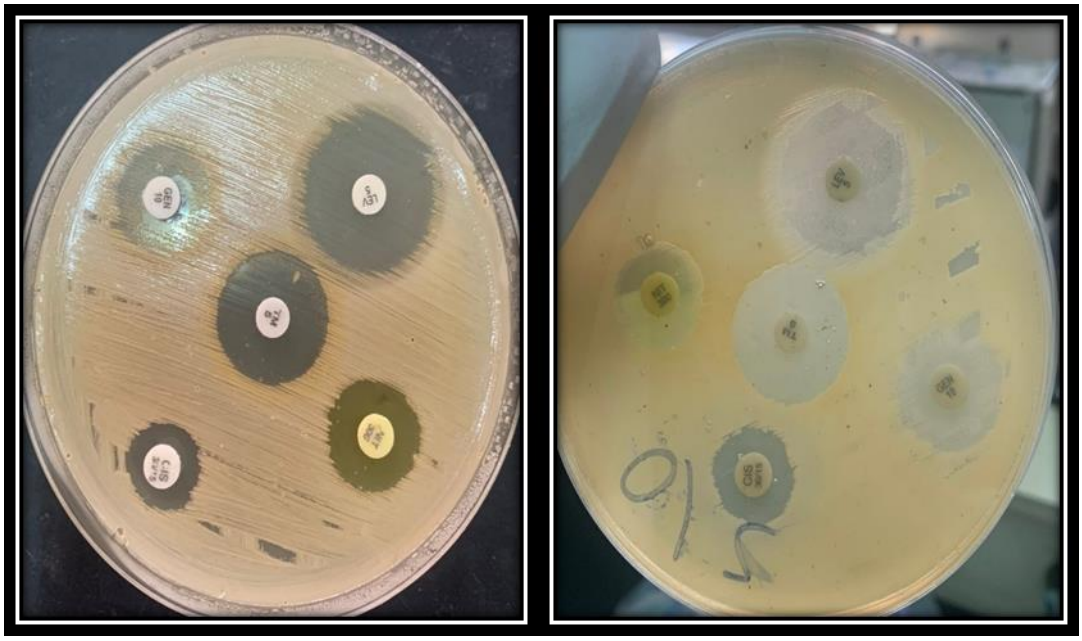

Figure 4. AST test of *S.aureus* on MHA.

Biosynthesis of CuONPs Synthesis and Characterization of CuNPs:

Were successfully synthesized CuNPs using reducing and stabilizing agents bacteria extracts. A rapid change from the blue color of the copper solution to a pale green with the addition of the extracts indicated in Figure 5.

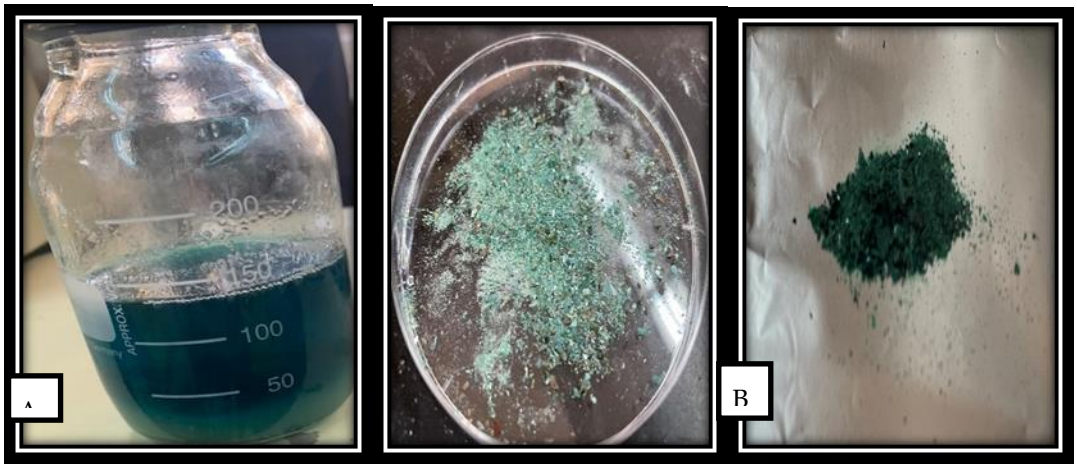

Figure 5. CuNPs solution biosynthesized by *S. epidermis* A: CuNPs with *S. epidermis* after 72hrs at room temp B. Synthesis CuNPs as Precipitate green metallic particles like powder.

UV–Vis Spectral Analysis:

The results showed that biosynthesized CuNPs exhibited a maximum peak (absorption peaks at 275 and 280 nm, as shown in Figure 6.

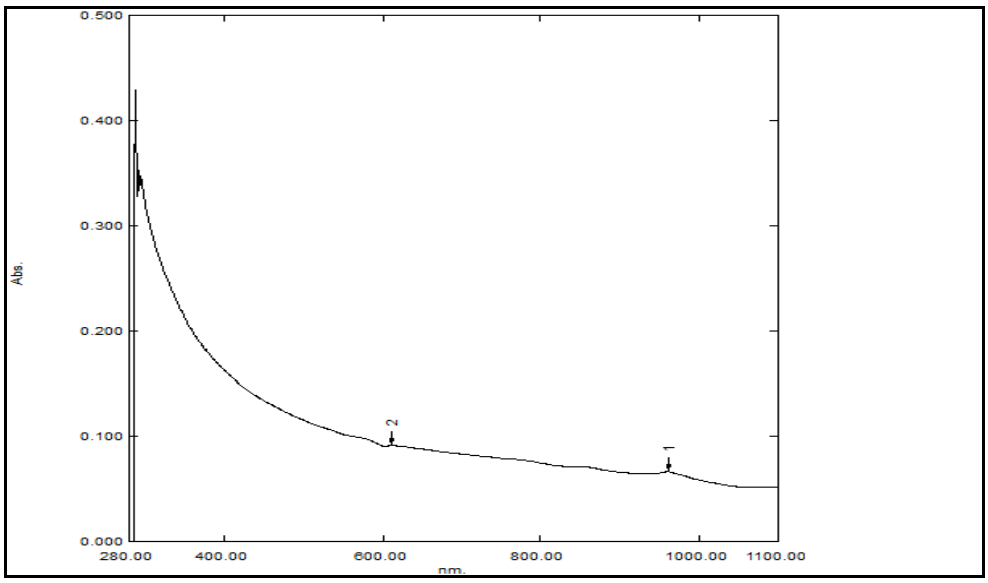

Figure 6. UV–Vis spectrophotometry of CuNPs.

As seen by increasing or reducing green synthesized CuO NPs, reduced absorption was extract volume. At 230 nm, the yellowish-green edge is responsible for the depth defects [16].

X-ray Diffraction (XRD):

The XRD spectra of CuO NPs, nanoparticle powder shown in Figure 7, confirmed the formation of hexagonal (wurtzite) structure of the CuNPs NPs by revealing prominent peaks corresponding to the diffraction peaks (100), (002), (101), (102), (110), (103) and (112).

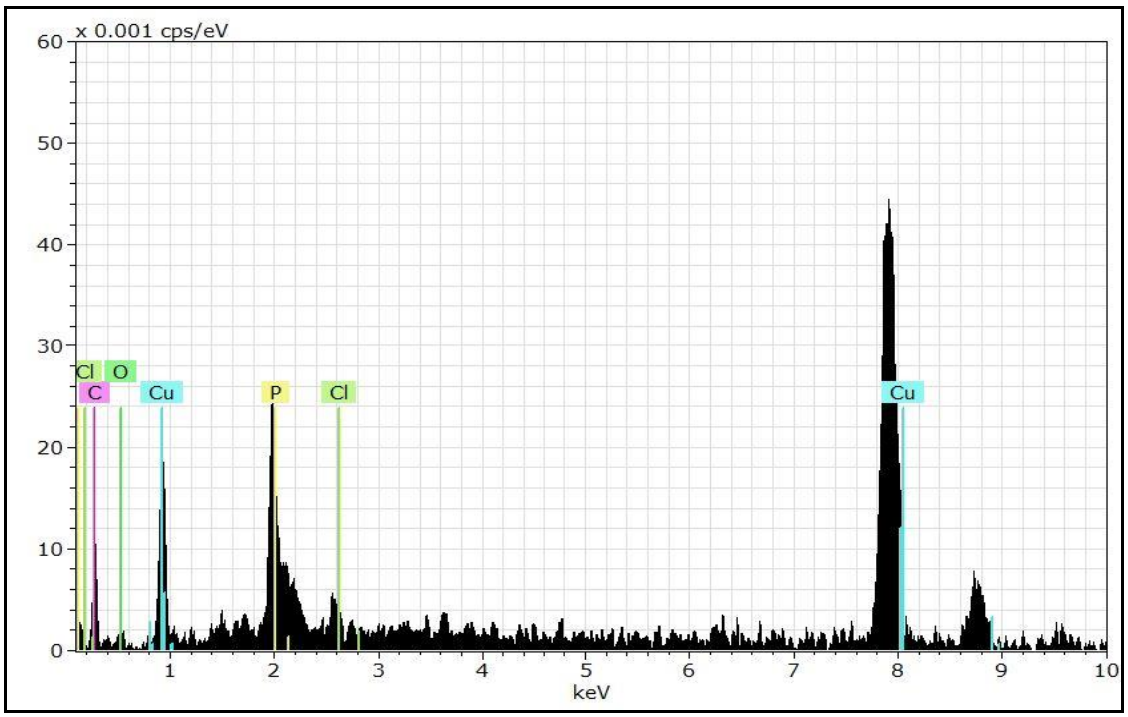

Figure 7. XRD analysis of synthesized CuoNPs.

Atomic force microscopy (AFM) analysis:

Characterize the biosynthesis of CuNPs by biological method detecting their average diameter to the morphology in both two- three dimensions. The results obtained in this study showed that the biosynthesized CuNPs by S. epidermis had an average diameter of (36) nm, as shown in the Table and Figure 8.

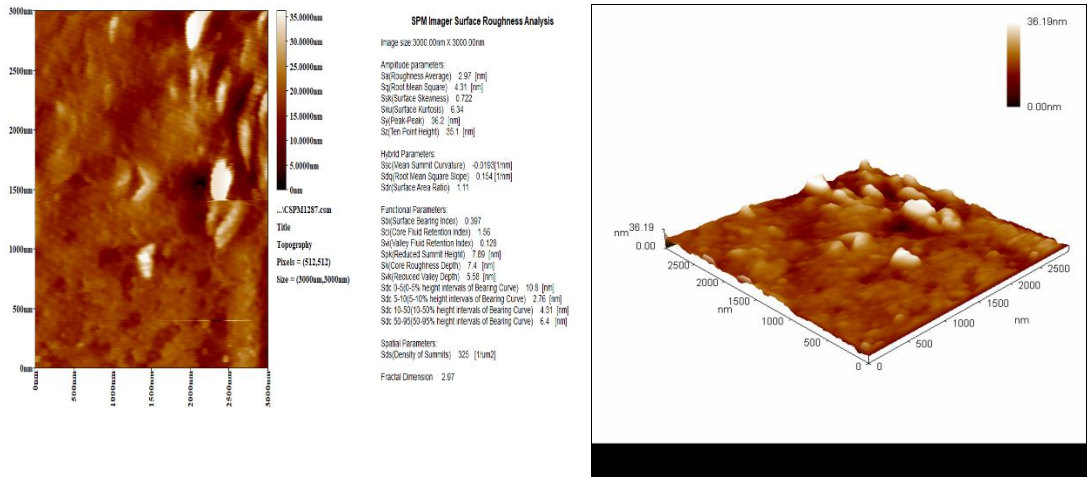

Figure 8. The biosynthesized CuNPs (A) 2D AFM of CuNPs (B) 3D AFM CuNPs.

Transmission Electron Microscopy analysis (TEM) of CuONPs:

Figure 9 is a spherical shape with crystalline of the prepared CNPs with 5-40nm diameter ranges. On the other hand, Morphological alternations in E. coli were observed using (SEM) by [18].

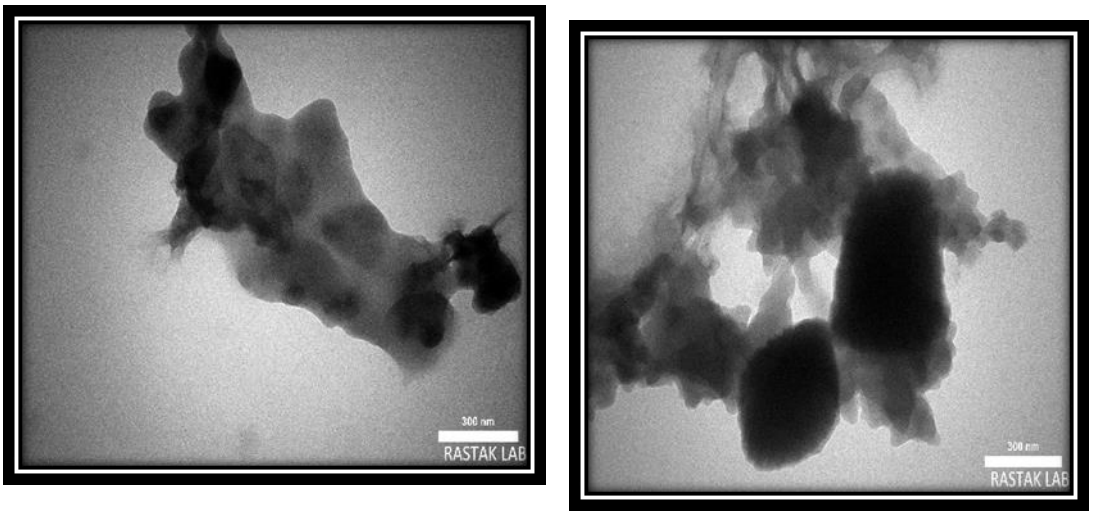

Figure 9. The transmission electron microscopic (TEM) images of the CuONPs.

Field Emission-Scanning Electron Microscope (FE-SEM):

CuONP spherical shapes exhibited clustered with size distribution ranging from 22 nm SEM nanoparticles clustered with a mean diameter of 6 nm.

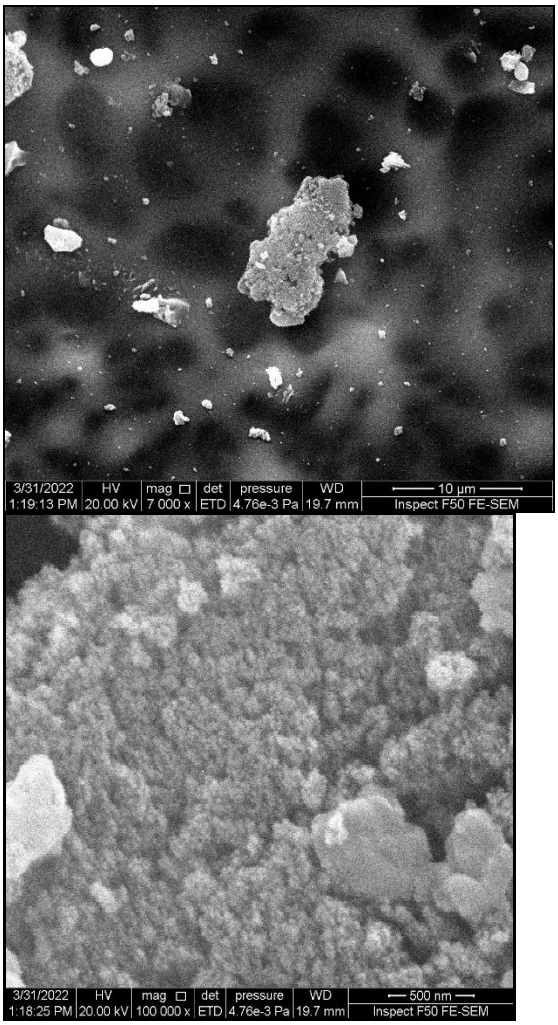

Figure 10. FE-SEM Image of CuoNPs.

Minimum Inhibitory Concentration (MIC) of CuNPs:

Well, diffusion was used to detect the activity of CuNP nanoparticles against (S. epidermidis). ZnO NPs in different concentrations (512, 256, 128, 64, 32 and 16) µg/ml showed antimicrobial activity, and the zone of inhibition reached (14) mm shown in Figure 11.

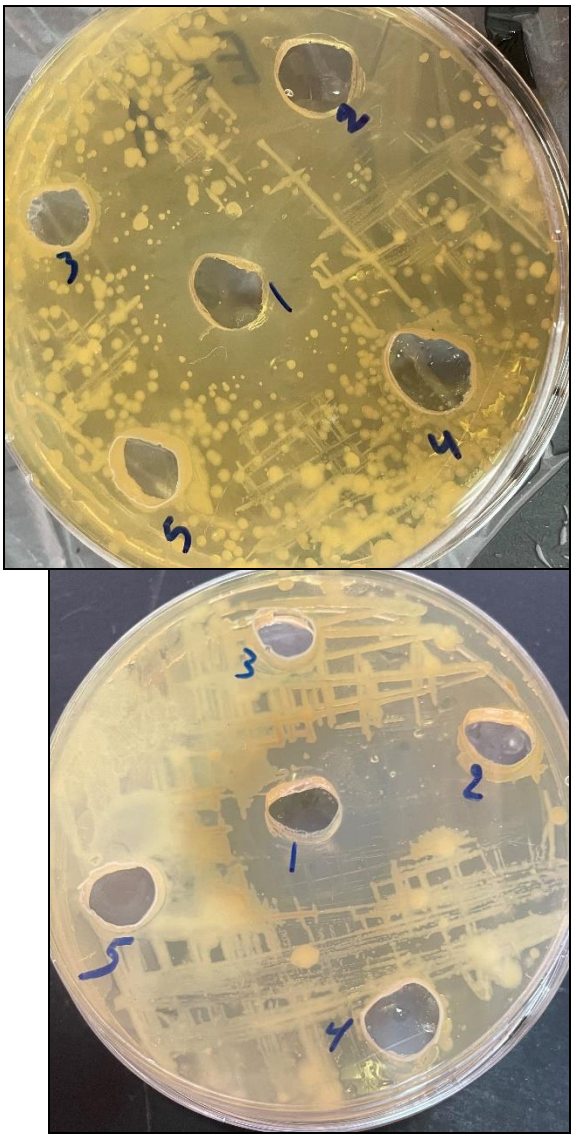

Figure 11. Minimum Inhibitory Concentration (MIC) of CuNPs con. 1) 512 µg/ml 2) 265 µg/ml 3) 128 µg/ml on MHA at 37°C for 24 hrs.

Toxicity testing of the cell line:

The MTT results in Figure 12 The concentration range between (16,32,64,256,512) µg/ml of CuNPs by chemical method reduced the number of HdFn cells. The cell viability was decreased with increasing the concentration of the CuNPs. It displayed a dose-dependent sequence of progressive cytotoxicity beginning at a lower concentration to its maximum inhibition (22)% inhibition of HdFn cells and (66)% inhibition of A375 cells.

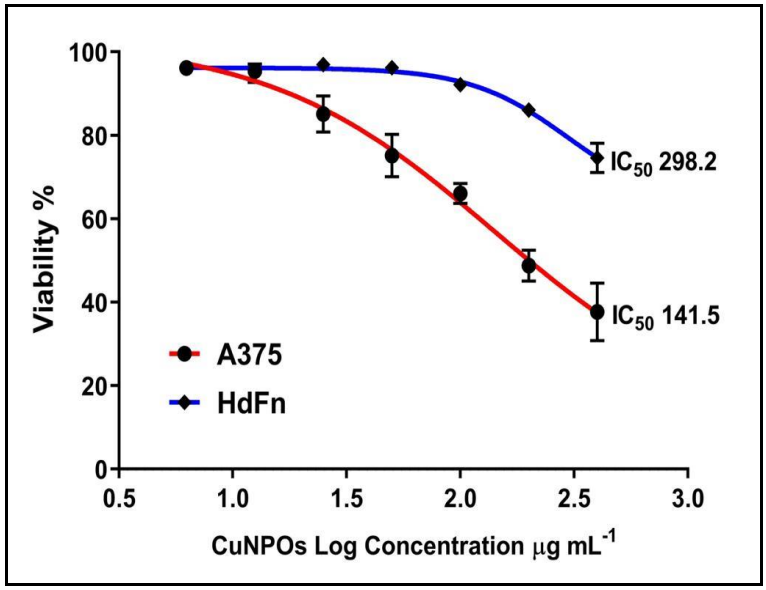

Figure 12. The MTT assay results of synthesized CuNPs on HdFn cells and normal A375.

	A375		HdFn	
Con-cen.	Mean	SD	Mean	SD
400.00	37.71	6.94	74.58	3.52
200.00	48.79	3.73	86.03	0.85
100.00	66.05	2.41	92.13	1.56
50.00	75.15	5.10	96.18	1.25
25.00	85.15	4.36	96.95	1.14
12.50	95.41	1.41	94.91	2.20
6.25	96.14	1.05	96.10	0.48

Discussion

The isolate was the source of isolation (wounds), according to [14]

The results agree with [15] most S. aureus MDR antibiotics: Gentamicin, Azithromycin, Cefoxitin, Trimethoprim and Levofloxacin. The XRD spectra of CuO NPs and nanoparticle powder confirmed the formation of the hexagonal (wurtzite) structure of the CuNP NPs by revealing prominent peaks corresponding to the diffraction peaks, which agree with [17]. Peaks assured the detected the presence of CuO monoclinic phase as synchronized. The Morphology and size reached 20nm of CuONPs were synthesized by the biology method of the field emission scanning electron microscopy Figure 10, The result according to [19]. The result agrees that [20] synthesis CuONPs have shown affection against microorganisms such as bacteria. The diameter of inhabitation bacterial growth inhibition reached more than 17mm. The CuNPs are connected to the toxic oxygen species of photocatalytic activity. CuO-NPs were found to cause significant cytotoxicity. The toxicity is often correlated with apoptosis, and a decrease in cell viability leads to cell death, as damage to cell membrane A375 [22].

Conclusion

The metal NPs synthesis by chemical was a significant use of hazardous environmental pollution concerns and expensive in vivo and invivo toxicity.

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