DOI: https://doi.org/10.53350/pjmhs22168594

ORIGINAL ARTICLE

The Synergistic Effect of Copper Nanoparticles and Vancomycin in Vitro and in Vivo

ZAHRAA H.KADHIM[1], MAIS E. AHMED[2], ILKER ŞIMŞEK[3]
[1]*Department of Biology / Institute of Natural Sciences / Çankiri Karatekin University*
[2]*Department of Biology /College of Science/ University of Baghdad*
[3]*Department of Biology / Institute of Natural Sciences / Çankiri Karatekin University*
Corresponding author: Mais E. Ahmed, Email: mais.emad@sc.uobaghdad.edu.iq

ABSTRACT
A new synergistic nanoparticle green synthesis from Vancomycin and CuoNPs techniques and evaluation effect invite anti-bacteria resistance methicillin-resistant S. aureus with zone of inhibition reached 20mm and characterization included UV-Visible, TEM , SEM and X-ray. rating at various concentrations (512, 256,128,64,32 and 16) µg/ml. The synergistic nanoparticle (CuoNPs) and are minimal toxicity, biology safety and antibacterial.the wound healing skin after 7 day after tretmant 64 µg/ml .
Keywords: Synergistic, Vancomycin, CuoNps

INTRODUCTION
New commercial applications of useing of nanoparticles has in interest in nanotechnology. Manufactured of NOPs fate and behavior of in the environment and features that they be treated more like complicated mixtures than small molecules(1). Green synthesis (Biological) of nanoparticles by using different type of cell included such as plant,seed extract or microorganisms like (bacteria, fungi) synthesizes included using different fruit, stem and leaf. (2). S. aureus is a majore human pathogenic cusese the ability to infection human body such like skine and chronic infections resprety tract , wound and injury (3). Prevalent in an increasing variety of species antibiotic resistance is becoming more widly the bacteria that resistantly most category of antibiotics and cuses formed strong biofilms (4). Human health care contributions to the newly technology using treatment infection and diagnosis,recent developments Nanobiotechnology (5) Against various types of microorganism the mode of action of NPs is have suggested that the disruption of cell membrane activity and cause of antibacterial activity. (6)

MATERIALS AND METHODS
S. aureus isolation and identification: Isolates were the sources of skin wounds confirmation identification on Mannitol salt agar (MSA), then incubated for 24-48 hrs at 37 °C and by morphological and biochemical tests to ensure their identity According to (7)
Synthesis of Copper oxide Nanoparticles: Green synthesis of CuoNps by supernatant of S. epidermids after centrifuged and removed the bacterial cell was mixed with 0.1 M of copper acetate dissolved in 100ml of de ionized water finaly flasks was incubated at 35°C and observed for color change.(8)
Preparation of Vancomycin and CuoNPs by Minimum Inhibition Concentration (MIC): Inoculated on nutrient broth S. aureus over night at 37°C. doing Six tubes were used containing 1ml bacteria suspension. Mixing with Six concentrations of each Vancomycin Ampule and CuoNps prepared between (512, 256,128,64,32 and 16) µg/ml using normal saline. 1ml of each concentration was putted in each tubes. The positive control was made without putting Vancomycin and CuoNps.. (9)
Synergistic of CuoNps with Vancomycin: The double dilution method used based on the method of (10) to preparation the MIC concentration determined from the previous step for each antibiotic and CuoNps was added to the first tube to study the synergistic effect of the Nanoparticles with the antibiotics.
Characterized Synergistic of CuoNps: UV-vis Spectroscopy .
X-Ray Diffraction (XRD) .
Transmittion Electron Microscopy (TEM) .
Atomic force microscope (AFM) .
Scanning Electron Microscope(SEM)
In vitro Antibacterial Activity
Well-Diffusion Assay (WDA): The plates agar cultured with S. aureus (1.5×10^8) cfu/ml . Wells cut into the plates with 5mm sterile cork borer were loaded with 100 µl of the way of synergistic CuoNPs the plates were incubated at 37°C for 24 hrs. (11)
Experimental design:
Laboratory animals: Twenty eight albino male Swiss white mice were obtained from Al-Nahrian University. Animals were housed in plastic cages at standard conditions of a climate controlled facility with a regular day–night cycle and Kept at temperature (20-25°C). In order to produce skin injury, the mice anesthetized with an intraperitoneal injection then the hair on the right side of the animals was shaved and the remaining hair and cleaned by soap and sterile with distilled water.

RESULTS
A primary identification as S. aureus isolates on differential media and confirmed identification (MSA) as shown Figure (1)

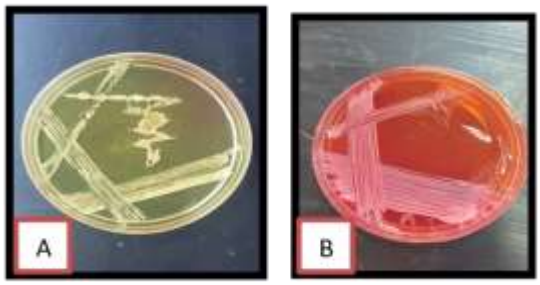

Figure 1 A: S. aureus (B) S .epidermidis on MSA at 37°C for 24 hrs

Biosynthesis of Copper Oxide Nanoparticles: Biosynthesized from S. epidermidis by copper Oxide Nanoparticles. The formation of Nanoparticles by change the color from yellow to blue, in addition to form a light blue precipitate. After centrifugation, the precipitate was shown in greenish blue color after drying with microwave we obtained shining blue powder, as shown in Figure (2)

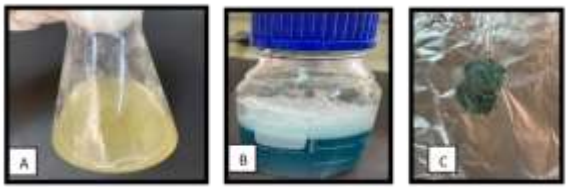

Figure 2 :A(bacteria activation in broth), B (countergauge bacteria to obtain suspension and remove cell), C) CuoNps as powder.

Synergistic of CuoNps with Vancomycin: When combined with nanoparticles and vancomycin show antibacterial activity against susceptible and resistant bacterial strains at significantly lower

concentrations. to date, no research study has examined the effect of the antibiotic mode of action and mechanism of bacterial resistance on the effectiveness of combined antibacterial treatment with nanoparticles . Shown in Figure (3)

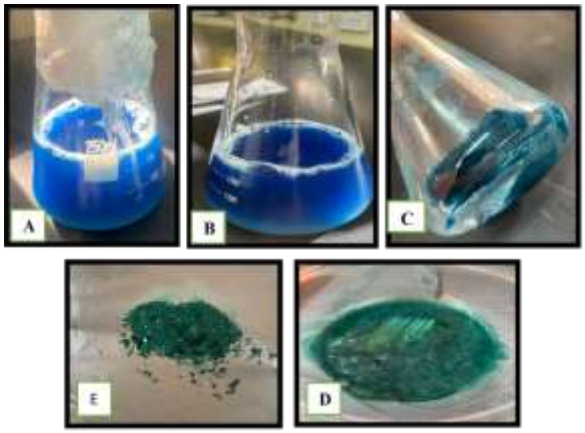

Figure 3: Synergistic of CuoNps with vancomycin

(A) Mixture of CuoNps with Vancomycin sealed tightly and kept in dark place at 37ºc for 72 hrs (B) synthesis of thick Precipitate (C) dark blue Precipitate formed (D)after drying with microwave (E) synergistic powder (metallic green color) .

Characterization of Synergistic CuoNps

UV–Vis Spectral Analysis: The results showed that biosynthesized CuoNps exhibited a maximum peak at (absorption peaks at 275 and 280 nm were) nm as shown in figure (4)

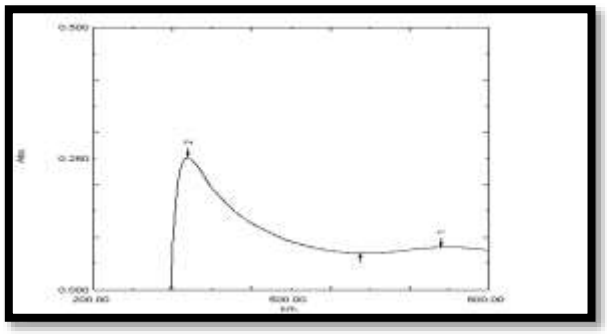

Figure 4: UV–Vis Spectrophotometry of Synergistic CuoNps .

Atomic Force Microscopy (AFM) analysis : Characterize the Biosynthesis of CuoNPs by biological method detecting their average diameter to the morphology in both two- three dimension. Figure (5).

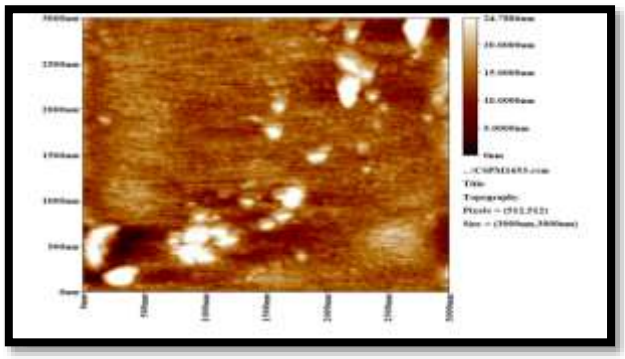

Figure 5: 2D AFM for Synergistic CuoNPS

Transmission Electron Microscopy analysis (TEM): The figure (6) spherical shape with crystalline of the prepared CNPs with diameter ranges 5-40nm.

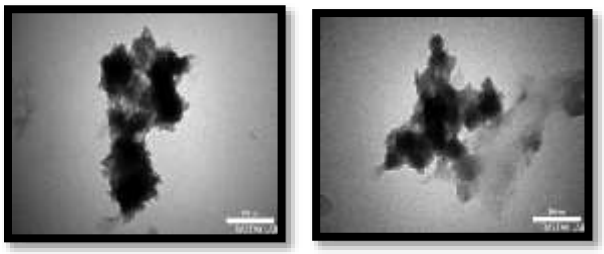

Figure 6: The Transmission Electron Microscopic (TEM) images of Synergistic CuoNps

Scanning Electron Microscope (SEM): The Morphology and size reached 20nm of CuoNps were synthesis by biology method of the field emission scanning electron microscopy Figure (7) Regarding CuoNps' shape, SEM images revealed that it was essentially spherical and uniform in appearance(15)

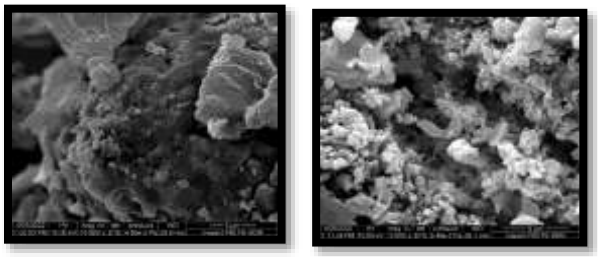

Figure 7: SEM Image of synergistic CuoNps

X-ray Diffraction (XRD) : The XRD spectra of CuoNps, nanoparticle powder confirmed formation of hexagonal (wurtzite) structure of the CuoNps NPs by revealing prominent peaks corresponded to the diffraction peaks (100), (002), (101), (102), (110), (103) and (112) Figure (8).

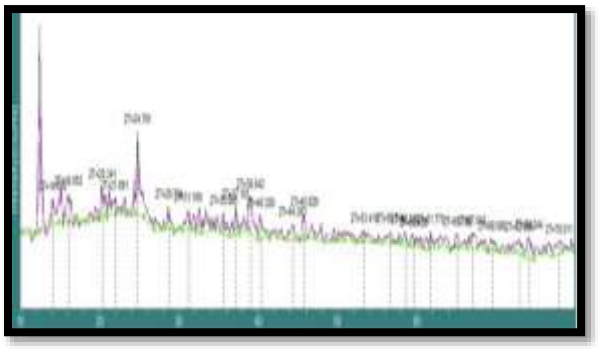

Figure 8: XRD analysis of synergistic CuoNps

In vitro Antibacterial activity by Well Diffusion (WDA): The synergistic synthesized formula were evaluated for antimicrobial activity via standard agar well-diffusion method aganist S. aureus inhibition zone reached 22mm . Figure (9) the antibacterial activity of various metal or metalloid NPs and their compounds, as well as their mode of action at various bacterial cellular levels, have been reviewed and described in the preceding section.

In vivo Antibacterial activity: histopathological normal kidney structure was observed with no morphological alterations. Following NP treatment, the liver tissue is also displaying a normal

structural makeup there was no reduction in the number of lymphocytes in the lymphoid nodules in the spleen.

Figure 9: WDA assay for Synergistic

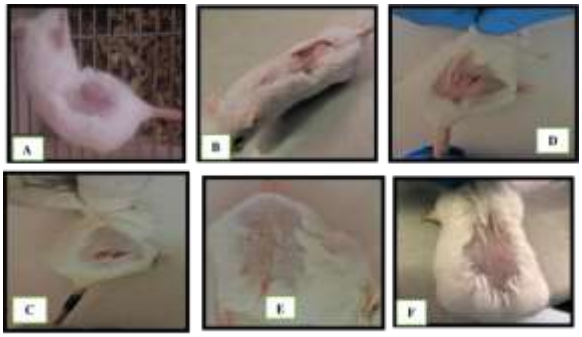

Figure 10: The Morphological of Healing Process to skin wound.

A) Negative control group
B) Positive control.
C) The mice skin wound infected with S. aureus and treated with the vancomycin .
D) The mice skin wound infected with S. aureus and treated with synergistic (CuoNps)

DISCUSSION

Characterization of Synergistic CuoNps Can seen by increasing or reducing in green synthesized CuoNPs, reduced absorption was extract volume At 230 nm, Synergistic as shown in figure 4 According to (12), CuoNps at about 250 the UV spectra for nm wavelength are consistent. Other findings demonstrated that CuoNps were successfully formed.while UV found that the average size of CuoNps was 80 nm. Because mistakes are more obvious in suspension due to the presence of dispersants of varied sizes, the other study by (13) .The AFM outcome as stated by (14) CuoNps nanoparticles in spherical forms were found to be clustered, with a mean diameter of 6 nm and a size distribution ranging from 22 nm. On other hand CuoNPs powder's XRD spectrum. This XRD pattern confirms the production of a peculiar centered cubic crystalline form of CuNPs, which is in good agreement with other findings by (19) . The synergistic interactions between metal oxide nanoparticles (NPs) and antibiotics that boost antibiotic activity while lowering nanoparticle toxicity to mammalian cells (17). It has been demonstrated that CuoNps interact with bacteria in a special way and that they are efficient against a variety of harmful bacteria .study in vivo As a result, in the kidneys, liver, lungs, and spleen of the treated animals, CNPs and CNP-CuoNps did not exhibit any influence of the histological abnormalities. (19).

CONCLUSION

Synergistic nanoparticles have a wide-ranging inhibitory activity, which was demonstrated by biologically purifying them from copper salts, and their effect was stronger after linking it with anti-vancomacin and studying cytotoxicity inside the body of an organism.

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