RESEARCH PAPER

A Green Synthesized of Zinc Oxide Nanoparticles Against MRSA Wound Healing *in Vivo*

Ayat E. Rasheed [1], *Mazin K. Hamid* [1], *Mais E. Ahmed* [2*]

[1] *Department of Physiology and Medical Physics, College Of Medicine, Al-Nahrain University, Baghdad, Iraq*

[2] *Department of Biology, College of Science, University of Baghdad, Iraq*

ARTICLE INFO

Article History:
Received 12 June 2023
Accepted 23 September 2023
Published 01 October 2023

Keywords:
Chemical method
Wound
ZnO nanoparticles

ABSTRACT

A new technique for nanoparticles with a chemical method and effect for bacteria resistance methicillin-resistant Staphylococcus aureus (MRSA), UV-Visible, TEM, EDX, SEM and X-ray diffraction pattern. rating antimicrobial excellent antibacterial activity against (MRSA) (with zone of inhibition of 11 ± 02mm, 9 ± 01mm,8 ± 03mm and 7.5 ± 02mm and 6.5 ± 02mm) at various concentrations (0.5 , 0.25, 0.125, 0.0625, 0.03125)mg /ml while good activity was 9 ± 01mm and 8 ± 03mm zone at 0.25, 0.125 mg/ml straight. The raise microorganism resistance to antibiotics a duo of have caused antimicrobial factors are widely recognized (ZnO NPs) and are minimal toxicity and biology safety. Evaluation MRSA by minimum restrained concentration (MIC) (0.5 ,0.25, 0.125, 0.0625, 0.03125) mg/ml of ZnO NPs. The goal of this research study zinc oxide (ZnO) nanoparticles synthesis and antibacterial. The wound healing skin after 12 days after tretmant 0.25 mg/ml.

How to cite this article
Rasheed A E., Hamid M K. , Ahmed M A. A Green Synthesized of Zinc Oxide Nanoparticles Against MRSA Wound Healing in Vivo. J Nanostruct, 2023; 13(4):989-998. DOI: 10.22052/JNS.2023.04.008

INTRODUCTION

Nano materials are known as the miracles of modern medicine. Metallic nanoparticles have different physical and chemical properties due to their wide surface ratio, while their bulk forms may not have these features [1,2]. nanomaterials contain superior chemical, biological, optical and mechanical properties which are often very different from the corresponding micro counterparts [3]. nanomaterials deal with sizes of 100 nanometers or smaller in at least one dimension. The material properties of nanostructures are different from the bulk due to the high surface area over volume ratio and possible appearance of quantum effects at the nanoscale. The study of size and shape effects on material properties has attracted enormous attention due to their scientific and industrial importance [4]. Liquid state synthesis methods, Gas phase methods, Biological methods) and top down methods (Mechanical milling, Laser ablation, Sputtering) [5]. Zinc oxide is an inorganic compound with the formula ZnO. It usually appears as a white powder, nearly insoluble in water and have broad energy band (3.37 meV), high bond energy (60 meV) and high thermal and mechanical stability at room temperature [6,7].Ther is a different nanostructures such as nanorods, nanosphere, nanotubes, nanowires, nanoneedles and nanorings[8,9].

* *Corresponding Author Email: mais.emad@sc.uobaghdad.edu.iq*

The most significant pathogens for humans *S. aureus* causes pathogensis such as carbuncles, boils, skin rashes, pneumonia, endocarditis, septicaemia and toxic shock syndrome [10,11]. Noval treatment alternative drug-resistant bacteria kill found that ZnO-NPs inhibit the effects of pathogenic bacteria such *as S. aureus*. [12,13]

MATERIALS AND METHODS

S. aureus isolation and identification

Fifteen isolates were the sources of isolation skin wounds and burns were obtained from (College of Science, Baghdad University). Confirmation identification on Mannitol salt agar (MSA), then incubated for 24-48 hrs at 37 °C under All the primary screened isolates then subjected to various morphological and biochemical tests to ensure their identity [14].and was preservation in 20% glycerol after identification by Vitic 2 system to chosen one strain multidrug resistant (MDR) antiontic.

Growth on Mannitol Salt Agar

A pure colony of tested bacteria were streaked agar and then this medium was used for the selective cultivation and isolation of bacteria [15]. Identification of *S. aureus* by using vitek2 system was done According to [16].

Antibacterial activity

For MRSA, the inhibition halo was present in concentrations 0.125mg mL^{-1} This results to detect the antibacterial effect of ZnO-NPs against MRSA. were performed as a qualitative well-Diffusion Assay (AWD) to observe and predict the ZnO-NPs. The result disagree with [17] antibacterial agents it is not able to determine the minimum inhibitory concentration (MIC) as it is impossible and by diffusion of the antimicrobial agent in the agar.

Preparation of ZnO NPs

Wet-chemical method used ZnO NPs were synthesized by in room temperature using diluted Zinc Nitrate Hexahydrate $Zn(NO_3)_2.6H_2O$ and Sodium hydroxide (NaOH). In this work, (0.2 mol) of $Zn(NO_3)_2.6H_2O$ was diluted in deionize water (25ml) about 14min at temperature (20 °C) by hotplate stirrer device and (0.4 mol)of NaOH was diluted in deionize water (25ml) about 5min at temperature (75 °C) after that mixed the NaOH and zinc nitrate by slowly added NaOH solution into zinc nitrate solution at temperature room under vigorous stirring which resulted in the formation of white suspension .We filtered the mixture by filter paper and put the prepared zinc oxide in oven about (2hour) in (100 °C).After that it was taken out from oven and grind it and put in filtering paper and washed in acetone followed by deionized water three times respectively .Finally we was placed in tubular furnace to calcination about 2hour and (500 °C) and calcinate ZnO NPs powder has been achieved [18]. as shown in (Fig. 1).

Characterization techniques

The synthesized ZnO NPs were characterized by: UV-vis Spectroscopy, X-Ray Diffraction (XRD), Transmittion Electron Microscopy (TEM), Atomic force microscope (AFM), Zeta potential device (ZP), Scanning Electron Microscope (SEM).

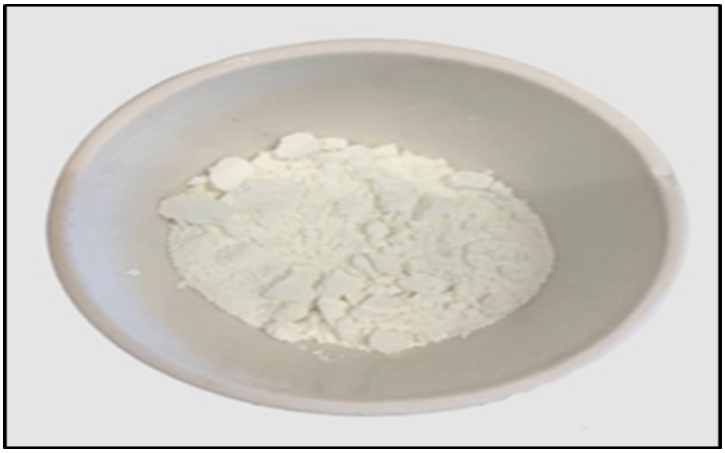


Fig. 1. ZnO nanoparticles powder.

Experimental design

Four major groups (each group contains 6 mice):

1- Group 1: (n=6) the mice without any treatment negative control

2- Group2: (n=6) the mice subjected to superficial wound on the dorsal area without treatment and consider as positive control

3- Group3:(n=6) the mice were subjected to superficial skin wound on back and infected with 0.1 ml of suspension 1.5×10^8 cfu/ml of *S aureus*

4- Group4:(n=4) the mice were subjected to superficial skin wound on back and infected with MRSA and treated with the ZnoNPs once daily for 12 days

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 ZnO NPs at different concentration, the plates were incubated at 37°C for 24 hrs. Hindrance

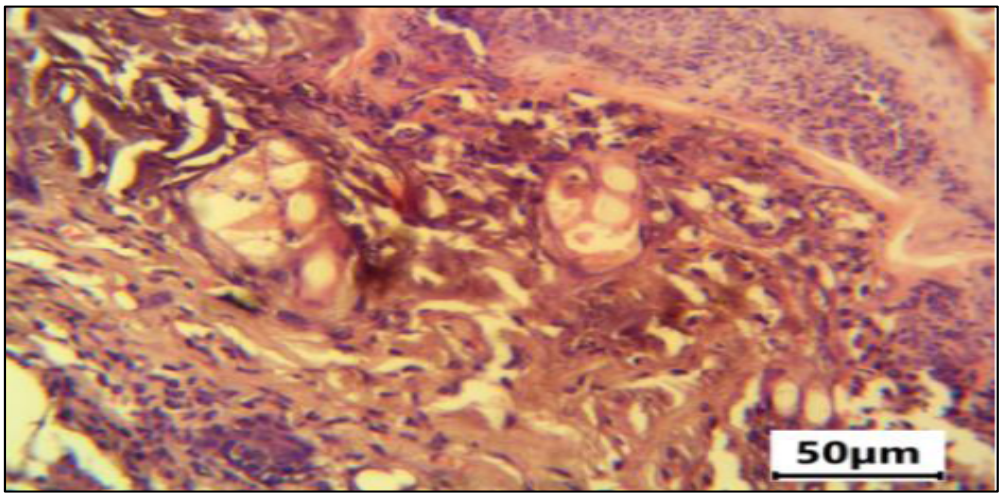


Fig. 2. skin in the control positive group after 48 hr post injury

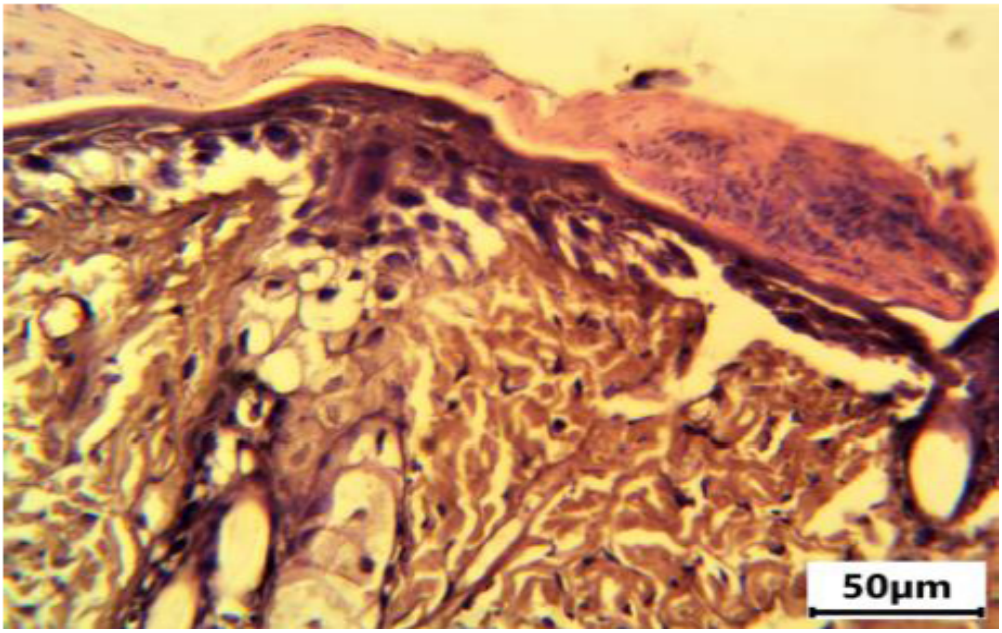


Fig. 3. skin in the control positive group after 6 days post injury

was identified by a zone of clearing around the supernatant well. As per the outcomes above, one isolate of *S. aureus* resistance was chosen and utilized as a part of the ensuing examinations [19].

RESULTS AND DISCUSSION

Post injury, characterized by complete loss of the epidermal layer .lesion in the skin of mice in the control positive 48 hr which alternative neutrophils by nflammatory cells, severe infiltration of neutrophils in the dermal layer (Fig. 2) while after day 6 post injury the healing represented by regeneration of lesion of the epithelia and collagen fiber in the dermis appear loos and irregular (Fig.

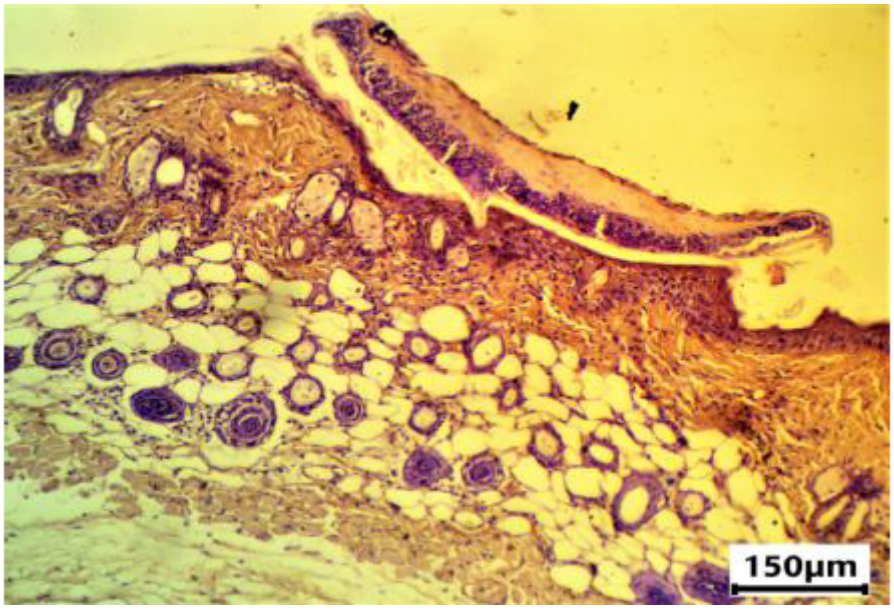


Fig. 4. skin in the control positive after 12 days post injury .

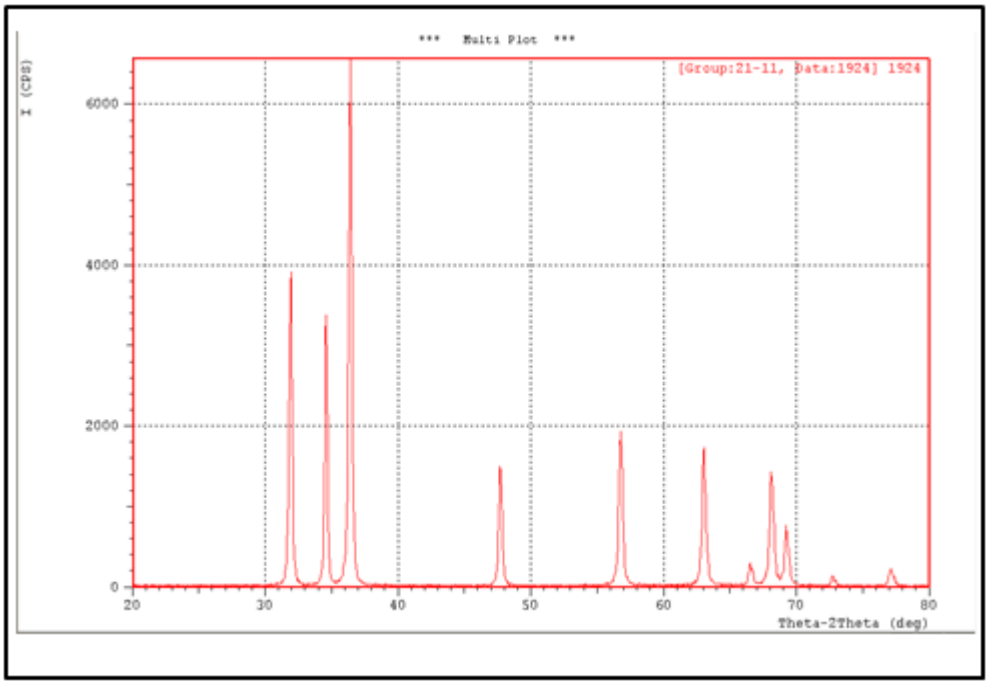


Fig. 5. X-ray diffraction of ZnO nanoparticles pattern.

3) and complete regeneration of the epithelial cells after 12 days post injury. (Fig. 4).

The 1st group showen wound healing by inflammatory cells especial PMNs with infiltration and few macrophages (14,20).In the 2 group the lesion to environmental factors and the metabolic

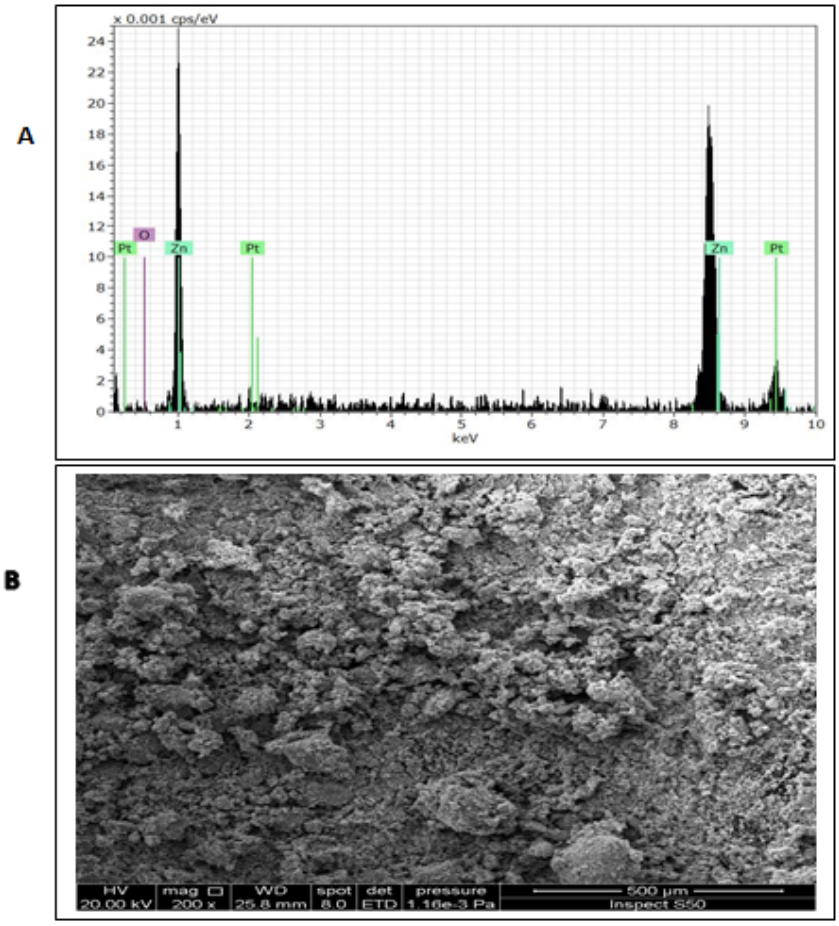


Fig. 6. (A) EDX and (B) SEM of ZnO nanoparticles.

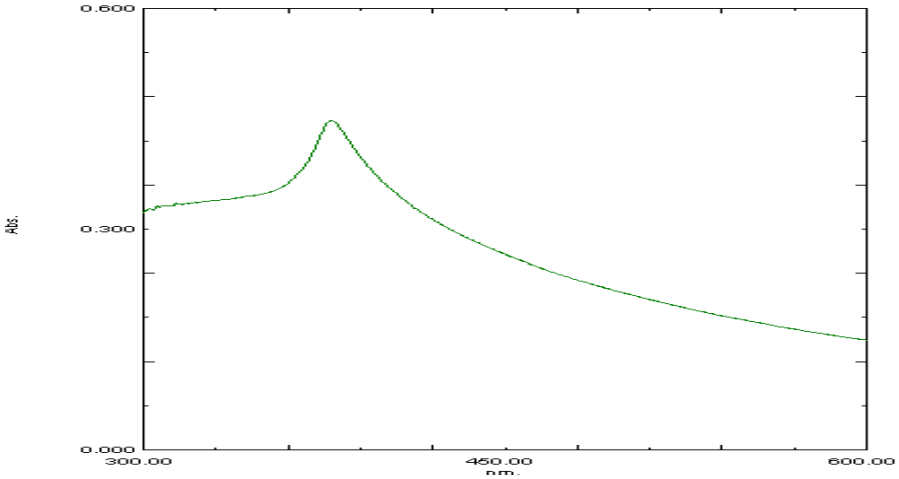
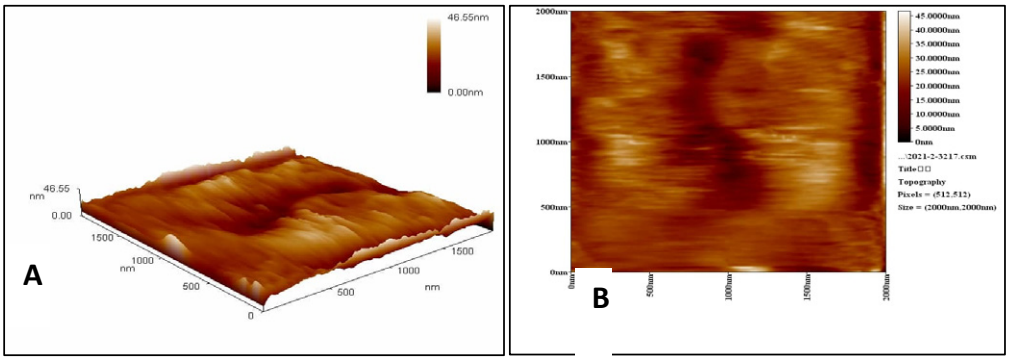


Fig. 7. The UV-visible absorption of ZnONPs.

shift to *S. aureus* protects the bacteria against the hosts immune system [21] while other tretmend depeded antibiotic [22,23].

ZnO-NPs characterization

X-ray diffraction analysis was done in the range 20˚-80˚ (2θ), diffraction results (Fig. 5) demonstrated with the standard of ZnO. This result showed the anatase phase indicates to ZnO NPs. This result almost agree with [20] synthrsis shape crystallite nanoparticles are crystalline and average size about 38.5 by using Debye- Scherrer



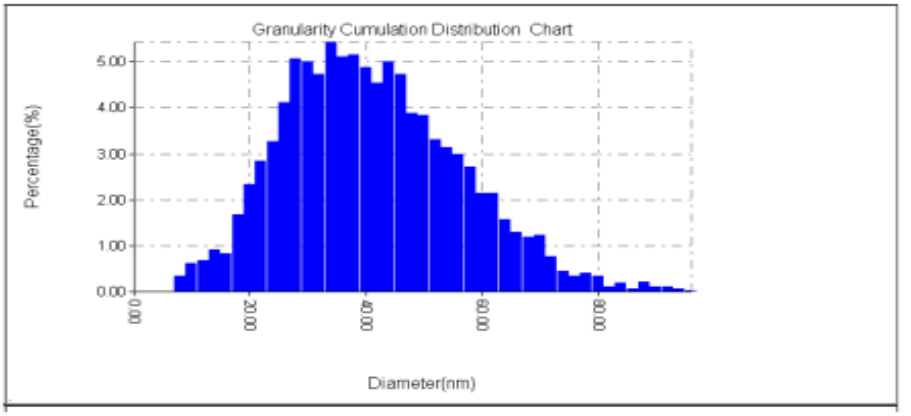
C

Granularity Cumulation Distribution Report

Sample: Sample Name **Code:**Sample Code
Line No.:lineno **Grain No.:**2605
Instrument:SPM **Date:**2021-11-21

Avg. Diameter:40.16 nm **<=10% Diameter:20.00 nm**
<=50% Diameter:38.00 nm **<=90% Diameter:60.00 nm**

Diameter(nm) <	Volume(%)	Cumulation(%)	Diameter(nm) <	Volume(%)	Cumulation(%)	Diameter(nm) <	Volume(%)	Cumulation(%)
8.00	0.35	0.35	38.00	5.14	48.10	68.00	1.19	95.47
10.00	0.61	0.96	40.00	4.88	52.98	70.00	1.23	96.70
12.00	0.69	1.65	42.00	4.53	57.50	72.00	0.77	97.47
14.00	0.92	2.57	44.00	4.99	62.50	74.00	0.46	97.93
16.00	0.84	3.42	46.00	4.72	67.22	76.00	0.35	98.27
18.00	1.69	5.11	48.00	3.88	71.09	78.00	0.42	98.69
20.00	2.34	7.45	50.00	3.84	74.93	80.00	0.35	99.04
22.00	2.84	10.29	52.00	3.30	78.23	82.00	0.12	99.16
24.00	3.26	13.55	54.00	3.15	81.38	84.00	0.19	99.35
26.00	4.11	17.66	56.00	2.99	84.38	86.00	0.08	99.42
28.00	5.07	22.73	58.00	2.73	87.10	88.00	0.23	99.65
30.00	4.99	27.72	60.00	2.15	89.25	90.00	0.12	99.77
32.00	4.72	32.44	62.00	2.15	91.40	92.00	0.12	99.88
34.00	5.41	37.85	64.00	1.57	92.98	94.00	0.08	99.96
36.00	5.11	42.96	66.00	1.31	94.28	96.00	0.04	100.00



file:///D:/Program%20Files%20(x86)/SPM%20Software/Image/report.htm 1/2

Fig. 8. AFM of ZnO nanoparticles

equation.

And the EDX and SEM image of ZnO NPs have showen in the Fig. 6.

Absorption spectroscopy UV is extensively used as a fashion to examine the optic Properties of certain nanoscale is examine it exhibits a strong absorption band at about (378 nm) showed Fig. 7. Other research of ZNOPs absorption UV is found rang 360 nm without calcined [21].

In this work, scanning probe microscope available at the department of Chemical Science - AL-Nahrain University AFM Measuring the granularity accumulation distribution, roughness. The results of AFM where featured the average roughness about 0.295nm and average size about 38.5nm. surface roughness it was conclude is veritably important for antibacterial exertion the properties ofsurface nanomaterial can overlap with the antimicrobial influence of ZnO NP showen Fig. 8. Result reported by Al-Taie is not consistent who show up 125.77 nm that the average particle size [22].

Fig. 9 show the TEM pictures of ZnO nanoparticles at various magnifications. Average size of rang 21-300 nme ZnO NPs shape are expand in a near-hexagonal shape, which indicate the good quality

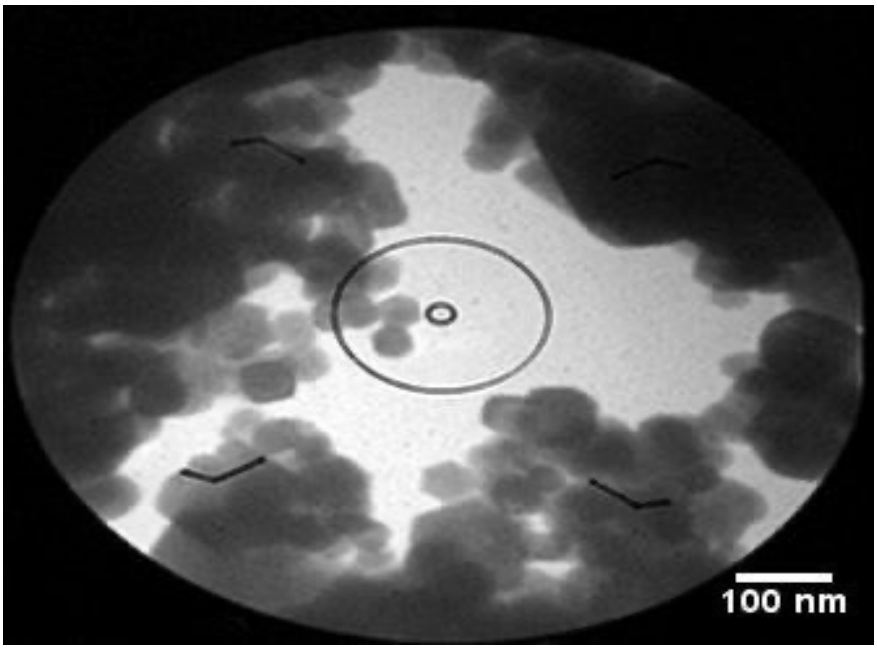


Fig. 9. TEM image of ZnO NPs.

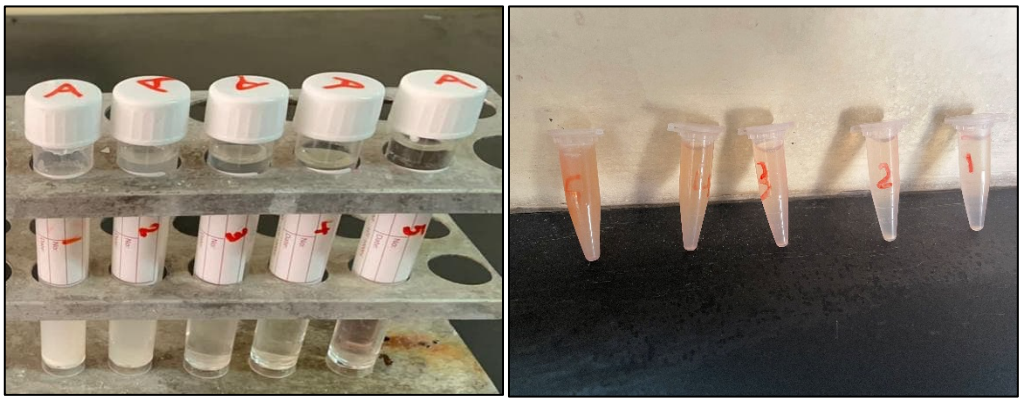


Fig. 10. MIC of ZnO-NPs at final concentrations (0.5 ,0.25, 0.125, 0.0625, 0.03125) mg/ml

of the ZnO-NPs and have good identitydiffraction of electron for the synthesized ZnO nanoparticles, the rings with a doted pattern in selected area diffraction of electron diffraction(SAED) confirms that nanoparticles of ZnO are poly crystalline. This isagree with the obtained results from powder XRD measurements..

Antibacterial activity

For MRSA, the MIC at 0.125mg mL-1 This result to detect the antibacterial effect of ZnO-NPs against MRSA. The result disagrees with [23] the MIC antibacterial agents to determine antibactreia agent in the agar.

Broth dilution

The result (Fig. 10). the concetration effect anti MRSA was 0.125 mg/ml,The result broth media assay agree nanoparticle-bacteria interactions the can be considered as confirmative [24].Different particle morphologies for ZnO-NPs antimicrobial activity [25] .

Fig. 11 It's a measure of the surface electrical charge of particles are that are suspended in liquid demonstrates the zeta potential of ZnO NPs was device is located at the ministry of Science and Technology. is the electric potential difference across the ionic layer around a charged. Zeta potential is 30.19mv for ZnO NPs [26].

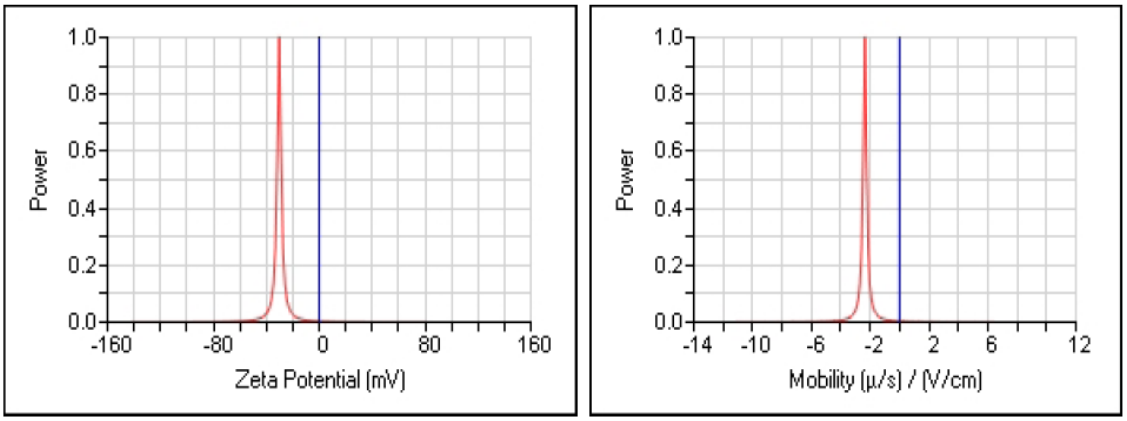


Fig. 11. Zeta potential of ZnO nanoparticles

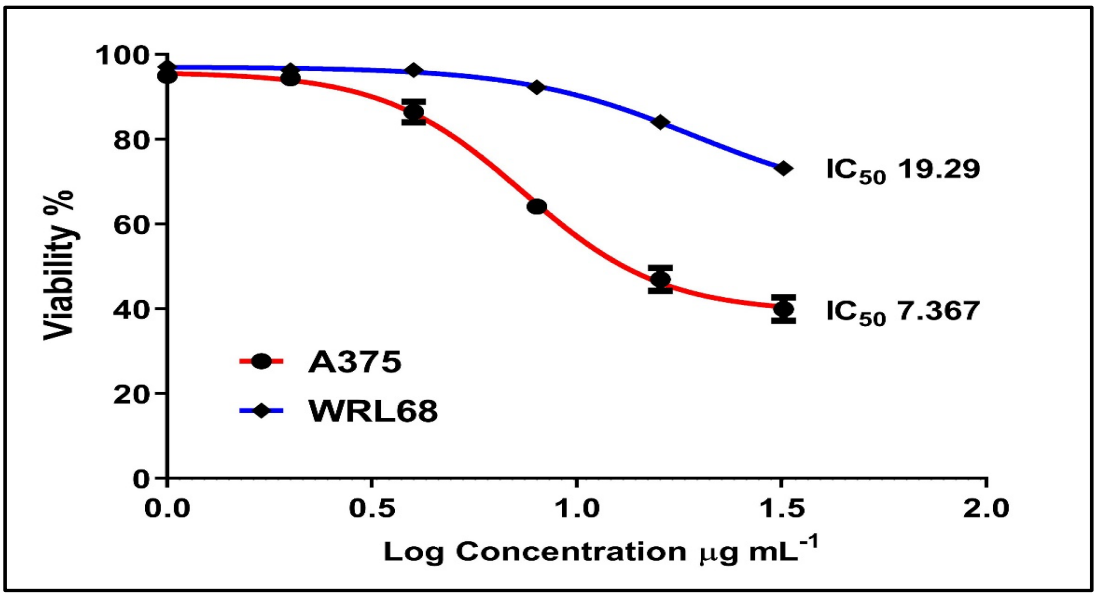


Fig. 12. The MTT assay results of synthesized ZnO NPs on WRL68 and normal A375

The MTT results (Fig. 12) The concentration rang between (0.5,1,1.5.2,2.5) mg mL of ZnO NPs by chemical method resulted reduction in the number of A375 cells, the nano-ZnO is connected to the toxic oxygen species of photocatalytic activity [27]. The toxicity is very often correlated with apoptosis and decrease in cell viability lead cell death such., damage cell membrane LDH [28].

CONCLUSION

The chemically synthesized zinc nanoparticles (ZnONPs) by the precipitation method are effective. As antibacterial activity against multidrug-resistant *S. aureus*. The heavy infiltration of neutrophils in the infected area, which extends to the dermis and subcutaneous tissue, al and neutrophil recruitment to the site of infection are required for an effective immune response against *S. aureus.* Which treated with nanoparticle synthesis showed good healing, and this may contribute to the antibacterial properties of the Zno-NPs since Nano-sized. ZnO cytotoxicity of ZnONPs by chemical method MTT assay and found minimum inhibition concentration at 0.25 mg/ml WRL 68 and tumor cell line (A375) and found minimum inhibition concentrations.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

REFERENCES

1. Ahmed ME, Al-Awadi AQ, Abbas AF. Focus on Synergistic Bacteriocin-Nanoparticles Enhancing Antimicrobial Activity Assay. Mikrobiolohichnyi Zhurnal. 2023;85(6):95-104.
2. Faiq NH, Ahmed ME. Inhibitory Effects of Biosynthesized Copper Nanoparticles on Biofilm Formation of Proteus mirabilis. Iraqi Journal of Science. 2024:65-78.
3. Seddiq SH, Zyara AM, Ahmed ME. Evaluation the Antimicrobial Action of Kiwifruit Zinc Oxide Nanoparticles Against Staphylococcus aureus Isolated from Cosmetics Tools. BioNanoScience. 2023;13(3):1140-1149.
4. Guisbiers G, Mejía-Rosales S, Leonard Deepak F. Nanomaterial Properties: Size and Shape Dependencies. Journal of Nanomaterials. 2012;2012(1).
5. Faiq NH, Ahmed ME. Effect of Biosynthesized Zinc oxide Nanoparticles on Phenotypic and Genotypic Biofilm Formation of Proteus mirabilis. Baghdad Science Journal. 2024;21(3):0894.
6. Satdev, Zinzala VJ, Saini LK, Chauhan AR. Synthesis and characterization of zinc oxide nanoparticles (ZnO-NPs) via precipitation method. Journal of Pharmacognosy and Phytochemistry. 2020;9(2):304-307.
7. Thakur S, Shandilya M, Guleria G. Appraisement of antimicrobial zinc oxide nanoparticles through Cannabis Jatropha curcasa Alovera and Tinosporacordifolia leaves by green synthesis process. Journal of Environmental Chemical Engineering. 2021;9(1):104882.
8. Sirelkhatim A, Mahmud S, Seeni A, Kaus NHM, Ann LC, Bakhori SKM, et al. Review on Zinc Oxide Nanoparticles: Antibacterial Activity and Toxicity Mechanism. Nano-Micro Letters. 2015;7(3):219-242.
9. Dobrucka R, Długaszewska J. Biosynthesis and antibacterial activity of ZnO nanoparticles using Trifolium pratense flower extract. Saudi J Biol Sci. 2016;23(4):517-523.
10. Holland TL, Arnold C, Fowler VG. Clinical Management of Staphylococcus aureus Bacteremia. JAMA. 2014;312(13):1330.
11. Saghalli M, Bidoki SK, Jamali A, Bagheri H, Ghaemi EA. Sub-minimum inhibitory concentrations of Zinc Oxide Nanoparticles Reduce the Expression of the Staphylococcus aureus ?-Hemolysin. Indian J Pharm Sci. 2016;78(6).
12. Sharma-Kuinkel BK, Wu Y, Tabor DE, Mok H, Sellman BR, Jenkins A, et al. Characterization of Alpha-Toxin hla Gene Variants, Alpha-Toxin Expression Levels, and Levels of Antibody to Alpha-Toxin in Hemodialysis and Postsurgical Patients with Staphylococcus aureus Bacteremia. J Clin Microbiol. 2015;53(1):227-236.
13. Berube BJ, Sampedro GR, Otto M, Bubeck Wardenburg J. The α Locus Regulates Production of Staphylococcus aureus Alpha-Toxin during Infection. Infection and Immunity. 2014;82(8):3350-3358.
14. Ahmed ME, Q. Al-Iam M, Ali dDMA. Evaluation of Antimicrobial Activity of Plants Extract Against Bacterial Pathogens Isolated from Urinary Tract Infection among Males Patients. Al- Anbar Medical Journal. 2021;17(1):20-24.
15. Morgan D. Manual of clinical microbiology (6th edn) edited by Patrick R. Murray, Euen J. Baron, Michael A. Pfaller, Fred C. Tenover and Robert H. Yolken ASM Press, 1995. $98.00 hbk (xxiii + 1482 pages) ISBN 1 55581 086 1. Trends Microbiol. 1995;3(11):449-450.
16. Prakash Singh M, Greenstein M. A simple, rapid, sensitive method detecting homoserine lactone (HSL)-related compounds in microbial extracts. J Microbiol Methods. 2006;65(1):32-37.
17. Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity: A review. Journal of Pharmaceutical Analysis. 2016;6(2):71-79.
18. Manzoor U, Tuz Zahra F, Rafique S, Moin MT, Mujahid M. Effect of Synthesis Temperature, Nucleation Time, and Postsynthesis Heat Treatment of ZnO Nanoparticles and Its Sensing Properties. Journal of Nanomaterials. 2015;2015(1).
19. Ahmed ME, Hasan HM, Kttafah AJ. Characterization and antibacterial activity of biogenic iron nanoparticles using Proteus mirabilis. Medical Journal of Babylon. 2024;21(1):39-45.
20. Katafa AJ, Hamid MK. Influence of ZnO Nanoparticles on Candida albicans of Human Male Pleural Fluid. Iraqi Journal of Science. 2020:540-549.
21. Talam S, Karumuri SR, Gunnam N. Synthesis, Characterization, and Spectroscopic Properties of ZnO Nanoparticles. ISRN Nanotechnology. 2012;2012:1-6.
22. Ahmed ME, Al-Awadi AQ. Enterococcus faecium BACTERIOCIN EFFLUX PUMP MexA GENE AND PROMOTE SKIN WOUND HEALING IN MICE. Journal of microbiology, biotechnology and food sciences. 2024;13(5):e10711.
23. Saad IK, Hassan B, Soumya E, Moulay S, Mounyr B. Antifungal Activity and Physico-chemical Surface Properties of the Momentaneously Exposed Penicillium expansum

Spores to Carvacrol. Res J Microbiol. 2016;11(6):178-185.
24. Negi H, Agarwal T, Zaidi MGH, Goel R. Comparative antibacterial efficacy of metal oxide nanoparticles against Gram negative bacteria. Ann Microbiol. 2011;62(2):765-772.
25. Bakhori SKM, Mahmud S, Ann LC, Sirelkhatim A, Hasan H, Mohamad D, et al. In-vitro antibacterial study of zinc oxide nanostructures on Streptococcus sobrinus. AIP Conf Proc: AIP Publishing LLC; 2014.
26. Zeta Potential. Definitions: Qeios; 2020.
27. Nosaka Y, Nosaka A. Understanding Hydroxyl Radical (•OH) Generation Processes in Photocatalysis. ACS Energy Letters. 2016;1(2):356-359.
28. Sruthi S, Ashtami J, Mohanan PV. Biomedical application and hidden toxicity of Zinc oxide nanoparticles. Materials Today Chemistry. 2018;10:175-186.