Cihan University-Erbil Scientific Journal (CUESJ)

Research Article

Antibacterial and Wound Healing Assessment of Silver Nanoparticles against Multidrug-Resistant *Klebsiella variicola*

Faraidun A. Ahmed, Khadijakhalil M. Barzani, Payman A. Hamasaeed

Department of Biology, College of Education, Salahaddin University-Erbil, Kurdistan Region, Iraq

ABSTRACT

The increase in antimicrobial resistance and the absence of novel antibiotic development cause problems in controlling infections for wound healing. Antibacterial properties of nanomaterials have emerged as a potentially effective approach in the pursuit of superior alternatives; nevertheless, the toxicity associated with higher concentrations has emerged as a significant obstacle. According to this study, green nanoparticle synthesis is demonstrated to be both economical and biocompatible on account of the bioactive compounds present. In the present work, silver nanoparticles (NPs) were prepared using *Pistacia khinjuk* gum; then, nanoparticle-based cream was prepared and compared with fucidin and Vaseline creams through assessment *in vitro* antibacterial and *in vivo* wound healing activity on Wistar albino rats infected with multidrug-resistant Gram-negative bacteria which were newly recorded in Iraq, namely, *Klebsiella variicola*. The findings revealed that the application of nanoparticle cream resulted in more rapid and effective wound healing (11 days), demonstrating a significant synergistic effect in comparison to fucidin (16 days), Vaseline (20 days), and the untreated control group rats (32 days). The results indicated that green NPs proved to be a significant strategy in the fight against multidrug-resistant bacteria, without any toxicity concerns.

Keywords: Multidrug resistance, *Klebsiella variicola*, biosynthesis of nanoparticle, antibacterial activity, wound healing

INTRODUCTION

The process of wound healing has four distinct phases: Hemostasis, inflammation, proliferation, and maturation. Impaired wound healing is mainly linked to bacterial infections, particularly those caused by multidrug-resistant (MDR) bacteria. These infections can lead to the development of chronic wounds, which, in turn, can result in severe problems that pose a risk to life.[1] Wound infection is an important category of nosocomial infection that has a global impact on a considerable number of patients. The potential risks and financial and medical burdens associated with bacterial contamination of a lesion are substantial, due to the rapid emergence of antibiotic-resistant bacteria.[2] MDR bacteria present a significant medical problem in our current period. These bacteria demonstrate the capacity to persist for prolonged durations and endure conditions with limited nutrients while proliferating. Moreover, they have the ability to establish colonies on compromised epidermis, thereby presenting a substantial worldwide peril to public health.[3,4] According to the World Health Organization reports, the rate of mortality associated with MDR infections exceeds that of all types of cancer combined. Infections caused by MDR bacteria in burn wounds are recognized as severe, widespread health hazards in a number of countries. As a result, novel nanoparticle-based materials have garnered significant interest within the domain of antimicrobial therapy for infections originating from burn wounds.[5,6] Nanoparticles (NPs) possess the capacity to emerge as a crucial and feasible therapeutic option for addressing MDR diseases. NPs consist of metallic compounds and their corresponding oxides, which have the greatest potential among all types of NPs and have generated significant interest among several experts. Moreover, the utilization of silver NPs (AgNPs) has generated significant attention.[7] Additional advancements in nanomaterials as a novel framework for biomedical applications through phyto-assisted synthesis offer numerous advantages, including cost-effective biocompatibility improvements. In addition, bioactive compounds present in plant extracts facilitate the reduction process and contribute to the stabilization of the resulting nanomaterials. Greenly synthesized AgNPs have been shown

Corresponding Author:
Faraidun A. Ahmed, Department of Biology, College of Education, Salahaddin University-Erbil, Kurdistan Region, Iraq.
E-mail: faraidun.ahmed@su.edu.krd

Received: May 25, 2024
Accepted: July 01, 2024
Published: August 20, 2024

DOI: 10.24086/cuesj.v8n2y2024.pp49-55

in recent studies to possess superior intrinsic antimicrobial and wound-healing properties while exhibiting minimal toxicity.[8,9]

This study investigates the combined antibacterial and wound-healing effects of AgNPs synthesized from *Pistacia khinjuk* gum extract on Wistar albino rats complicated with *Klebsiella variicola* infection. Cream formulations with optimal qualities were created for conducting *in vitro* and *in vivo* antibacterial and wound healing tests.

METHODOLOGY AND EXPERIMENTAL DESIGN

Isolation and Identification of *K. variicola*

A total of 157 clinical samples were collected in different hospitals in Erbil city-Iraq in the summer of 2022. Specimens were inoculated on blood agar and MacConkey agar and incubated at 37°C for 24 h. Gram-negative bacteria were identified by studying molecular identification using polymerase chain reaction (PCR) (16S rRNA) gene and sequencing. A web-based submission tool (https://submit.ncbi.nlm.nih.gov) to assist with the submission procedure was utilized. Each sequence has been submitted to GenBank and accession numbers for isolates were generated. Then, an antimicrobial sensitivity test was used to assess the sensitivity of isolated bacteria against 19 antimicrobials.

Plant Extract Preparation

Fresh *P. khinjuk* gum was bought from local markets in Halabja city-Kurdistan Region-Iraq and verified the *P. khinjuk* category in the College of Education, Salahaddin University-Erbil, Iraq. To dissolve *P. khinjuk* gum, dimethyl sulfoxide (DMSO) was used as an organic solvent. One gram of *P. khinjuk* gum was added to 100 mL of DMSO, then the solution was sonicated for 1 h at 60°C, and the extract was collected and stored at 4°C for further experiments.[6,10]

Biosynthesis of AgNPs

For the production of AgNPs, 100 mL of *P. khinjuk* gum extracts were added dropwise to 400 mL of 1 mM of silver nitrate ($AgNO_{3)}$ solutions under stirring. The obtained solutions were stirred for 2 h at 1000 rpm and at 5°C. The pH of solutions was controlled between 8 and 10 by adding sodium hydroxide (NaOH). The solution was incubated for 72 h at 37°C. For preparation powder of NPs, the solution was boiled to evaporation of the solvents, then, put in the oven for 2 h at 200°C for full drying and sterilization.[10-12]

Characterization of AgNPs

Characterization of AgNPs was screened by double-beam ultraviolet (UV)-visible spectrophotometer for confirming the formation of AgNPs and Fourier-transmission infrared spectroscopy (FTIR) analysis was conducted to identify the potential functional groups in the biomolecules found in the plant extract. X-ray diffraction (XRD) measurements and the chemical composition of the prepared NPs were measured by (energy-dispersive X-ray [EDX] spectroscopy) performing in scanning electron microscopy (SEM). The SEM was used to investigate the AgNPs' size and surface shape.[13-15]

Antibacterial Activity of AgNPs

The antibacterial assessment of AgNPs against MDR *K. variicola* was detected *in vitro* by the well diffusion method. A known concentration (0.1%) of 10 mg/mL of AgNPs was prepared by dissolving 0.05 g of NPs in 5 mL ethanol, the solution sonicated for 1 h at 50°C. The suspension (0.5 McFarland standard 1.5×10^8 colony forming unit/mL) was inoculated on the Mueller–Hinton agar. Plates containing wells of 7 mm diameter were filled with extract of NPs. After 24 h of incubation at 37°C, the inhibition zones on the plates were measured in mm.[16]

In vivo Wound Healing Activity of AgNPs

Animal preparation and burning rats

The excision wound healing model was selected to evaluate the wound healing impact of AgNPs in Wistar albino rats (200 ± 10 g). Two rats were housed in an individual cage measuring 40 × 25 × 20 cm. Standard care conditions, including a 12-h light-dark photoperiod, temperature control set at 24 ± 2°C, and continuous access to food and water, were consistently maintained. The experimental protocol adhered to ethical guidelines for animal experimentation outlined in Directive 2010/63/EU and was approved by the ethics committee.[6,17-19] Initially, 8 rats (n = 2) were anesthetized using a combination of 2% xylazine and 10% ketamine (10 + 90 mg/kg body weight) administered intramuscularly. Subsequently, professional hair clippers were used to remove the hair. Burn wounds of a specific thickness were created by applying heat to a solid stainless steel bar [Figure 1a] on specified sections of the animal's dorsal proximal region for a duration of 5 s [Figure 1b].[20,21] To demonstrate the inhibitory effects of the synthesized AgNPs on antimicrobial and wound healing activity, the burned rats were infected with activated pure cultures of *K. variicola*. The activated culture was applied to the wounds of each burned rat [Figure 1c] and allowed to grow for 24 h.[6,21,22]

Creams formulation and treatment of wounds

The nanoparticle-based cream was prepared in a concentration of (1% w/w) by dissolving 0.05 g of AgNPs 2 mL DMSO, then, 5 g of pure Vaseline wax was added, and heated on the magnetic stirrer hot plate with gentle stirring at 500 rpm for 1 h to liquidity the Vaseline and mixing Vaseline with NPs, and also to eliminate the DMSO from the mixture; then, the mixture was cooled to room temperature while being stirring to produce a homogeneous cream, which was then sterilized in an autoclave typically 15 min. The sterilized cream was, then, cooled with continuous stirring.[21,23] The burned rats were randomly divided into four major groups; test group treated with NPs cream, positive control (pure Vaseline wax), negative control (no treatment), and standard group (Fusidin 2%) (n = 2). Following 24 h of infection, treatment started, the wound area being swabbed by mentioned creams in a single dose during 24 h [Figure 2].[21,24] Healing times were recorded daily until complete wound closure.

Oral Acute Toxicity/Single-Dose Toxicity Study

To evaluate the toxicity of each nanoparticle in a living system, female Wistar albino rats (weighing 200 ± 10 g) were chosen

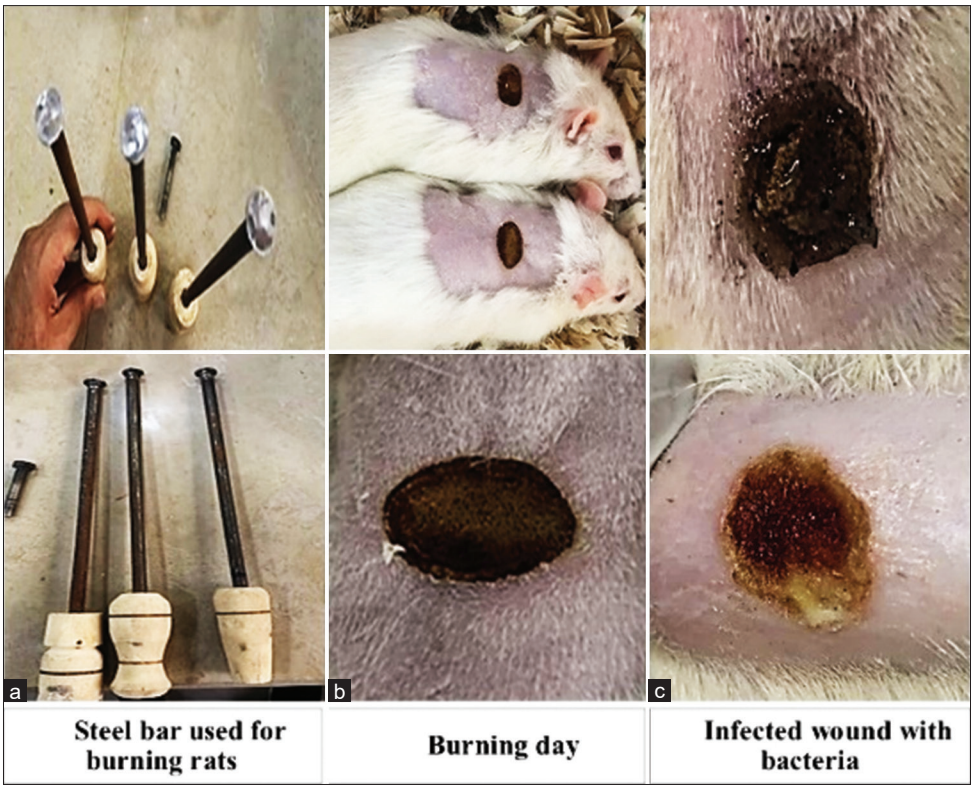

Figure 1: Appearance of different wounds

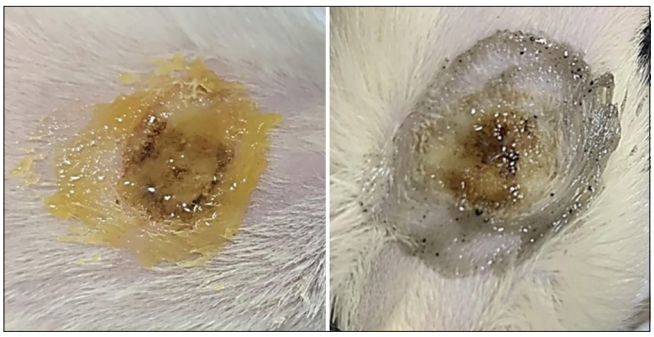

Figure 2: Treatment of burned rats

for an acute oral toxicity test. The test followed as outlined in the OECD Test Guidelines 425 (OECD, 2008). The rats underwent a 1-week acclimatization period and were fasted overnight before the dosing.[25]

The required quantity of the AgNPs (mg/kg) relative to body weight was dissolved in ethanol. Subsequently, corn oil was added as a vehicle, and the mixture was gently heated with stirring to achieve homogenization and evaporation of the ethanol [Table 1].[21] Depending on (OECD 425–27), four rats were weighed, and the limited test dose (2000–5000 mg/kg) relative to body weight was administered orally either in a single dosage or twice within a 24 h period by gavage through a stomach tube for each nanoparticle. Following dose administration, the animals were individually monitored periodically within the first 24 h and daily for the next 14 days. Throughout the study, additional observations were conducted on each rat, comparing their condition to the control group, including behavioral alterations and modifications to the eyes, skin, and fur.[26] On the 15th day, the rats were anesthetized with a combination of xylazine 2% and ketamine 10%, sacrificed, and exterior surface and abdominal organs macroscopically investigated.[26,27]

RESULTS

In the present study, *K. variicola* was identified and recorded for 1st time in Iraq depending on the molecular study (PCR) using 16S rRNA, sequencing and submitted to NCBI, and the accession number was acquired (OQ380694). However, the antimicrobial susceptibility test showed that out of 19 tested antimicrobials, *K. variicola* was 100% resistant to 12 antimicrobials.

Biosynthesis of AgNPs

Biological active AgNPs were successfully produced using *P. khinjuk* gum. Color change observed during the initial stages of the process and synthesis of NPs was confirmed, and the color of $AgNO_3$ and *P. khinjuk* gum changed from colorless to dark brown [Figure 3].

Characterization of AgNPs

The generation of NPs was examined using a UV-visible spectrophotometer. A strong plasmon absorbance band was observed at 441 nm and confirmed the presence of AgNPs [Figure 4a]. The FTIR spectra revealed the stabilization of AgNPs by *P. khinjuk* gum, which showed characteristic metal NPs bands at 617.22, 835.18, 1107.14, 1384.89, 2924.09, and 3452.58 [Figure 4b]. The band ta 617.22 is due to C-Br

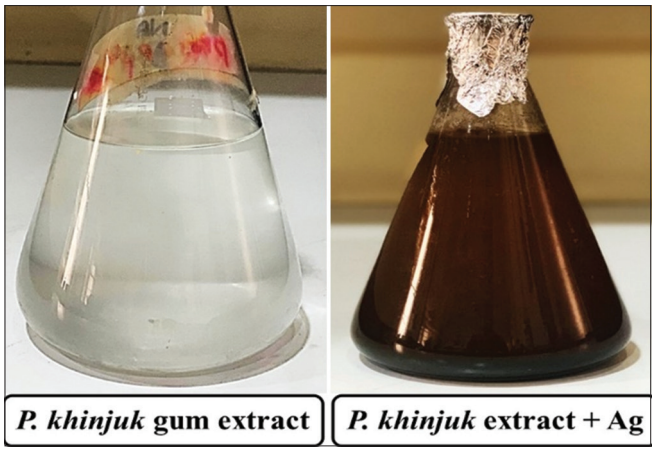

Figure 3: Visual observation of the color change of silver nanoparticles

stretching of the halo compound. At 835.18, absorption band revealed the existence of C = C bending of the alkene. The presence of C-N stretching (amine) and S = O stretching (sulfate) was confirmed by peaks at 1107.14 and 1384.89, respectively. The existence of C-H stretching functional group of alkenes was confirmed by the peak at 2924.09. The presence of O-H stretching of alcohol was revealed by the absorption band at 3452.58. XRD analysis of AgNPs exhibited six diffraction peaks at 28.805, 37.675, 43.949, 46.981, 54.472, and 64.098 represents to reflections of (100), (111), (020), (211), (220), and (311) of face-centered cubic silver [Figure 4c]. The EDX spectroscopy of AgNPs [Figure 4d] illustrated the existence of notable absorption peaks at 2 keV, signifying the creation of metallic AgNPs with a crystalline structure. The form and size of AgNPs were examined using SEM, revealing a regular and spherical morphology [Figure 4e].

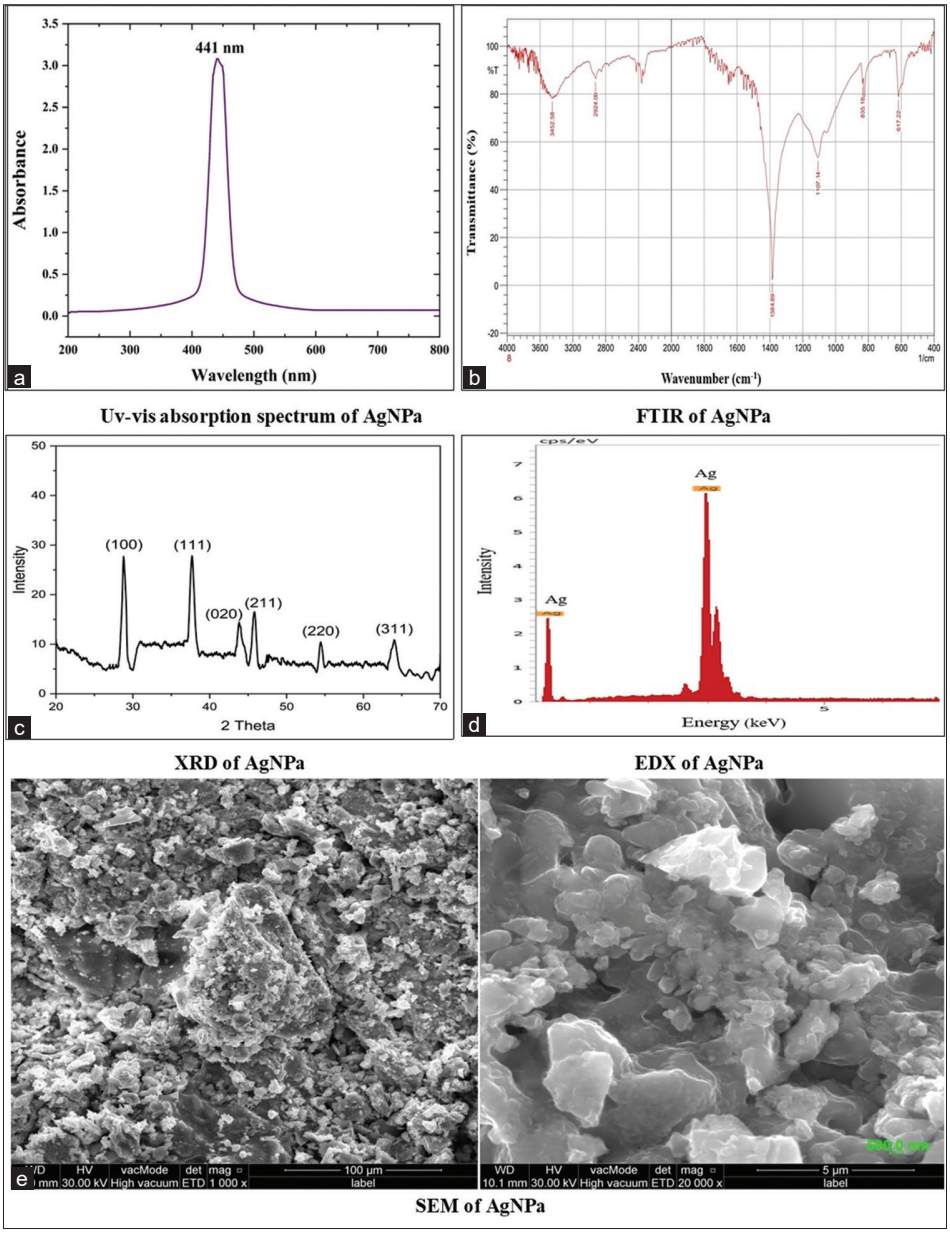

Figure 4: Characterization of silver nanoparticles from Pistacia khinjuk gum

In vitro and *in vivo* Antimicrobial and Wound Healing Activity of AgNPs

The antibacterial activity of AgNPs at a concentration of 0.1% was investigated against *K. variicola*. The result displayed the inhibition zone with 11 mm. Furthermore, healing times were recorded daily until complete wound closure and were varied among the treatment groups. Wounds treated with nanoparticle creams were notably revealed shorter healing time (11 days) compared to standard group treatment (16 days), positive control (20 days), and non-treated group (32 days), as displayed in [Table 2 and Figure 5].

In vivo Toxicological Study (Acute Oral Toxicity)

Throughout the experimental period, no fatalities were reported, and the rats did not exhibit any substantial indications of toxicity. These observations suggest that the tested NPs can be characterized as possibly nontoxic. At the end of the study, the rats were sacrificed after being anesthetized. The abdominal organs were tested macroscopically to evaluate any potential changes associated with toxicological effects. Importantly, no notable differences were detected between the test groups and control group, indicating the absence of toxicological concerns related to the administration of AgNPs. This robust evaluation supports the safety profile of the NPs, reinforcing their potential for further biomedical applications.

DISCUSSION

Results of nanoparticles demonstrated that AgNPs may suppress MDR bacterial growth. Various proposed mechanisms suggest how NPs can influence rates of bacterial survival. The NPs penetrate bacterial cells; moreover, silver metal or ions trigger the production of free radicals

Table 1: Dose preparation and administration

Conc. mg/kg	Ethanol (mL)	Vehicle (mL)	No. of doses in 24 h	No. of used rat	Lethality
2000	2	3	1	1	No
3000	2	3	1	1	No
4000	4	5	2	1	No
5000	4	5	2	1	No

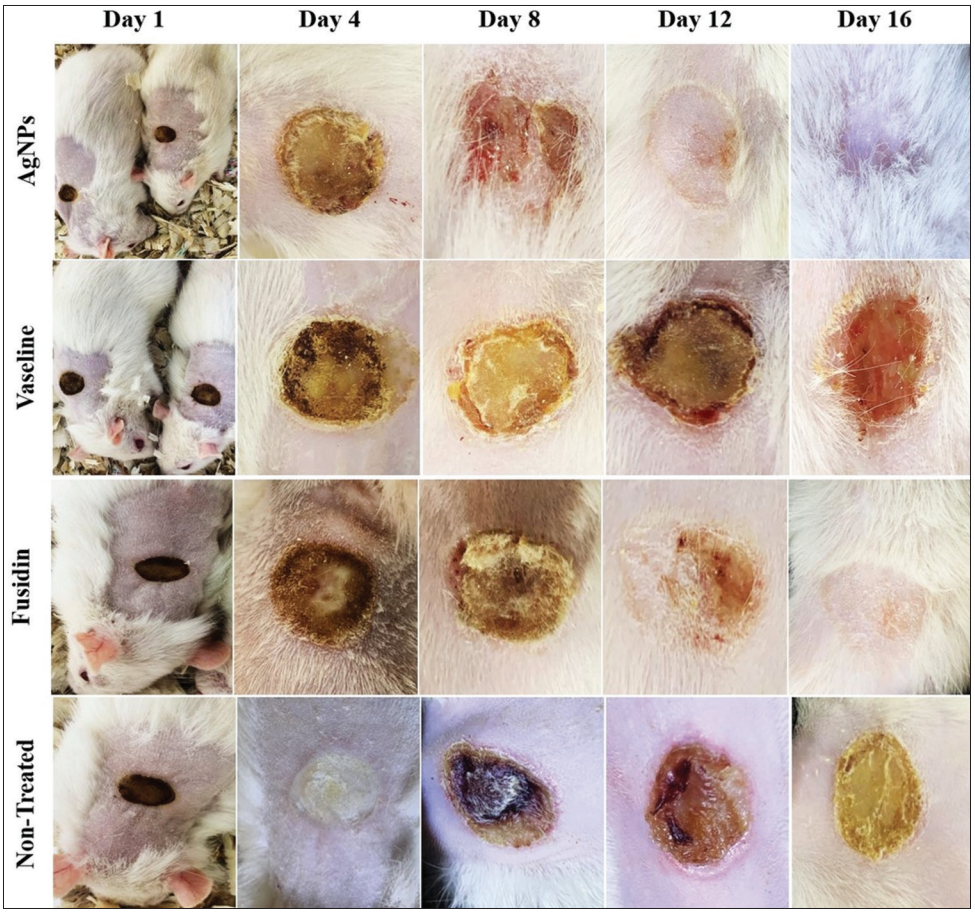

Figure 5: Wound healing on different days with nanoparticles, positive control, standard control, and negative control

Table 2: Healing time of treated rat wounds

Study creams	Healing time (days)
Silver nanoparticles	11
Vaseline (positive control)	20
Fusidin (standard control)	16
Non-treated (negative control)	32

and reactive oxygen species, which can harm DNA and denature proteins, ultimately resulting in bacterial death.[16] The findings reveal how nanoparticle-based creams may improve wound healing in complex *K. variicola* infections. NPs lowered healing times compared to controls. Similar findings were obtained by El-Banna *et al*.,[28] The study found that nanoparticle-treated groups healed faster than non-treated groups. Each nanoparticle's size, composition, and wound environment interactions affect healing timeframes. NPs may speed healing with antibacterial and regenerative characteristics.[1] NPs kill bacteria and regenerate skin. AgNPs are highly efficient. Their unique qualities prevent wound infections and speed tissue healing compared to topical therapies.[28]

Scientific studies have demonstrated that the *P. khinjuk* gum extract-mediated AgNPs had potent antibacterial properties and the capacity to promote wound healing.[10,16] The study assessed the capacity of plant extract-derived AgNPs to promote wound healing in Wistar albino rats, demonstrating accelerated re-epithelialization compared to conventional medication.[6] Green synthesis of antibacterial metal NPs using plant extracts as reducing and stabilizing agents is possible. Current pharmacology and toxicology need new effective, non-toxic antibacterial treatments for infectious disorders caused by diverse bacterial strains, especially MDR in clinical settings.[29]

The *K. variicola*, a new Gram-negative bacteria found in Iraq in gunshot wounds, is a novel observation with significance for clinical care and microbiology. Similar to the present study, Nakamura-Silva *et al*.[30] reported the first case in Brazil infected by *K. variicola*.

CONCLUSION

The present study has taken forward to biosynthesis of AgNPs using *P. khinjuk* gum. The investigation evaluated their *in vitro* antimicrobial properties and *in vivo* excision wound healing activities on Wistar rat models. The results indicated that AgNPs effectively impede the growth of MDR *K. variicola*. This study highlighted the combined benefits of NPs as a safe, economical, and promising choice for applying to promote efficient wound healing. Furthermore, the NPs demonstrated the ability to heal excision wounds in a timeframe of 11 days. In addition, the present study represents a substantial challenge in developing novel NPs for antibacterial drugs and wound creams, offering improved alternative applications. It contributes to an enhanced understanding of the biological impacts induced by these valuable metals, paving the way for a new paradigm in medical research.

REFERENCES

1. A. Pormohammad, N. K. Monych, S. Ghosh, D. L. Turner and R. J. Turner. Nanomaterials in wound healing and infection control. *Antibiotics*, vol. 10, no. 5, p. 473, 2021.
2. A. Shariati, S. M. Hosseini, Z. Chegini, A. Seifalian and M. R. Arabestani. Graphene-based materials for inhibition of wound infection and accelerating wound healing. *Biomedicine and Pharmacotherapy*, vol. 158, p. 114184, 2023.
3. B. Nițescu, D. Pițigoi, D. Tălăpan, M. Nițescu, S. Ș. Aramă, B. Pavel, A. Streinu-Cercel, A. Rafila and V. Aramă. Etiology and multi-drug resistant profile of bacterial infections in severe burn patients, Romania 2018-2022. *Medicina*, vol. 59, no. 6, p. 1143, 2023.
4. A. A. Al-Naqshbandi, H. A. Hassan, M. A. Chawsheen and H. H. A. Qader. Categorization of bacterial pathogens present in infected wounds and their antibiotic resistance profile recovered from patients attending rizgary hospital-erbil. *Aro-the Scientific Journal of Koya University*, vol. 9, no. 2, pp. 64-70, 2021.
5. M. A. Khalil, G. M. El Maghraby, F. I. Sonbol, N. G. Allam, P. S. Ateya and S. S. Ali, Enhanced efficacy of some antibiotics in presence of silver nanoparticles against multidrug resistant *Pseudomonas aeruginosa* recovered from burn wound infections. *Frontiers in Microbiology*, vol. 12, p. 648560, 2021.
6. S. V. N. Pammi, V. S. Padavala, T. S. K. Karumuri, C. S. Kommavari, M. Shaik, V. R. M. Kolapalli and L. K. Ruddaraju. Wound healing synergy in Wistar albino rats via green synthesized nanoparticles and topical antibiotic neomycin. *OpenNano*, vol. 11, p. 100135, 2023.
7. A. R. Alnsour, R. M. Daghmash, M. M. Masadeh, K. H. Alzoubi, M. M. Masadeh, N. H. Bataineh, H. H. Batayneh and M. S. Al-Ogaidi. The pharmaceutical role of silver nanoparticles in treating multidrug-resistant bacteria and biofilms. *Current Nanoscience*, vol. 20, no. 4, pp. 471-494, 2024.
8. A. W. Alshameri and M. Owais. Antibacterial and cytotoxic potency of the plant-mediated synthesis of metallic nanoparticles Ag NPs and ZnO NPs: A review. *OpenNano*, vol. 8, p. 100077, 2022.
9. F. Khan, M. Shariq, M. Asif, M. A. Siddiqui, P. Malan and F. Ahmad. Green nanotechnology: Plant-mediated nanoparticle synthesis and application. *Nanomaterials* (*Basel*), vol. 12, no. 4, p. 673, 2022.
10. A. Fattahi, T. Sakvand, M. Hajialyani, B. Shahbazi, M. Shakiba, A. Tajehmiri and E. Shakiba. Preparation and characterization of *Pistacia khinjuk* gum nanoparticles using response surface method: Evaluation of its anti-bacterial performance and cytotoxicity. *Advanced Pharmaceutical Bulletin*, vol. 7, no. 1, p. 159, 2017.
11. F. E. Meva, M. L. Segnou, C. O. Ebongue, A. A. Ntoumba, P. B. E. Kedi, V. Deli, M. A. Etoh and E. M. Mpondo. Spectroscopic synthetic optimizations monitoring of silver nanoparticles formation from *Megaphrynium macrostachyum* leaf extract. *Revista Brasileira de Farmacognosia*, vol. 26, pp. 640-646, 2016.
12. S. Zafar, A. Ashraf, M. U. Ijaz, S. Muzammil, M. H. Siddique, S. Afzal, R. Andleeb, K. A. Al-Ghanim, F. Al-Misned, Z. Ahmed and S. Mahboob. Eco-friendly synthesis of antibacterial zinc nanoparticles using *Sesamum indicum* L. Extract. *Journal of King Saud University-Science*, vol. 32, no. 1, pp. 1116-1122, 2020.
13. B. A. Othman and N. M. Nanakali. The preventive effects of *Urtica dioica* extract and nanoparticle on oxidative stress and lipid profile in hyperlipidemic male rats. *Cihan University-Erbil Scientific Journal*, vol. 6, no. 2, pp. 125-132, 2022.
14. F. Ameen, E. E. Altuner, R. N. E. Tiri, F. Gulbagca, A. Aygun, F. Sen, N. Majrashi, R. Orfali and E. N. Dragoi. Highly active iron (II) oxide-zinc oxide nanocomposite synthesized *Thymus vulgaris* plant as bioreduction catalyst: Characterization, hydrogen evolution and photocatalytic degradation. *International Journal*

of Hydrogen Energy, vol. 48, no. 55, pp. 21139-21151, 2023.
15. F. M. Moghaddam, A. Jarahiyan, M. H. Haris, P. Y. Pazoki and B. Aghamiri. High catalytic performance of CoCuFe2O4/ZIF-8 (Zn) nanocatalyst for synthesis of new benzimidazole derivatives. *Journal of Molecular Structure*, vol. 1285, p. 135496, 2023.
16. A. H. Hamad, M. A. Chawsheen and A. A. Al-Naqshbandi. Role of laser produced silver nanoparticles in reversing antibiotic resistance in some multidrugresistant pathogenic bacteria. *Aro-the Scientific Journal of Koya University*, vol. 10, no. 1, pp. 104-110, 2022.
17. M. H. Homady, L. O. ALquraishi, T. S. Salih and A. S. M. Juma. The intra gastric effects of grape juice on histological structure of liver tissue in castrated treated mice. *Journal of Human Anatomy*, vol. 4, p. 000147, 2020.
18. M. H. Homady, L. O. ALquraishi, R. D. Thanoon and T. S. Salih. Immunohistohemical and molecular studies of mouse liver in castrated subjects treated with grape juice. *American Journal of Clinical Anatomy and Physiology*, vol. 2, pp. 13-17, 2020.
19. K. A. Marinou and I. A. Dontas. European union legislation for the welfare of animals used for scientific purposes: Areas identified for further discussion. *Animals* (*Basel*), vol. 13, no. 14, p. 2367, 2023.
20. J. Y. Kim, D. M. Dunham, D. M. Supp, C. K. Sen and H. M. Powell. Novel burn device for rapid, reproducible burn wound generation. *Burns*, vol. 42, no. 2, pp. 384-391, 2016.
21. M. K. Samad and F. E. Hawaiz. Synthesis, characterization, antioxidant power and acute toxicity of some new azo-benzamide and azo-imidazolone derivatives with *in vivo* and *in vitro* antimicrobial evaluation. *Bioorganic Chemistry*, vol. 85, pp. 431-444, 2019.
22. M. Zhu, P. Liu, H. Shi, Y. Tian, X. Ju, S. Jiang, Z. Li, M. Wu and Z. Niu. Balancing antimicrobial activity with biological safety: Bifunctional chitosan derivative for the repair of wounds with Gram-positive bacterial infections. *Journal of Materials Chemistry B*, vol. 6, no. 23, pp. 3884-3893, 2018.
23. S. Kantipudi, J. R. Sunkara, M. Rallabhandi, C. V. Thonangi, R. D. Cholla, P. Kollu, M. K. Parvathaneni and S. V. N. Pammi. Enhanced wound healing activity of Ag-ZnO composite NPs in Wistar Albino rats. *IET Nanobiotechnology*, vol. 12, no. 4, pp. 473-478, 2018.
24. D. Dwivedi, M. Dwivedi, S. Malviya and V. Singh. Evaluation of wound healing, anti-microbial and antioxidant potential of *Pongamia pinnata* in wistar rats. *Journal of Traditional and Complementary Medicine*, vol. 7, no. 1, pp. 79-85, 2017.
25. B. Olela, J. Mbaria, T. Wachira and G. Moriasi. Acute oral toxicity and anti-inflammatory and analgesic effects of aqueous and methanolic stem bark extracts of *Piliostigma thonningii* (Schumach.). *Evidence-Based Complementary and Alternative Medicine,* vol. 2020, p. 5651390, 2020.
26. M. H. Zarei, Z. Lorigooini, H. A. Khoei and E. Bijad. Acute oral toxicity assessment of galbanic acid in albino rat according to OECD 425 TG. *Toxicology Reports*, vol. 11, pp. 111-115, 2023.
27. H. Wati, R. Muthia, K. Kartini and F. Setiawan. Acute toxicity study of the ethanolic extract of *Eleutherine bulbosa* Urb in Wistar rats. *Pharmacy Education*, vol. 21, no. 2, pp. 143-147, 2021.
28. A. H. El-Banna, F. S. Youssef, H. Y. Elzorba, A. M. Soliman, G. G. Mohamed, S. H. Ismail, M. R. Mousa, H. A. Elbanna and A. S. Osman. Evaluation of the wound healing effect of neomycin-silver nano-composite gel in rats. *International Journal of Immunopathology and Pharmacology*, vol. 36. https://doi.org/10.1177/03946320221113486
29. C. E. Escárcega-González, J. A. Garza-Cervantes, A. Vazquez-Rodríguez, L. Z. Montelongo-Peralta, M. T. Treviño-Gonzalez, E. Díaz Barriga-Castro, E. M. Saucedo-Salazar, R. M. Chávez Morales, D. I. Regalado-Soto, F. M. Treviño-González, J. L. Carrazco Rosales, R. Villalobos Cruz and J. R. Morones-Ramirez. *In vivo* antimicrobial activity of silver nanoparticles produced via a green chemistry synthesis using *Acacia rigidula* as a reducing and capping agent. *International Journal of Nanomedicine*, vol. 13, pp. 2349-2363, 2018.
30. R. Nakamura-Silva, L. M. D. Macedo, L. Cerdeira, M. Oliveira-Silva, Y. T. C. Silva-Sousa and A. Pitondo-Silva. First report of hypermucoviscous *Klebsiella variicola* subsp. Variicola causing primary endodontic infection. *Clinical Microbiology and Infection*, vol. 27, no. 2, pp. 303-304, 2021.