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RESEARCH ARTICLE

Treatment of methicillin resistant *Staphylococcus aureus* wound infection using vancomycin-loaded nanoparticles: An *in vitro* and *in vivo* study

HASSAN THOULFIKAR A. ALAMIR[1], MUSTAFA GHENI TAHER[2], MAZIN RAZOOQI MOHAMMED[3], SAJA HAMEED KAREEM[4] and ABDULADHEEM TURKI JALIL[5]*

[1] College of Pharmacy, University of Al-Ameed, Karbala, Iraq

[2] Department of Pathology and Forensic Medicine, College of Medicine, University of Diyala, Baquba, Diyala, Iraq

[3] Bilad Alrafidain University College, Diyala, 32001, Iraq

[4] National University of Science and Technology, Dhi Qar, Iraq

[5] Medical Laboratories Techniques Department, Al-Mustaqbal University College, Babylon, Hilla, 51001, Iraq

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ABSTRACT

Background: Due to the rise of bacteria that are resistant to multiple drugs, treating infections in burn wounds has become a worldwide problem. It is important to find new ways to treat these infections. Lipid-based drug delivery systems have emerged as a promising type of carrier for antibiotics as well as antibacterial agents, such as antimicrobial peptides and nucleic acids, for wound infections. To this end, the current study assessed the antibacterial effects and wound healing properties of vancomycin-loaded solid lipid nanoparticles (SLN) against methicillin-resistant *Staphylococcus aureus* (MRSA) surgical wound infections. *Material and methods:* vancomycin-SLN was synthesized by double emulsion solvent evaporation techniques, and then *in vitro* antibacterial activity was evaluated by well diffusion and broth microdilution methods. A surgical wound was inflicted on 36 male rats; the infection was caused by MRSA, and free vancomycin and vancomycin-SLN were topically applied. *Results:* Particle size, PDI, zeta potential, drug loading, and encapsulation efficiency of the optimum vancomycin-SLN were 350 ± 24 nm, 0.402 ± 0.014, −16.3 ± 2.5 mV, 13.9 ± 1.4%, and 92.14 ± 3.1%, respectively. *In vitro* antibacterial assays showed lower MICs for free vancomycin and killed bacteria more efficiently than vancomycin-SLN. However, the synthesized nanoparticles indicated long-term antibacterial activity against MRSA. Animals that were treated with vancomycin-SLN showed the best wound healing procedure. Treatment of rats with vancomycin-SLN increased re-epithelialization and decreased granulation tissue development, as seen by histological analysis. *Conclusion:* Antibiotic-loaded SLN could not only manage wound infection but also enhance wound healing. Therefore, this kind of treatment should be considered by scientists as an applicable treatment for wounds.

KEYWORDS

vancomycin, MRSA, wound infection, solid lipid nanoparticles

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*Corresponding author.
E-mail: abedalazeem799@gmail.com

1. INTRODUCTION

Healing a wound is a dynamic and intricate process that requires the replacement of dead cells and tissues [1, 2]. Noteworthy, wound infections now account for 60% of burn patient

mortality and 300,000 deaths globally each year, despite the great breakthroughs in infection control and wound healing [3, 4]. Necrotic tissues lower the presence of immune cells in the wound area and make it easier for pathogens to penetrate the underlying tissues and disseminate. Additionally, wounds affect the integrity of the skin, which in turn attenuates cellular and humoral immunity [5, 6]. Due to the aforementioned problem, different wounds, such as burn, diabetic, and surgical wounds, are prone to infection and may develop into an infected ulcer or even an invading infection like sepsis [7, 8]. *Staphylococcus aureus*, especially methicillin-resistant *S. aureus* (MRSA), is considered a bacterial pathogen that is frequently detected in burn wound infections [9, 10]. This bacterium gets into eschar and enters subcutaneous, unburned tissues, which may lead to abscesses of different sizes. These abscesses protect *S. aureus* from the body's immune system and antibiotics. They also make it possible for this bacteria to get into the bloodstream [11].

Vancomycin is one of the best choices for managing infections caused by MRSA. However, the prevalence of vancomycin-resistant strains is increasing, and there is an essential need for alternative antibiotics [12]. In addition to antibiotic resistance, the continuous presence and release of antibacterial agents are essential for managing wound infections. To this end, recently published studies used different local antibacterial therapies, such as natural compounds, bacteriophage therapy, and nanoparticles, for managing burn wound infection, enhancing wound healing, and reducing the toxicity of antibiotics [13, 14].

In this regard, the use of nano-platforms loaded with commonly used antibiotic has been considered by scientists to enhance the activity of antibiotics at the site of infections. Not only can these drug delivery vehicles target and regulate drug release, but they can also carry a substantial dose of medicine [15–18]. For the treatment of burn wound infection, researchers have also examined the use of drug carriers that can overcome some of antibiotics' drawbacks, such as limited solubility and survivability in water and tissue [19–21]. One such drug carrier that allows for regulated drug release and improved chemical stability of entrapped medicines is the solid lipid nanoparticle (SLN). The development of SLN as a carrier system has led to the effective incorporation of several medications with a wide range of potential uses [22]. Despite having some limitations, SLNs have advantageous properties. For instance, SLNs would shield drugs from enzymatic and chemical breakdown. Since organic solvents are not required, the production of SLNs is simpler than that of other categories of drugs [23]. Therefore, SLN regulates the drug's release and improves the chemical stability of trapped antibiotics. To this end, in the present study, we used the SLN for the slow and steady release of vancomycin in the site of infection, enhancing the performance of this antibiotic in the management of surgical wound infections caused by MRSA.

2. MATERIAL AND METHODS

2.1. Research materials

Vancomycin and all chemicals and solvents were purchased from Sigma-Aldrich (Munich, Germany). All of the cultural media utilized in this investigation were purchased from the German company Merck. Additionally, Male Wistar rats were used for *in vivo* studies.

2.2. Synthesis and evaluation of Van-SLN

According to a recently published study, the double emulsion/melt dispersion method was used for the production of nanoparticles [24]. To determine the optimal formulation, various fat, surfactant, and vancomycin concentrations were evaluated. Using Ben-Marie, stearic acid was heated to 70 °C, 5 °C above its melting point. Next, pre-dissolved and warmed poloxamer and lecithin, as well as vancomycin, were added to the molten lipid, followed by magnetic agitation (150 rpm) to combine the ingredients. After 60 s of sonication at 45 °C, the mixture was homogenized with warm distilled water (DW). To create the second emulsion, we added heated tween-80 to the first and homogenized the mixture with ultrasonic equipment at 45 °C for 2 min (15 s on, 5 s off). The resulting mixture was slowly introduced to a solution of cold DW (5 °C) containing the stretched quantum dot cadmium telluride for 5 min in order to stabilize the generated nanoparticles. The identical conditions (drug-free solid lipid nanoparticles) were seen during the synthesis of drug-free SLN. Finally, a high-speed centrifuge (25,000 rpm for 15 min) was used to thoroughly wash the nanoparticles three times with DW. We then lyophilized it at −80 degrees Celsius using a vacuum pump (lyophilizer). The nanoparticles were first filtered via a 450 nm membrane to remove impurities before being used in biological studies.

Noteworthy, the PDI, mean particle size, and zeta potential of synthesized nanoparticles were evaluated. Additionally, a FTIR spectroscope (PerkinElmer, Spectrum 400, United States of America) and the dialysis bag method (cutoff of 12,000 to 14,000 Da) were used for spectroscopic evaluation and drug release assay of samples, respectively. The thermal behavior of the nanoparticle and its components were assessed using Differential Scanning Calorimetry (DSC) (Mettler Toledo, Hong Kong) device. To investigate the nanoparticle's shape, we employed an emission scanning electron microscope (Fe SEM). Finally, according to the kit's instructions (Kiazist Kit/Iran), fibroblast cell lines were utilized to test the cytotoxicity of the manufactured nano-drug. The concentrations of nanoparticles and free vancomycin employed in the experiment ranged from 25 mg mL^{-1} to 400 mg mL^{-1}.

2.3. Bacterial strains

MRSA ATCC 33591 was used in the present study. The isolated bacterium was grown overnight in Brain Heart Infusion (BHI) broth medium and then put in incubators

with new BHI medium for 18–24 h until it reached the mid-logarithmic phase. After centrifuging the culture at 1,000 g for 15 min, the supernatant was collected. The bacterial pellet was then washed twice with phosphate-buffered saline (PBS) and resuspended in cold PBS to get a concentration of about $1^{*}10^{8}$ CFU mL^{-1}. The bacterial mixture was kept at −20 °C until the next steps were taken.

2.4. Antibacterial effect (well diffusion and minimum inhibitory concentration (MIC))

Clinical and Laboratory Standards Institute (CLSI) 2021 suggested using the well diffusion and microbroth dilution methods to evaluate antibacterial effect of synthesized nanoparticles. The bacterial suspension was diluted to make 10^{4} CFU mL^{-1} for the well diffusion test, which was then used in the antibacterial studies. With a clean cotton swab, a lawn of the bacteria culture made above was spread evenly on the Muller–Hinton agar (MHA) plates. Several 6 mm holes were punched into the bacteria-covered MHA plates with a circular glass tube. In each well, 100 µL of nanoparticle sample solution was added, and the plates were kept at 37 °C for 18 h. We measured the width of the inhibition zone, which shows how susceptible bacteria are to the nanoparticles [24, 25]. Notably, we used a microbroth dilution method to determine the MIC of free vancomycin and synthesized nanoparticles according to the recently published study [13].

2.5. Wound infection

In this study, a surgical wound was made on male Wistar rats that were 6–8 weeks old. Noteworthy, each group had 12 rats, and rats with infectious wounds were treated with vancomycin-SLN, free vancomycin, and SLN free, as well as a negative control that contained untreated rats. On the day of the wounding, electric scissors were used to shave the rats' back hairs, and the area where the wound was made was cleaned very well. Then, 10 mg kg^{-1} of xylazine hydrochloride and 50 mg kg^{-1} of ketamine were injected into the rats' muscles to induce anesthesia. To make a wound in this location, a one-centimeter-long groove was made, and $1.5^{*}10^{8}$ of MRSA was put into the wound. The rat's survival was checked every day. On Days 4, 7, and 14, after the experiment, photos were taken and the size of the wound was measured to see how well it was healing. Additionally, five rats from each group were killed on Days 4 and 7 post-infections, and biopsy samples were taken aseptically. The biopsies were then weighed, homogenized in 10 mL of PBS, and inoculated with MHA. Additionally, colony counts were presented as log10 CFU per gram of tissue. Importantly, on day 14, punch biopsies were taken from the wound site, fixed in 10% formalin, paraffin-embedded, and stained with hematoxylin and eosin (H&E). A pathologist (who didn't know which animals were treated and which ones weren't) used re-epithelialization and the thickness of granulation tissue to compare the two groups of animals [26, 27].

2.6. Statistical analysis

When the data didn't follow a standard pattern, the Mann-Whitney test was used to look at the results. GraphPad Prism (version 8.3.0) and SPSS (version 20.0) were used for statistical analysis. When the *P*-value was less than 0.05, the difference between the treatment and control groups was statistically significant.

3. RESULTS

Particle size, PDI, zeta potential, drug loading, and encapsulation efficiency of the optimum vancomycin-SLN were 350 ± 24 nm, 0.402 ± 0.014, −16.3 ± 2.5 mV, 13.9 ± 1.4%, and 92.14 ± 3.1%, respectively. There was no statistically significant change in nanoparticle size ($P > 0.05$) from the first to the ninth month. However, the nanoparticles' diameter grew from 350 to 420 nm, or 16.6%, after a year. A morphological study of synthesized nanoparticles using FE-SEM revealed that the majority of the particles were round, with a smooth surface and a homogeneous distribution (Fig. 1).

Furthermore, the FTIR data indicated that the production of nanoparticles did not include a chemical reaction that resulted in the formation of a novel chemical compound. The DSC results demonstrated that the drugs were not crystalline and were bound inside the lipid matrix. According to the results of a drug release investigation, the manufactured nanoparticles released 85% of the drugs within 72 h.

3.1. Microbiology results

The results of the well diffusion and MIC tests for the antibacterial activity of synthesized nanoparticles are presented in Table 1. Vancomycin alone proved more effective than vancomycin-SLN at killing MRSA bacteria in the well diffusion method. Additionally, the free vancomycin showed a lower MIC in comparison to the vancomycin-SLN. On the other hand, in both well diffusion and microbroth dilution,

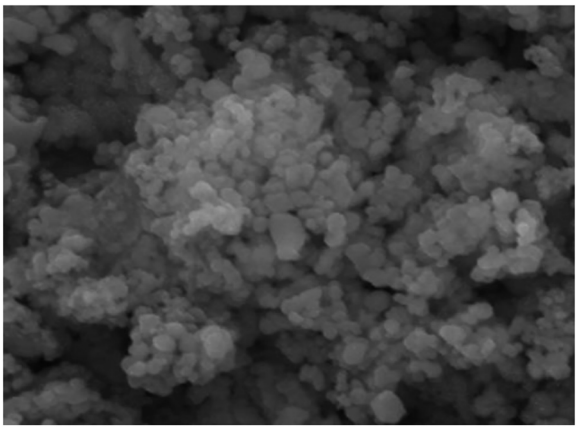

Fig. 1. Emission scanning electron microscope images of vancomycin-SLN nanoparticles

Table 1. Results of well diffusion and MIC tests after treatment with free drugs and synthesized nanoparticles (vancomycin-SLN)

Bacterial strain	Methods	Antibacterial activity, Zone of inhibition (mm) in different incubation time (hours) and three concentrations (μg ml^{-1})											
MRSA	Well diffusion	Free vancomycin (2.5–1.25–0.625 μg ml^{-1})			Van-SLN (2.5–1.25–0.625 μg ml^{-1})								
		24h			24h			48h			72h		
		27	20	18	16	14	13	20	18	16	25	21	17
	MIC	Free vancomycin 0.625						Van-SLN 1.25					

the bacteria in direct contact with the vancomycin demonstrated a rise in the diameter of the growth inhibition zone over the course of 72 h of incubation, indicating persistent release of the drug from the vancomycin-SLN.

3.2. Cytotoxicity assay using MTT

To test the effects of free vancomycin, vancomycin-SLN, and free SLN on the cells, we incubated them at 37 °C with varying doses of each. All studies were performed three times, and as a negative control, the same cells were incubated in culture medium (without any treatment). The MTT assay, read at 570 nm, determined cell viability. The comparisons were based on the fact that the control samples all survived (Fig. 2).

3.3. *In vivo* antibacterial activity of vancomycin-SLN

On Days 4 and 7, we took wound biopsies and homogenized them in PBS. There was a report of log10 CFU g^{-1} of tissue for the bacterial count. There were the most bacteria in the control group of rats, while the highest rate of bacteria reduction was observed in mice treated with vancomycin-SLN (*P*-value 0.001) (Fig. 3). Noteworthy, this treatment showed a significant antibacterial effect on day 7 in comparison to free vancomycin.

3.4. Wound healing after infection of rats

The wound healing process in the vancomycin-SLN treated rats and untreated animals during two weeks is shown in Fig. 4. The capacity of the manufactured nanoparticles to decrease bacterial numbers at the infection site led to faster wound healing.

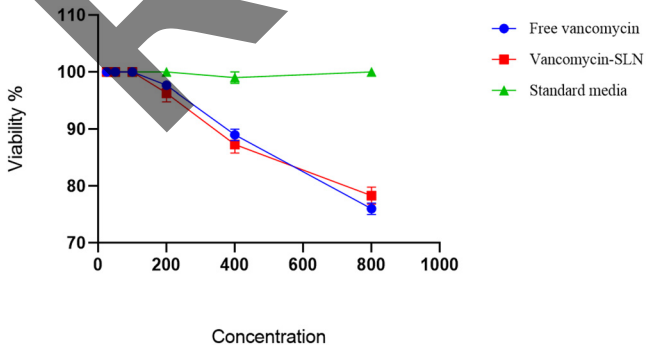

Fig. 2. Cytotoxicity assay of vancomycin-SLN nanoparticles at different concentrations on human fibroblasts cells cell lines

3.5. Histological analysis

All of the treated groups exhibited quicker skin layer renewal and repair compared to the control group. Figure 5 displays a comparison of the rates of epithelial cell development between the untreated and treated groups. It has been confirmed that vancomycin-SLN has about 50% greater epithelial diameter, confirming faster healing than other groups.

4. DISCUSSION

Wound infection is a big problem when it comes to treating different wounds, such as surgical and burn wounds, and it causes illness and death all over the world. Multi-drug-resistant (MDR) bacteria are becoming more common, which makes it harder to use conventional antibiotics to manage wound infections [28–30]. Also, systemic antibiotic treatment may not be able to keep wounds from getting infected because of things like necrosis, granulation tissue, limited blood supply, and fibrosis, which stop antibiotics from getting into the infected tissues [27, 31, 32]. Therefore, using antibacterial agents on the surface of a wound area can help prevent infection. To this end, in the present study, we used SLN nanoparticles coated with vancomycin for inhibition of MRSA surgical wound infection. The SLN gel was more active for 11 days, while the free vancomycin was only more active for one day. Additionally, vancomycin-SLN not only inhibited wound infection in infected rats but also improved wound healing processes.

The results of a recently published study also showed that high-dose curcumin SLN increased angiogenesis and remarkably enhanced burn wound closure [33]. In another study, the authors also used the emulsion solvent evaporation technique to produce fluoxetine-SLN and used these nanoparticles for the treatment of rats with diabetic wounds. The results indicated that fluoxetine-SLN enhanced cellular proliferation, angiogenesis, and the level of collagen synthesis [34]. A study that was published in 2022, El-Ezz et al. applied a hydrogel loaded with Cu (II) Schiff base 8-hydroxy quinolone complex SLN for management of excision wound healing in rats. These nanoparticles significantly modified wound healing, angiogenesis, and intact re-epithelization

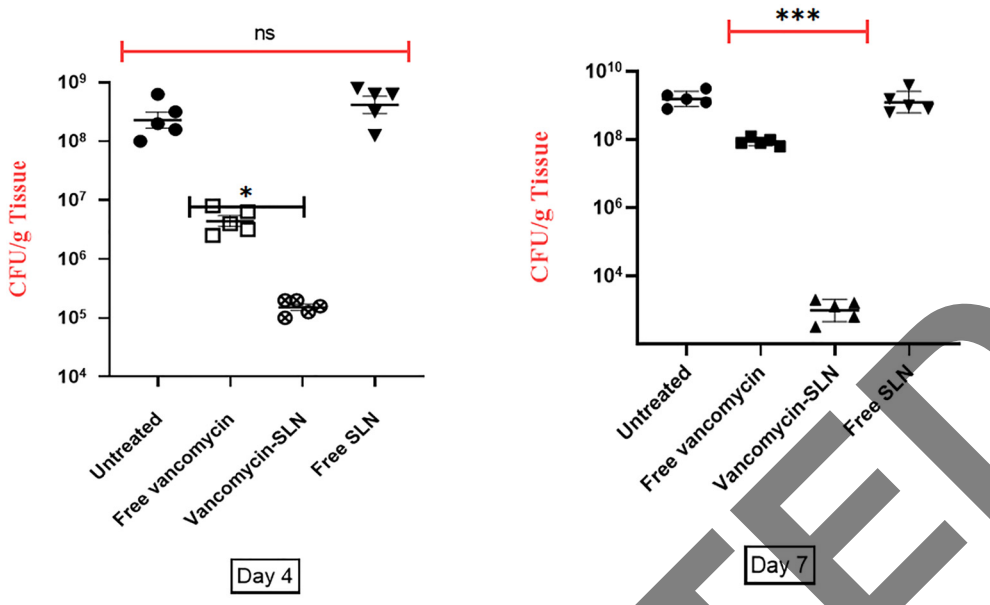

Fig. 3. MRSA wound burden on Days 4 and 7 in rats were infected with 1×10^8 cells of bacteria by the amount of CFU growth ($n = 5$ wounds per group). Treatment of rats with vancomycin-SLN remarkably reduced the MRSA number in the treated group compared to the untreated animals ($^{*}P$ value < 0.05, $^{***}P$ value < 0.001)

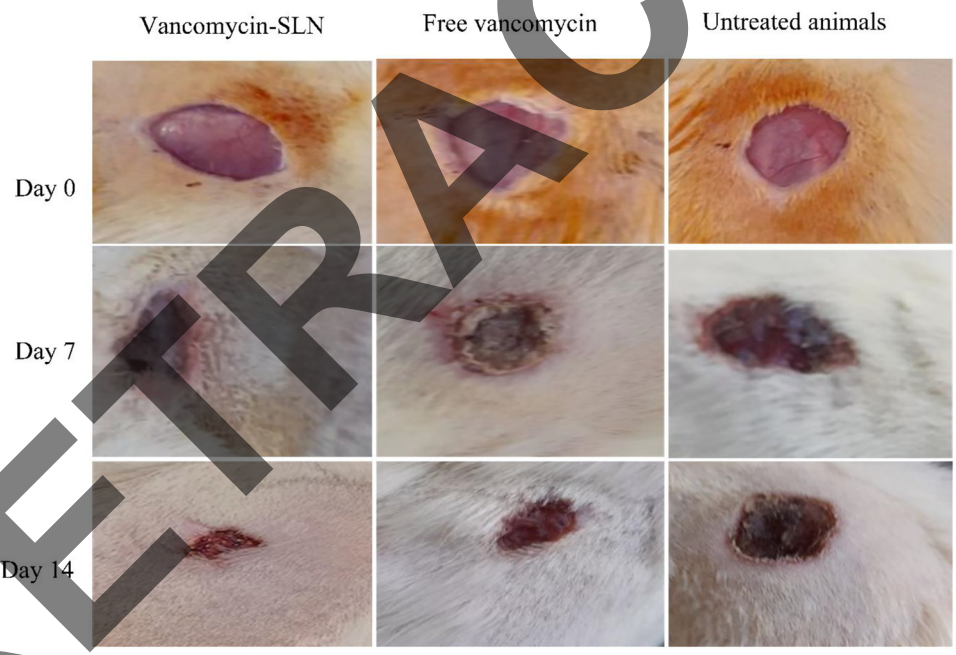

Fig. 4. Wound healing procedure of treated rats with free vancomycin and vancomycin-SLN and control rats on days 0, 7, and 14

[35]. Collectively, SLN could be a potential carrier for the delivery of different compounds for enhancement of wound healing processes in different wound types, including surgical, burn, and diabetic wounds.

It should be noted that, because of their superior biocompatibility, durability, and skin adhesion, lipid nanoparticles have taken a commanding lead over other nanoparticles in the field of wound healing. Lipid nanoparticles' adhesive properties allow them to stick to the stratum corneum, where they produce a layer that blocks the evaporation of moisture. These have a major physiological benefit because their lipid composition is so comparable to that of the lipid bilayers between corneocytes and keratinocytes in the stratum corneum. Therefore, compared to other drug delivery systems, this allows the lipid nanoparticles and their encapsulated drugs to penetrate the epidermis and come into closer contact with the wound site [36–38]. Furthermore, due to the lipidic bilayers and aqueous core of lipid nanoparticles, they are capable of carrying a desired payload that includes both lipophilic and

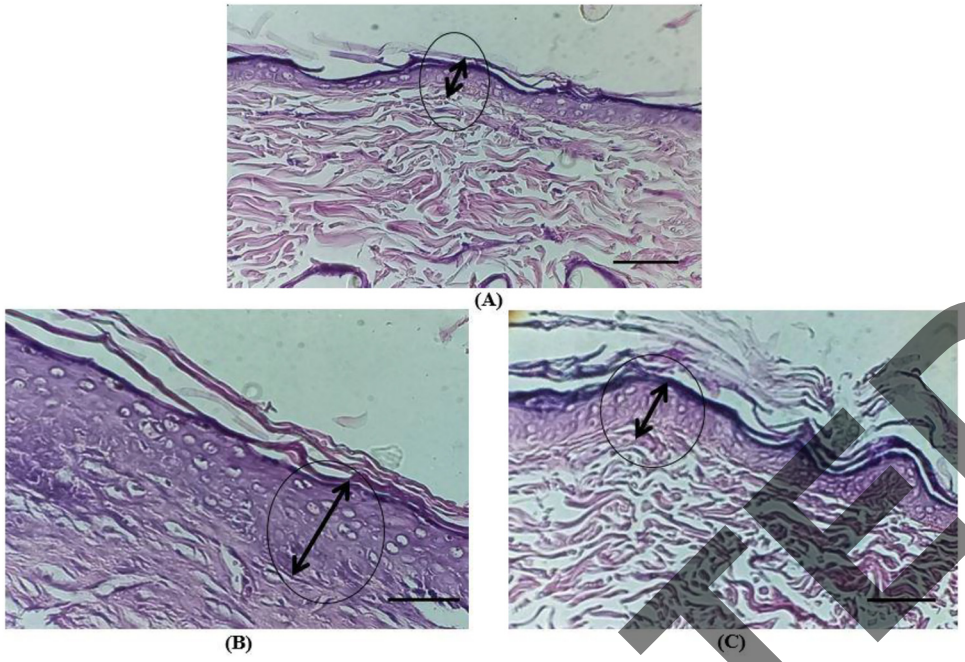

Fig. 5. Histopathology of skin at day 14 stained with H&E Epithelial layer thickness in treated groups with (A); untreated animals (B); vancomycin-SLN and (C); free vancomycin

hydrophilic drugs [37]. Altogether, SLNs' biocompatible and biodegradable composition, which is similar to that of the human body, is a major contributor to their usefulness as drug delivery systems in wound treatment.

In addition, as mentioned, our results showed that vancomycin-SLN nanoparticles released vancomycin for a long time and significantly inhibited MRSA infection in *in vitro* and *in vivo*. In this regard, in a recently published study, the authors designed a neomycin sulfate gel loaded with SLN. The synthesized nanoparticles not only showed excellent *ex vivo* permeability but also indicated more significant antibacterial activity against *S. aureus* than the pure neomycin gel [39]. In another study, *Ho* et al. developed a hydroxyethyl cellulose-based gel containing metronidazole loaded into SLN. After 24 h of treatment, nanoparticles exhibit sustained *in vitro* drug release, excellent *ex vivo* permeability, and improved *in vitro* antibacterial efficacy [40]. Additionally, studies have shown that antibacterial drugs such as LL37 peptide, mupirocin, and levofloxacin can have a greater impact when delivered via lipidic nanocarriers, speeding up the wound healing process [36].

Noteworthy, the positive zeta potential of antibiotic-loaded lipid-based nanocarriers may increase their effectiveness by enhancing their contact with the negative charge surfaces of bacterial cells, enabling antibiotic penetration into the bacterium [41]. Moreover, these carriers are also capable of penetrating bacterial cells by causing damage to their membranes, hence decreasing the quantity of ATP that is present in the cell, and ultimately leading to membrane disruption [42, 43]. Therefore, vancomycin-SLN can improve current clinical drug therapy, bring innovative products, and enhance vancomycin's application in wound infections.

It's noteworthy to mention that, vancomycin is one of the best choices for the management of MRSA infections; however, nowadays, the prevalence of vancomycin-resistant *S. aureus* (VRSA) is increasing. To this end, the performance of vancomycin-SLN nanoparticles should be evaluated against VRSA wound infections. In addition, the anti-biofilm characteristics of vancomycin-SLN should be evaluated against bacterial biofilm in the wound site because infections caused by bacterial biofilm communities are extraordinarily resistant to eradication by the host immune system and antibacterial agents.

5. CONCLUSIONS

Our results showed vancomycin-SLN as a promising modern wound therapy, especially for infected wounds. The formulated SLNs have a nanometer range, a spherical structure, high encapsulation efficiency, drug loading, and a sustained release profile. The advantages mentioned introduce SLN-based drug delivery platforms as a promising candidate for inhibition of wound infection and enhancement of wound healing procedures. Noteworthy, the cost of putting antimicrobial agents into lipid nanocarriers will be higher than the cost of using antibacterial agents directly. However, the benefits of lipid nanoparticles may outweigh the cost concerns and have commercialization potential if clinical trials could be done to rule out any toxicity issues before they are used to treat diabetic wounds. Unfortunately, the data about SLN-based nanocarriers usage for wound infection management is limited. Therefore, this kind of treatment should be considered by scientists in future studies as an applicable treatment for wound infection.

DECLARATIONS

Author contribution: HA and AJ conceived and designed the study. MM, MT, and SK performed the microbiologic workup of the samples. HA and AJ wrote the paper and performed the statistical analysis. Notably, all authors approved the definitive version of the manuscript.

Conflict of interest: The authors declare that there are no potential conflicts of interest in the present study.

Ethical approval: The study protocol and animal use were reviewed and approved by the local ethics committee on animal experimentation (Al-Mustaqbal University College (approval no. H12REA183)).

Consent for publication: Not applicable.

Availability of data and materials: Data sharing not applicable to this article as no datasets were generated during the current study.

ACKNOWLEDGMENTS

Not applicable.

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