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Evaluation of glycine as a monotherapy and in combination with neomycin in promoting wound healing in rabbits

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Abstract

Background: Skin wounds infected with *Staphylococcus aureus* pose a significant challenge in veterinary medicine due to their delay in healing and impairment of tissue repair. Therefore, this study aims to evaluate the therapeutic effect of neomycin and glycine, both individually and in combination, on accelerating the healing of infected skin wounds experimentally. **Methodology:** The study included two parts: in vitro and in vivo. In the in vitro part, the minimum inhibitory concentration (MIC) for both neomycin and glycine were determined, and a checkerboard test was performed to evaluate the interaction between them. In the animal study, twenty adult rabbits were randomly divided into four groups, five rabbits for each: a positive control group (untreated), a neomycin ointment group (5% in white petrolatum), a glycine ointment group (2.5% in white petrolatum), and a combination group treated with neomycin (2.5%) + glycine (2%) formulated in a white petrolatum base. Surgical wounds measuring 2 cm² were created on the backs of the rabbits, and then all of them were injected with a bacterial suspension containing *S. aureus*. The wound area was assessed using photographs and analyzed using ImageJ software on the days 7, 14, and 21 after treatment. **Results:** Neomycin exhibited a higher inhibitory activity against *S. aureus* than glycine. The interaction test between the two compounds also showed a fractional inhibitory concentration index (FICI) value of 2, indicating neither a synergistic nor an antagonistic interaction (no interaction). In contrast, animal results showed that all treatment groups had a significant reduction in wound area compared to the control group, with the combination group (neomycin + glycine) achieving the fastest healing rates during the observation period. **Conclusions:** The combination of neomycin and glycine improves wound healing in vivo, although no synergistic interaction between the two compounds was found in the *in vitro* study. This suggests that the positive effect in animals may be related to local physiological mechanisms related to the inflammatory response and accelerated tissue regeneration.

Keywords: Glycine, neomycin, wound healing, *Staphylococcus aureus*, combination

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Introduction

Skin wounds are very common in small animals and are often colonized or infected by bacteria, leading to delayed healing and increased treatment costs (Kožár *et al*., 2018). Traditional veterinary treatment relies on broad-spectrum antibiotics to control bacterial infections. However, their frequent and indiscriminate use has contributed to the rise of antibiotic resistance among pathogenic and commensal skin bacteria (Wendall *et al*., 2015). This problem is serious because resistant genes or resistant bacteria can be transmitted to humans through direct contact with pets (Bomba *et al*., 2017).

Staphylococcus aureus is one of the most dangerous pathogens, causing infections in traumatic and surgical wounds, as well as in the bloodstream, respiratory, urinary, reproductive systems, bones, conjunctiva, and skin. These infections often lead to purulent discharge, tissue necrosis, and sepsis (Maali *et al*., 2018; Franca *et al*., 2021). Antibiotic-resistant strains, particularly methicillin-resistant *S. aureus* (MRSA), are highly resistant to many antibiotics, increasing mortality rates and hospitalization, and representing a global clinical threat (Ibrahim, 2012; Moazen *et al*., 2022; Tasneem *et al*., 2022). Given the challenges of bacterial resistance, recent studies have focused on alternative approaches using low-toxicity natural compounds such as amino acids and sugars to modify bacterial metabolism. Certain amino acids—like lysine, glycine, serine, and valine—have been shown to enhance antibiotic effectiveness and reprogram bacterial phenotypes from resistant to susceptible (Stokes *et al*., 2019; Zhao *et al*., 2021; Hong *et al*., 2023). Glycine, serine, and threonine can also boost the bactericidal activity of antibiotics against pathogens such as *Escherichia coli* and *Edwardsiella piscicida* (Ye *et al*., 2018; Cheng *et al*., 2019; Kou *et al*., 2022). Glycine, the simplest amino acid, is a major component of structural proteins such as collagen and elastin (Wu, 2010). Although considered non-essential, endogenous production may be insufficient for metabolic needs (Wu *et al*., 2013). Therefore, this study aimed to evaluate the therapeutic potential of neomycin alone and combined with glycine in treating wounds infected with multidrug-resistant *S. aureus* in rabbits, seeking to develop novel therapeutic strategies to improve wound healing in veterinary medicine.

Materials and Methods

Ethical approval

Ethical approval for this study was obtained from the Local Committee on Animal Care and Use at the College of Veterinary Medicine, University of Baghdad (Approval No. 944, dated 22/4/2025).

Isolation and identification of *S. aureus*

The bacterium *S. aureus* was isolated from 10 samples collected from skin wounds of dogs and cats showing signs of infection at Al-Noor Veterinary Clinic, Al-Aziziya District, Wasit. Bacterial detection procedures were performed at the Microbiology Department, College of Veterinary Medicine, University of Baghdad. A single bacterial colony was cultured in Brain Heart Infusion (BHI) broth (Oxide, UK) and incubated for 24 hours to obtain a fresh overnight culture. Subsequently, the bacteria were streaked on Mannitol Salt Agar (MSA) (Hi-Media, India) for primary identification. The VITEK 2 system, an automated platform designed for the identification of microorganisms and the determination of their antimicrobial susceptibility, was used to confirm the identification of *S. aureus*.

Minimum inhibitory concentration

According to CLSI (2016), the minimum inhibitory concentration (MIC) of the antibacterials against *S. aureus* was determined using the broth macrodilution technique. After the microbe was inoculated into Muller-Hinton broth (company Oxoid, UK) for 24 hours, the inoculum was adjusted to a 0.5 McFarland tube, containing roughly 1.5x108 CFU/ml. Neomycin and glycine were then serially diluted (two folds) in tubes containing the culture, which was incubated at 37°C for a full day. The tubes' opacity was visually examined at the end of the incubation period. Turbidity indicated that the bacterial growth had not been prevented by the medium containing antimicrobial agents. Using the lowest possible antibacterial concentration, the MIC was determined.

Study the interaction between neomycin and glycine

The experiment adhered to established guidelines and used neomycin and glycine in Mueller Hinton broth at concentrations of 256, 128, 64, 32, 16, 8, 4, 2, 1, and 0.5 µg/ml. The antimicrobial solutions (180 µl) were added to a 96-well plate, followed by 20 µl of a bacterial suspension adjusted to 5×10^6 CFU/ml to achieve a final inoculum of approximately 5×10^5 CFU/ml per well, and the incubation was carried out at 35 ± 2°C for 3 hours. Afterward, 22 µl of 10% resazurin dye (BDH, UK) was added and the plates were incubated for 2 more hours. Bacterial growth was indicated by red or rose color, while the blue dye indicated no growth. The fractional inhibitory concentration index (FICI) was calculated as:

FICI = (MIC of compound A in combination / MIC of compound A alone) + (MIC of compound B in combination / MIC of compound B alone)

FICI ≤ 0.5 denotes synergy, 0.5–4 indicates no interaction, and FICI > 4 signifies antagonism (Maali *et al*., 2018).

Surgical wound establishment

In this experiment, twenty rabbits were anesthetized with a mixture of xylazine (5 mg/kg) and ketamine (35 mg/kg) administered intramuscularly (Bayer, Germany), a dosing protocol commonly recommended for surgical anesthesia in rabbits (Hassan *et al*., 2024). The dorsal area on both sides was prepared by shaving the hair and disinfecting the skin with chlorhexidine–alcohol, then 2 cm^2 full-thickness excision wounds were created using a sterile blade (Sinus Medical, Iraq). Each wound was injected with 0.2 mL of a 10^6 CFU/ml *S. aureus* suspension to induce infection. Animals were divided into four equal groups: infected positive control (no treatment); neomycin ointment in white petrolatum (5%) twice daily for two weeks; glycine in white petrolatum (2%) at the same dose; and a combination therapy of neomycin (2.5%) + glycine (2%) prepared in a white-petrolatum base.

Assessment of the wound closure rate

Photographs were taken for all wounds, using a ruler next to the wound edge on days 7, 14, and 21 of initial wound treatment. The images were processed using a morphometric measurement program (Image J®, National Institutes of Health, Bethesda, Maryland, USA) to assess wound areas during each period for both groups.

Results

Identification of *S. aureus*

Based on the data obtained from the VITEK 2 system, the tested bacterium was Staphylococcus aureus, which was multi-drug resistant (Figure 1).

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Patient Name: Patient ID:
Location: Physician:
030412063363234
Otganism Quantity:
Selected Organisn: Staphylococcus aureus
Source:
Comments: 98% probability Staphylococcus aures
Bionumber 030412063363230

Susceptibility Information	**Analysis Time:** 8.25 hours			**Status:** Final	
Antimicrobial	**MIC**	**Interpretation**	**Antimicrobial**	**MIC**	**Interpretation**
Cefolixin Screen	POS	+	Clindamycin	<= 0.25	S
Benzylpenicillin	>= 0.5	R	Linezolid	2	S
Amoxicillin		R	Teicoplanin	-	R
Oxacillin	>= 4	R	Vancomycin	TRM	S
Amikacin	-	R	Tetracyeline	10	S
Gentamicin	>= 16	R	Tigecycline	<= 0.12	S
Tobramymin	>= 8	R	Fosfomycenin	-	S
Levofloxacin	NEG	R	Nitrofurantoin	<= 16	S
Inducible Clindamycin	-	R	Fusidic Acid	<= 0.5	S
Erythromycin	>= 8	R	Rifampicin	-	S
Erythromycin	> 8	R	Trimethoprim/ Sulfamethoxazole	<= 10	S
AES Findings	Consistent				

Figure 1: The VITEK 2 system report shows that the tested bacterium is multi-drug-resistant *S. aureus*

Determination of MIC of the neomycin and glycine

The lowest concentrations of the neomycin and glycine that inhibited *S. aureus* growth were 64 µg/ml and 128 µg/ml, respectively (Figure 2). This seems very high compared with breakpoints fixed by CLSI (2020) (Lescat *et al*., 2019), that means this bacterium is resistant to these antibacterials.

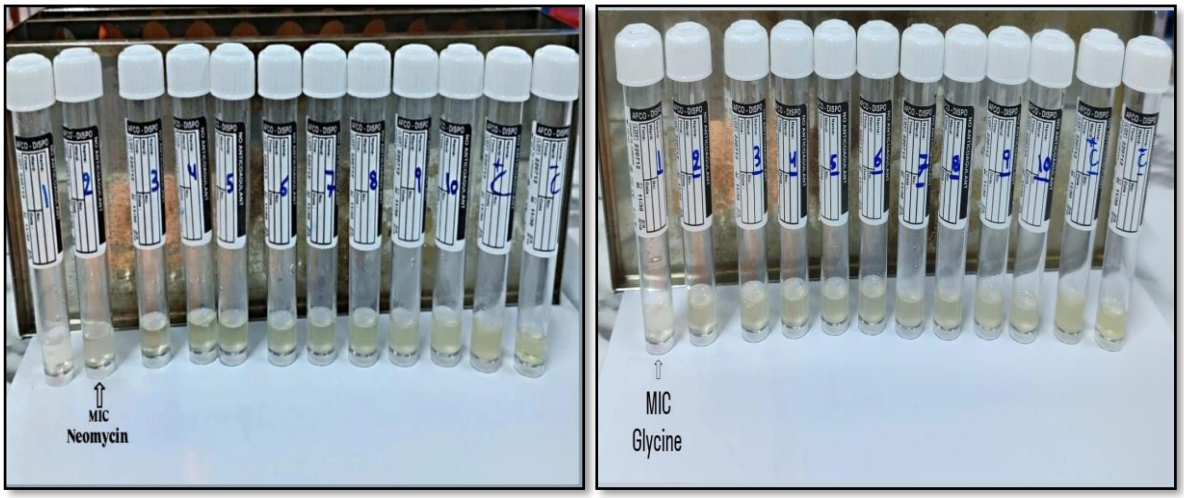

Figure 2: Determination of MIC for neomycin (64 µg/ml) and glycine (128 µg/ml) against *S. aureus*

The MIC of the combination between neomycin and glycine was 64 µg/ml and 128 µg/ml, respectively, where the blue color refers to the lack of any bacterial growth, while the red color refers to the growth occurrence. The FICI value of 2 indicated to no interaction between neomycin and glycine when used in combination (Figure 3).

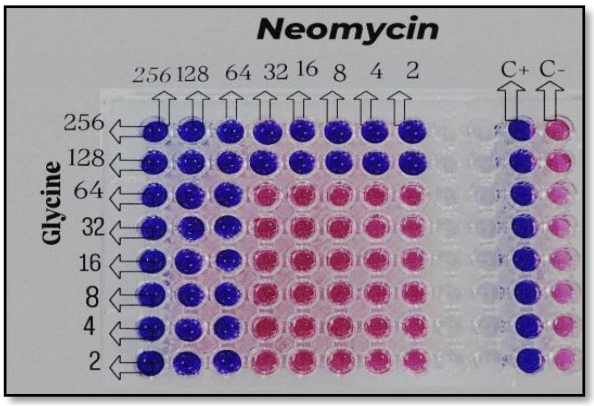

Figure 3: Checkerboard showing the MIC of the combination between neomycin and glycine is 46 µg/ml and 128 µg/ml, respectively, blue color no bacterial growth, while the red color indicates growth

Surgical wound measurement

The wound healing outcomes showed significant differences ($p < 0.05$) among the experimental groups throughout the observation period (days 7, 14, and 21). The combination therapy group exhibited the smallest wound area, reflecting the fastest healing rate, with significantly greater improvement compared to both the neomycin group and the glycine group, as well as the untreated control group. The neomycin-treated group demonstrated more rapid healing than the glycine-treated group across all time points. In contrast, the control group showed markedly

delayed wound closure during the same periods. By day 21, no significant differences ($p < 0.05$) were detected among the groups, as nearly all wounds contracted to a small residual area (<13 mm²). This confirms that while untreated wounds eventually healed, the healing process was considerably slower compared to all treatment groups (Table 1).

Table 1: Wound area in mm² on the days 7, 14 and 21 in rabbits infected with resistant *S. aureus* and treated topically with neomycin, glycine and combination

Group	Periods			
	Initial Wound	7 Days	14 Days	21 Days
Positive Control	200±0.00 Aa	196.00±2.98 Aa	37.00±2.77 Ba	13.00±2.46 Ca
Neomycin	200±0.00 Aa	118.00±2.73 Bc	24.00±1.51 Cb	12.00±1.73 Da
Glycine	200±0.00 Aa	136.00±2.82 Bb	32.00±2.77 Ca	13.00±1.41 Da
Combination	200±0.00 Aa	82.00±3.36 Bd	21.00±2.28 Cb	9.00±1.14 Da
LSD	6.92			

Means with a different small letter in the same column are significantly different (P<0.05) Means with a different capital letter in the same row are significantly different (P<0.05). Values represent Means ± SE, N= 5

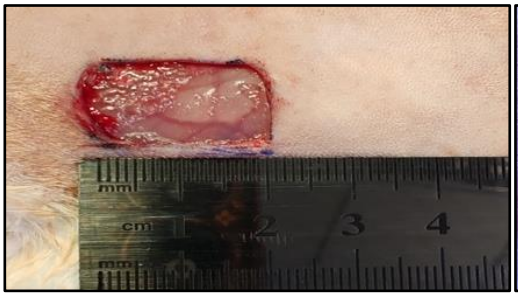

Figure 4: A wound sized 2 cm² on the back of a rabbit

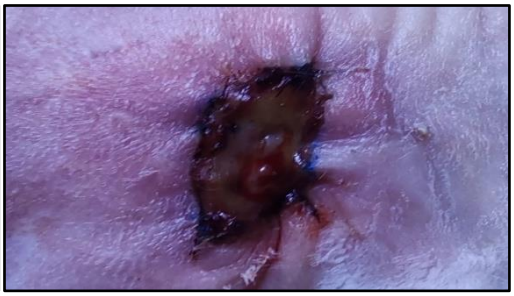

Figure 5: The wound after a bacterial infection occurred

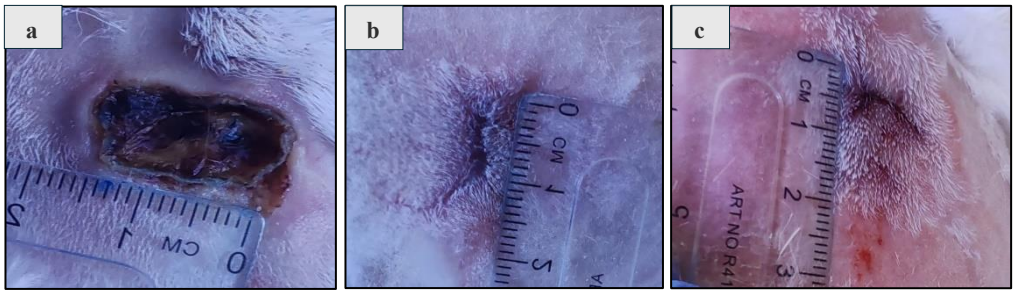

Figure 6: A wound in a positive control animal without treatment on: a- day 7, b- day 14, and c- day 21

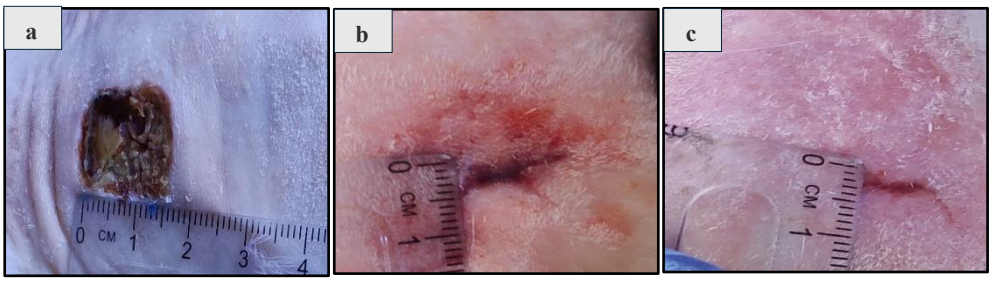

Figure 7: A wound in a rabbit treated topically twice daily with neomycin on: a- day 7, b- day 14, and c- day 21

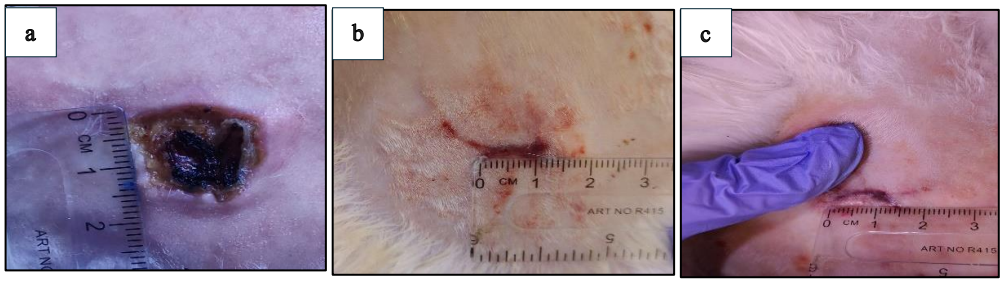

Figure 8: A wound in a rabbit treated topically twice daily with glycine on: a- day 7, b- day 14, and c- day 21

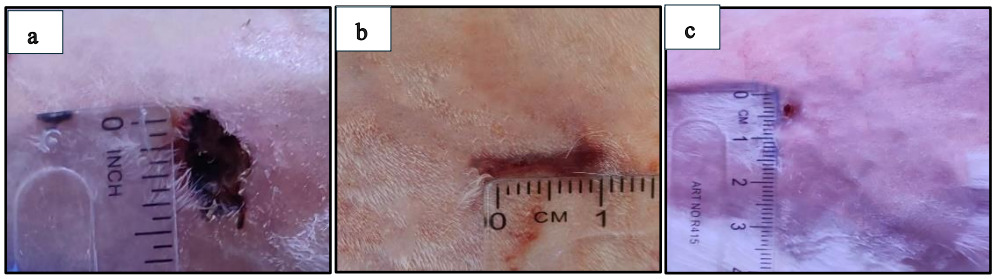

Figure 9: A wound in a rabbit treated topically twice daily with a combination (neomycin +glycine) on: a- day 7, b- day 14, and c- day 21

Discussion

The FICI value of 2 indicated no interaction between neomycin and glycine when used in combination. A FICI value between 0.5 and 4 denotes no interaction, values below 0.5 indicate synergy, and values above 4 indicate antagonism. The lack of interaction implies that the combined antimicrobial effect of neomycin and glycine is not greater than the sum of their individual effects. Therefore, the combination acts independently, showing neither synergy nor antagonism. These findings have clinical relevance for the potential use of neomycin and glycine combination therapy against *S. aureus*.

The results presented in Table 1 show that the wound area decreased significantly within each group between days 7 and 21 ($P < 0.05$). This pattern is consistent with the normal transition from the inflammatory phase to the proliferative and remodeling phases, characterized by granulation tissue formation, collagen deposition, and myofibroblast-mediated re-epithelialization and contraction (Gurtner *et al*., 2008; Wilkinson & Hardman, 2020). Between groups, on days 7 and 14, the combination treatment group exhibited the smallest wound area, followed by neomycin and then glycine, whereas the control group showed the largest wound area. This indicates that reducing the local bacterial burden while supporting cellular repair processes promotes faster wound contraction and re-epithelialization (Mingeot-Leclercq & Tulkens, 1999; Razak *et al*., 2017). The superiority of the combination therapy can be attributed to two complementary mechanisms. Neomycin inhibits the growth of *S. aureus* and suppresses bacterial toxins, thereby reducing prolonged inflammation that delays epithelial proliferation. Meanwhile, glycine enhances extracellular matrix formation through its structural role in collagen synthesis and reduces pro-inflammatory cytokines, creating a favorable environment for tissue contraction and

regeneration (Mingeot-Leclercq & Tulkens, 1999; Razak *et al*., 2017; Zhong *et al*., 2020). Moreover, the presence of glycine may improve bacterial membrane permeability and potentiate the efficacy of aminoglycosides at low topical doses—a synergistic effect previously reported against resistant staphylococci. By day 21, the wound areas among all groups converged, showing no significant differences ($P > 0.05$).

Conclusions

Neomycin treatment demonstrated marked efficacy in reducing *S. aureus* bacterial load, while glycine contributed significantly to enhancing tissue regeneration and wound closure. The combination of neomycin and glycine resulted in faster wound contraction and improved recovery compared to either treatment alone or the untreated control. Although the in vitro interaction analysis (FICI = 2) indicated no interaction existed between the two compounds, their combined in vivo application produced superior healing outcomes. This effect likely reflects the complementary actions of neomycin's antimicrobial activity and glycine's anti-inflammatory and collagen-stimulating properties. These findings suggest that glycine may serve as a valuable adjunct to conventional antibiotics in promoting wound healing, warranting further investigation into its clinical potential.

Acknowledgments

N/A

Authors' Contributions

The authors independently performed the study design, animal experimentation, laboratory procedures, statistical analysis, data interpretation, and preparation of the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

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تقييم الجلايسين كعلاج وحيد وبالاشتراك مع النيوميسين في تعزيز التئام الجروح في الأرانب

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المستخلص

الخلفية: تُعدّ الجروح الجلدية المصابة بجراثيم *Staphylococcus aureus* من التحديات المهمة في الطب البيطري، لما تسببه من تأخير في عملية الالتئام وضعف في ترميم الأنسجة. لذلك، يهدف هذا البحث إلى تقييم التأثير العلاجي لكلٍّ من النيومايسين والجلايسين، بشكل منفرد وبالاشتراك، في تسريع التئام الجروح الجلدية المصابة تجريبيًا. **المنهجية:** تضمنت الدراسة جزأين: جزءًا مختبريًا (*in vitro*) وجزءًا حيوانيًا (*in vivo*). في الجزء المختبري، تم تحديد الحد الأدنى للتركيز المثبط للنمو (MIC) لكل من النيومايسين والجلايسين، كما أُجري اختبار Checkerboard لتقييم التفاعل بينهما. أما في الجزء الحيواني، فقد استُخدمت عشرون أرنبًا بالغة، وُزعت عشوائيًا إلى أربع مجموعات (خمسة أرانب لكل مجموعة): مجموعة السيطرة المصابة (بدون علاج)، مجموعة عُولجت بمرهم النيومايسين (٥٪ في الفازلين الأبيض)، مجموعة عُولجت بمرهم الجلايسين (٢,٥٪ في الفازلين الأبيض)، ومجموعة عُولجت بالمزيج (نيومايسين ٢,٥٪ + جلايسين ٢٪) في قاعدة فازلين أبيض. أُحدثت جروح جراحية بمساحة ٢ سم^2 على ظهر كل أرنب، ثم حُقنت جميع الجروح بمعلق بكتيري يحتوي على *S. aureus*. جرى قياس مساحة الجرح بالتصوير وتحليلها باستخدام برنامج ImageJ في الأيام ٧ و١٤ و٢١ بعد بدء العلاج. **النتائج:** ظهرت النتائج المختبرية أن النيومايسين يمتلك فعالية تثبيطيه أعلى ضد الجرثومة مقارنةً بالجلايسين. كما بيّن اختبار التفاعل أن قيمة مؤشر التركيز المثبط الكسري (FICI) بلغت ٢، مما يدل على عدم وجود تآزر أو تعارض بين المركبين. أما في التجربة الحيوانية، فقد لوحظ انخفاض معنوي في مساحة الجروح في جميع المجموعات المعالجة مقارنةً بمجموعة السيطرة، وكانت أسرع نسبة شفاء في مجموعة العلاج المزدوج (نيومايسين + جلايسين). **الاستنتاجات:** أن الجمع بين النيومايسين والجلايسين ضمن قاعدة الفازلين الأبيض يحسّن من عملية التئام الجروح الجلدية المصابة في الجسم الحي، على الرغم من غياب التآزر بينهما في الاختبارات المختبرية. ويُحتمل أن يكون هذا التحسن ناتجًا عن تأثيرات فسيولوجية محلية تتعلق بالاستجابة الالتهابية وتسريع تجديد الأنسجة.

الكلمات المفتاحية: جلايسين، نيوميسين، التئام الجروح، *Staphylococcus aureus*، مركب