

Clinical and microbiological outcomes of topical mupirocin-corticosteroid treatment for infected wounds in Wistar rats

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ABSTRACT

Background Wound infection poses major challenges in clinical practice and can be associated with high morbidity or mortality in some instances.

Hypothesis/aim This study aimed to determine the effects of different corticosteroid-mupirocin cream combinations on wound infection, wound re-epithelialisation and contraction in male Wistar rats.

Methods A randomised controlled study was carried out to compare the effects of different corticosteroid-mupirocin cream combinations on the healing of infected wounds in male Wistar rats. Forty-two male Wistar rats weighing 150–200g were randomly divided into seven groups, with six rats each. Group 1 (negative control) had no wounds. Wounds were made on the lower backs of the rats and infected with bacteria. During each dressing change, the wounds were assessed for signs of infection via the Southampton wound grading, and the percentage re-epithelialisation was also calculated. On day 24 of the experiment, wound swabs were taken and sent for microscopy and culture. The data were analysed using analysis of variance, and $p < 0.05$ was taken as the accepted level of significant difference.

Results/findings There was no significant difference in the contraction rate across the groups ($p = 0.502$), however, the 2:1 mupirocin-betamethasone group had the highest degree of wound contraction (77.9%) and a lower infection rate ($p = 0.0001$).

Conclusions This study revealed that the combination of mupirocin and betamethasone is more efficacious in promoting wound epithelialisation and wound bacteria.

Implications for clinical practice The use of mupirocin-betamethasone combination is effective in the healing of infected wounds.

Keywords mupirocin, topical steroids, wound epithelialisation, wound infection

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KEY MESSAGES

- Wound infection results from the invasion of wounds by microorganisms, which can lead to the chronicity of the wound, thus preventing its timely closure. The use of combined topical corticosteroids and antibiotics has been shown to facilitate healing in such wounds. A combination of mupirocin, a topical antibiotic which also has wound healing properties, with corticosteroid was used to manage infected wounds in this study.
- The aim of the study was to determine the effects of different corticosteroid-mupirocin cream combinations on the percentage of wound re-epithelialisation and reduction in wound size (wound contraction) in male Wistar rats.

- The 2:1 mupirocin-betamethasone group had the highest degree of wound contraction (77.9%) and a lower infection rate. Wound epithelialisation was significantly lower in the mupirocin-triamcinolone groups

INTRODUCTION

A wound is a loss of integrity of the tissue with associated dysfunction. The pathological processes that lead to wounding either start from the exterior or from the internal organ.¹ The etiology can be accidental or intentional, and it can also result from an ongoing disease process that disrupts the local environment. The common causes of wounds include trauma, surgery, infection, extremes of temperature and chemicals.¹ The time to healing of wounds also varies depending on

several factors. A completely healed wound can, therefore, be defined as one that has been restored to normal architecture and function within a reasonable time frame.¹

Wound infection results from the invasion of wounds by microorganisms, which can lead to the chronicity of the wound, thus preventing its timely closure. Wound infection poses major challenges in clinical practice and can be associated with early and late sequelae, with high morbidity or mortality in some instances. The immense social and economic impact of chronic wounds worldwide is a consequence of their high rate of occurrence in general and their increasing frequency, especially in the aging population.² According to Fila et al,³ for every 100,000 people aged between 45 and 65 years, 120 have chronic wounds. In addition, 800 of every 100,000 people who are older than 75 years have chronic wounds.³

The processes involved in the initiation, maintenance, and completion of re-epithelialisation are essential for successful wound closure, and the inability to re-epithelialise is a clear indicator of chronic nonhealing wounds.⁴ Re-epithelialisation involves covering a skin wound with new epithelium. It progresses from the surrounding wound margins toward the center, creating a continuum in the regeneration of a differentiated epidermis over the dermoepidermal junction (DEJ).⁵ The re-epithelialisation of human partial-thickness wounds has also been documented to occur primarily from stem/progenitor cells in the eccrine sweat glands and pilosebaceous units.

Furthermore, hypergranulation tissue, on the other hand, is an aberrant healing process that is characterised by the overgrowth of granulation tissue beyond the wound surface. It impairs the migration of peripheral keratinocytes and re-epithelialisation.⁶ This further increases the time at which wounds are exposed to microbial colonisation and infection. Topical corticosteroids have been found to dampen excessive inflammation and suppress hypergranulation tissue formation.⁶ They are also known to preserve epithelial appendages, which are responsible for wound re-epithelialisation.

The effectiveness of corticosteroids has been shown to be enhanced when combined with topical antibiotics, especially in the treatment of granulation tissue and acceleration of re-epithelialisation.⁶⁻⁸ The topical antibiotic combined with corticosteroids used in this study is mupirocin, a short-chain fatty acid and the primary fermentation metabolite of *Pseudomonas fluorescens*, which displays antimicrobial effect by inhibiting isoleucyl-transfer RNA.⁹

In addition to the antibacterial property, it has also been shown that mupirocin stimulates the proliferation of human keratinocytes and the release of several growth factors which promote wound healing.¹⁰ Furthermore, combining the topical corticosteroids and antibiotics may possibly help reduce the risks of bacterial resistance, allergic reactions and potential delay in wound healing that has been associated with sole topical antibiotics application on wounds.¹¹⁻¹³

Two different corticosteroids (triamcinolone and betamethasone) with varying combination ratios with mupirocin were used in this study.

This study aimed to determine the effects of different corticosteroid-mupirocin cream combinations on the

percentage of wound re-epithelialisation and reduction in wound size (wound contraction) in male Wistar rats. They also considered their possible ability to reduce wound infection. At the time of the literature search, no previous research had investigated the effect of mupirocin-corticosteroid combinations on wound infection and re-epithelialisation.

METHODS

Drugs

The topical antibiotics and corticosteroids employed in this experiment included 15g of 2% mupirocin ointment (Mupiderm®, Yash Medicare, India), 25g of 0.1% triamcinolone acetonide cream (Fidson Healthcare Plc, Nigeria) and 25g of 0.1% betamethasone valerate cream (Fidson Healthcare Plc, Nigeria).

Animals

The male Wistar rats used in the study were placed in plastic cages at a temperature between 25°C±2.5°C. They had standard laboratory diet and water *ad libitum*. They were also acclimatised to the new environment over a period of 14 days before starting the experiment. Ethical approval was obtained from the Faculty of Basic Medical Sciences, LAUTECH Ethical Review Board, and the identification code on the ethical clearance certificate was ERC/FBMS/054/2024. All procedures were carried out in accordance with the protocols of the faculty and within the provisions of the European Council Directive (EU2010/63) for animal care and use.

Experimental design

In all 42 male Wistar rats whose weights ranged between 150 and 200grams were used in the study. The rats were randomly assigned to seven groups of six rats each. The animals were separated into seven groups as shown below:

Group 1 – Rats with no wounds during the experiment

Group 2 – The rats whose wounds were dressed with only Vaseline gauze

Group 3 – Rats who had a wound dressing with mupirocin ointment only.

Group 4 – Rats who were dressed with a combination of mupirocin and betamethasone at a ratio of 1:1.

Group 5 – Rats who were dressed with a combination of mupirocin and triamcinolone at a ratio of 1:1.

Group 6 – Rats who were dressed with a combination of mupirocin and betamethasone at a ratio of 2:1.

Group 7 – Rats who were dressed with a combination of mupirocin and triamcinolone at a ratio of 2:1.

Study protocol

Wound creation was performed on Day 0 for Groups 2–7 after anaesthetising the rats with intraperitoneal ketamine at a dose of 50mg/kg. A 2x2cm area was marked on the right lower back of each rat via a transparent film template. A razor blade was used to shave the hair, and the skin was prepared with 5% povidone iodine solution. The skin and subcutaneous tissue within the marked area were excised with a scalpel, but the panniculus carnosus muscle was spared. Hemostasis was ensured by using a pressure pack, and the wounds were dressed with Vaseline gauze or Medipore adhesive dressing and further fastened with zinc oxide plaster.

The rats were monitored until they regained consciousness after the procedure. The dressings were left in place for three days to allow the wound to granulate. On the third day postoperative, the wounds were inoculated with a prepared bacterial solution. Dressing of the wounds under sterile conditions and regular disinfection of the environment were helpful in preventing cross contamination. At each dressing change, the wounds were cleaned with normal saline after the old dressing was removed and before a new dressing was placed. Additionally, the wounds were assessed for signs of infection every week via the Southampton wound grading system.

The wound diameter and area were also measured every week via a sterile meter and the imitoMeasure App, respectively.¹⁴ The percentage of re-epithelialisation was assessed on Days 7, 10, 17 and 24. On Day 24, wound swabs were taken and sent for microscopy and culture. On the last day of the experiment, the rats were sacrificed via cervical dislocation and buried.

Wound dressing protocol

The dressing protocol for each experimental group post wound infection is highlighted below:

Group 2 – (Positive control) Vaseline gauze, Medipore adhesive dressing, zinc oxide plaster.

Group 3 – (Mupirocin ointment only) Mupirocin ointment (0.3ml), Vaseline gauze, Medipore adhesive dressing, zinc oxide plaster.

Group 4 – (Mupirocin-betamethasone combination 1:1) 0.15ml of mupirocin ointment/0.15ml of betamethasone cream, Vaseline gauze, Medipore adhesive dressing, zinc oxide plaster

Group 5 – (Mupirocin-triamcinolone combination 1:1) 0.15ml of mupirocin ointment/0.15ml of triamcinolone cream, Vaseline gauze, Medipore adhesive dressing, zinc oxide plaster.

Group 6 – (Mupirocin-betamethasone combination 2:1) 0.2ml of mupirocin ointment/0.1ml of betamethasone cream, Vaseline gauze, Medipore adhesive dressing, zinc oxide plaster.

Group 7 – (Mupirocin-Triamcinolone combination 2:1), 0.2ml of mupirocin ointment/0.1ml of triamcinolone cream, Vaseline gauze, Medipore adhesive dressing, zinc oxide plaster.

The volume of cream applied to the wounds was measured by removing the plunger from the 2ml syringe and pressing the cream from the tube directly into the syringe until it reached the 2ml mark. The plunger was then replaced gently, avoiding spilling cream from the tip of the syringe. The appropriate volume was dispensed through the tip of the syringe according to the group protocol.

Preparation and induction of the bacteria colony forming unit

Microorganisms isolated from clinical specimens were obtained from the microbial stock of the Medical Microbiology laboratory, LAUTECH Teaching Hospital. The stored bacteria were subcultured on MacConkey Agar and blood agar media and incubated at 37°C for 24 hours. The incubated culture media were checked for bacteria growth and the isolates were identified as *Staphylococcus aureus* and *Pseudomonas aeruginosa* through colonial morphology, Gram staining and biochemical tests. A colony of each organism was picked from the agar plate via a 3mm wire loop and diluted in 5ml of normal saline in different universal bottles. Each wound

was therefore inoculated with 0.1ml of *Staphylococcus aureus* solution and 0.1ml of *Pseudomonas aeruginosa* solution respectively.³

OUTCOME MEASURES

Clinical measures

The primary outcomes measured included the following:

A) The diameter and area of the wounds on days 7, 10, 17 and 24 were measured via a sterile meter rule and the smartphone-based imitoMeasure App (imito; imito AG, Zurich, Switzerland).

The take measurements using the App, photographs of the wounds were taken with a Redmi smartphone via the imitoMeasure App.¹⁴ The camera of the smartphone was positioned parallel to the wound. The calibration marker (sticker) was positioned next to and in the same plane as the wound, and a photograph was taken after recognition of the marker by the application. The operator's finger was used to encircle the borders of the wound, and the imitoMeasure App reported the results of the area, width, length and circumference, which were recorded via screenshots and saved.

B) Percent re-epithelialisation. The percent re-epithelialisation (REP) was calculated for weeks 1, 2, 3 and 4 by measuring the length of the neoepidermis on the wound (using a sterile steel meter rule) and dividing it by the diameter of the wound multiplied by 100.

C) The percent wound contraction was calculated by subtracting the current wound area (A₂) from the previous area (A₁), dividing the result by the previous wound area and multiplying by 100. $(A_1 - A_2 / A_1 \times 100)$.^{15,16}

D) Wound infection. Wound infection was assessed via both clinical and microbiological methods. For the clinical assessment, the Southampton wound scoring system was used.¹⁷ as stated below:

Grade 0 – Normal healing

Grade I – Normal healing with mild erythema

Grade II – Erythema with other signs of inflammation

Grade III – Clear or hemoserous discharge from the wound

Grade IV – Purulent discharge from the wound

Grade V – Deep wound infection with or without dehiscence

Microbiological analysis

The swab samples taken from the wound site were subjected to microscopy and culture to detect the presence of microorganisms, identify the microorganism type and to determine the microbial growth density.

DATA ANALYSIS

Data analysis was performed via SPSS for Windows (SPSS Inc, Chicago, US) software version 23. Continuous variables were compared across groups via one-way analysis of variance (ANOVA) and Tukey's post hoc test. The level of significance was set at a p value of <0.05.

RESULTS

The results revealed a significant increase in the rate of epithelialisation for all groups from the first week to the third week. After the third week, there was a gradual reduction in the percentage of epithelialisation in the mupirocin-triamcinolone groups (both 1:1 and 2:1). The mupirocin-betamethasone 2:1 was observed to have the highest percentage epithelialisation. At the end of the experiment, all the other groups, including the control, presented maximal percentage epithelialisation (Figure 4.1a).

Wound contraction was significantly maximal at the second week of the experiment for all the groups. At the third week, the mupirocin-triamcinolone 2:1 group had the maximum contraction, followed by the mupirocin-triamcinolone 1:1 group. At the end of the experiment, the mupirocin-betamethasone 2:1 group presented the highest wound contraction rate (Figure 4.1b).

Compared with the other groups, the mupirocin-betamethasone groups (1:1 and 2:1) presented the lowest bacterial density. This means that the combinations could be more efficacious against the bacteria. This difference was, however, not statistically significant (Table 4.2).

In the first week of the experiment, the control, mupirocin-betamethasone 1:1 and mupirocin-triamcinolone 1:1 groups presented the highest Southampton scores, which indicated a relatively high rate of infection, but this difference was not statistically significant. At week 2, the control still had the highest score, followed by the mupirocin-triamcinolone 1:1 and mupirocin-triamcinolone 2:1 groups, which was statistically significant, with a p value of <0.0001. At the third week, the mupirocin-triamcinolone 2:1 group had the highest Southampton score, followed by the control and mupirocin-triamcinolone 1:1 groups. This difference was also statistically significant, with a p value of 0.015. By the last week of the experiment, only the mupirocin-triamcinolone groups (1:1 and 2:1) had high Southampton scores. This difference was also statistically significant, with a p value of <0.0001 (Table 4.2).

DISCUSSION

The results of this study showed a significant increase in the rate of epithelialisation for all the test groups when compared

with the control at the second week of the experiment. At the third week of the experiment the mupirocin-betamethasone 2:1 combination had the highest percentage of epithelialisation. This combination showed a possibly good efficacy in the stimulation of epithelial tissue migration for the healing of the wounds. The effect of the control group and the different combinations on wound epithelialisation was noticed to balance up at the 4th week of the experiment except in the triamcinolone groups which had significantly reduced epithelialisation.

The possible reason why the control group eventually balanced up with the other three test groups could be adduced to the intrinsic ability of the rat to clear wound microbes and heal its wound over time without topical dressing application.¹⁸ The rat exhibits a robust inflammatory response which helps to clear the wound of debris and bacteria, it also has a high capacity for cell proliferation which enables it to produce new epithelial cells to cover the wound¹⁸ The mupirocin-triamcinolone combination groups were possibly associated with more occlusion and excessive moisturising of the wound with attendant worsening of infection.

As a potent corticosteroid, triamcinolone can suppress the immune system, potentially impairing the natural defense against infection. On the other hand, the immunosuppressive effect of betamethasone, a more potent corticosteroid, is balanced by its anti-inflammatory properties which can help reduce inflammation and promote a conducive environment for wound healing.¹⁹

Betamethasone's common salts, like dipropionate and valerate, enhance its anti-inflammatory profile up to 450 times.²⁰ In addition, betamethasone valerate has a more favorable therapeutic index when compared with triamcinolone acetonide.²¹ The less favorable therapeutic index of triamcinolone most likely may have accounted for the death of two rats in the mupirocin-triamcinolone 2:1 group, one at the beginning of week 3 and one in week 4 of the experiment.

Concerning the effect of the topical applications on wound contraction, there were no significant differences at the end of the experiment but the mupirocin-betamethasone 2:1

Table 1. Effects of combinations of corticosteroids and mupirocin on wound infection parameters

Groups	Wound infection parameters (Mean±SEM)				
	Bacteria density	Southampton score (week 1)	Southampton score (week 2)	Southampton score (week 3)	Southampton score (week 4)
Group 2 (Control)	2±0.258	1±0.683	3.167±0.307	0.833±0.307	0±0
Group 3 (Mup)	1.667±0.211	0±0	0.333±0.211*	0±0	0±0
Group 4 (Mup1/Beta1)	1.333±0.211	1±0.632	0.333±0.211*	0±0	0±0
Group 5 (Mup1/Triam1)	1.833±0.307	1±0.632	1±0.447*	0.833±0.543	1.333±0.615*
Group 6 (Mup2/Beta1)	1.333±0.211	0±0	0.667±0.211*	0±0	0±0
Group 7 (Mup2/Triam1)	1.5±0.289	0.833±0.654	1±0.258*	1±0	1.5±0.5*
ANOVA F test	1.251	0.882	13.719	3.433	5.431
Significance	0.313	0.505	0*	0.015*	0.001*





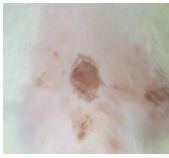







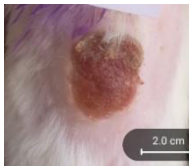


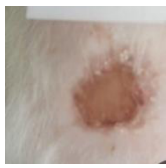








	Week 1	Week 2	Week 3	Week 4
Group 2				
Group 3				
Group 4				
Group 5				
Group 6				
Group 7				

Figure 1. Progression of wound healing in each group

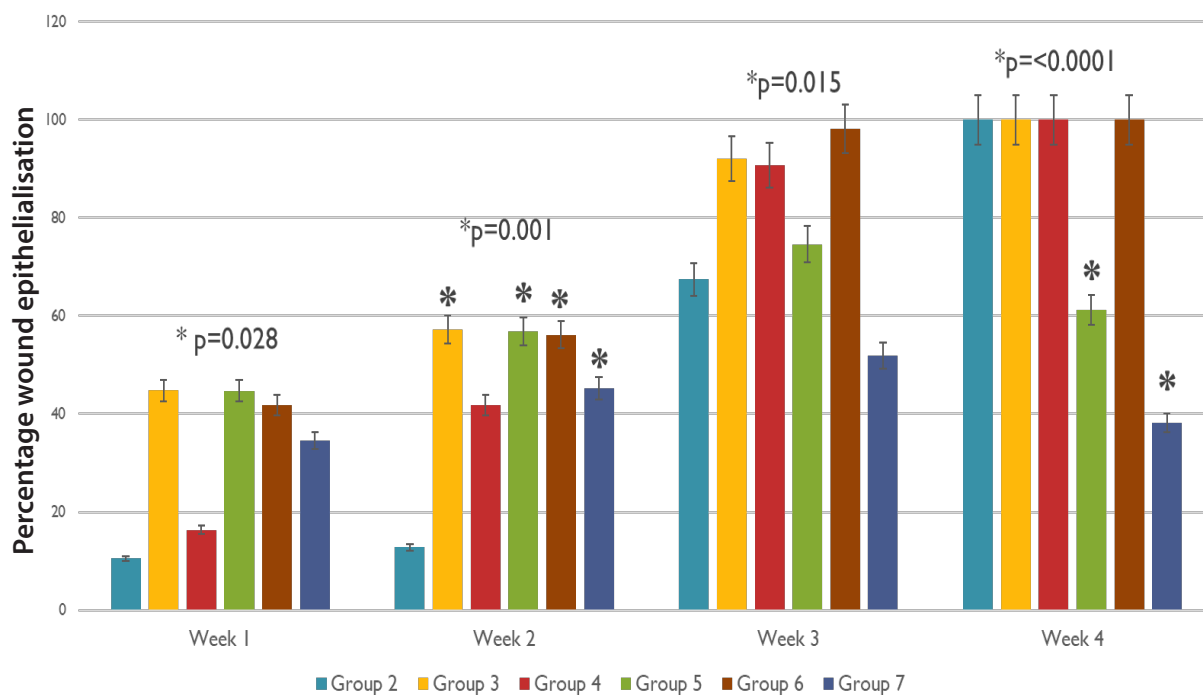


Figure 2. The effects of different cream combinations on percentage re-epithelialisation. Each bar represents the mean \pm SEM; * p = a significant difference, $p < 0.05$ vs the control. Note the number of rats per group was six in weeks 1 and 2, but at Weeks 3 and 4, the number of rats in Group 7 was 5 and 4, respectively.

combination was, however, found to be associated with the highest wound contraction rate. Comparatively, in the study by Bosanquet et al²², where antibiotic-corticosteroid cream combination was used for the treatment of chronic wounds, prior to treatment, all the wounds presented with signs of delayed healing, with 17 of them enlarging in size. However, following treatment, there was significant overall contraction of the wounds, which suggests that the antibiotic-corticosteroid combination may be helpful in reducing the wound size.²² In addition, a faster rate of healing and contraction has been reported in rat wounds dressed with mupirocin topical spray over in those dressed with only normal saline solution,²³ though rats are known to have faster wound contraction rate which may be due to their carnosus muscle.^{1,24,25}

Our study also revealed that the maximum reduction in the bacteria density occurred in groups 4 and 6, which involved combinations of mupirocin and betamethasone at ratios of 1:1 and 2:1, respectively. The control group, however, had the least suppression effect on the bacteria density. Furthermore, at the beginning of our study 34 wounds were inoculated with both *Pseudomonas aeruginosa* and *Staphylococcus aureus* and the infection was confirmed using Southampton's wound infection scoring system. At the end of the experiment however, *Pseudomonas aeruginosa* was only cultured from five wounds (two in the mupirocin only group, none in mupirocin-betamethasone 1:1 group, one in mupirocin-triamcinolone 1:1 group, one in mupirocin-betamethasone 2:1 group and one in mupirocin-triamcinolone 2:1 group), both *Pseudomonas aeruginosa* and *Staphylococcus aureus* were cultured on one wound (mupirocin-triamcinolone 1:1 group) and *Staphylococcus aureus* was cultured on the remaining 28 wounds (with the highest number in the control group).

Both *Staphylococcus aureus* and *Pseudomonas aeruginosa* were considered in this study in order to simulate mixed bacterial infection that is commonly seen in chronic wounds. *Staphylococcus aureus* was the choice of the Gram-positive

organisms because it is the most commonly cultured bacteria on chronic wounds,²⁶ while *Pseudomonas aeruginosa* was also chosen because it is the most commonly cultured Gram-negative organism on chronic wounds.^{26,27}

The topical antibiotics used in our study were chosen because mupirocin is known to have high anti-staphylococcal activity and is effective in eradicating *Staphylococcus aureus*.²⁸ It is generally not effective against most Gram negative bacteria, due to its inability to penetrate their outer membrane. However, Savage et al²⁹ noticed improved penetration of mupirocin through the wall of Gram negative bacteria when it is combined with cationic steroid antibiotics.²⁹

At the time of the literature search no study has considered the effect of mupirocin-corticosteroid combination on gram negative organism thus using *Pseudomonas aeruginosa*, a gram-negative organism in our study helped to know if there is any improvement in the penetration of the bacteria's outer cell membrane by the different combinations. Although it was observed in our study that mupirocin-betamethasone 1:1 combination was the most effective against *Pseudomonas aeruginosa*, further research with larger sample size will be needed to establish this.

Limitations

The use of an acute wound model, which differs significantly from chronic wound conditions used for comparison in this study is a major limitation.

CONCLUSION

The results of this study revealed that there was no significant difference in the wound contraction rate across the groups; however, the 2:1 combination of mupirocin and betamethasone had the highest wound contraction rate and percentage of epithelialisation. Wound epithelialisation was significantly lower and the infection rate was greater in the triamcinolone groups than in the other treatment groups and the control.

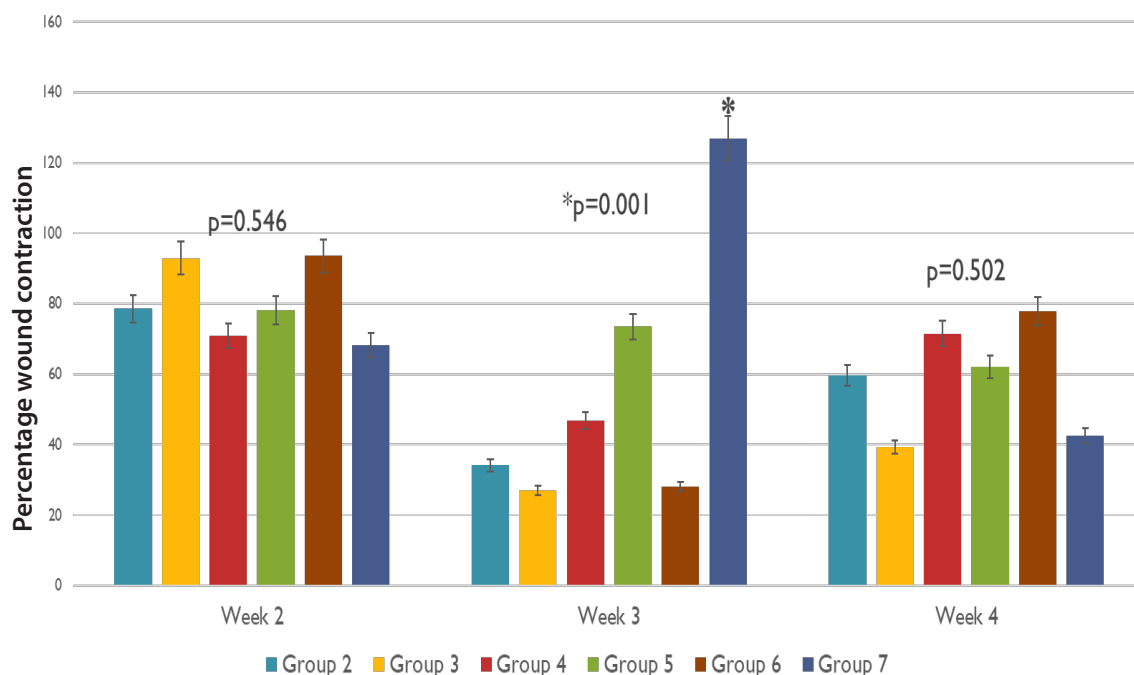


Figure 3. Effects of different cream combinations on wound contraction. Each bar represents the mean \pm SEM; * p = a significant difference, $p < 0.05$ vs the control. Note the number of rats per group was six in Weeks 1 and 2. At Weeks 3 and 4, the number of rats in Group 7 was 5 and 4, respectively.

IMPLICATION FOR CLINICAL PRACTICE

It is recommended that the 2:1 mupirocin-betamethasone cream combination should be used in the management of infected wounds to improve wound healing.

FURTHER RESEARCH

Further studies will be needed in human to further establish the findings in this work.

DECLARATIONS

Ethical approval and consent to participate- The Ethical Review Board of the Faculty of Basic Medical Sciences, LAUTECH, granted ethical approval with identification code ERC/FBMS/054/2024. All the experimental procedures were carried out according to the approved protocols of the Faculty of Basic Medical Sciences, LAUTECH, and as prescribed by the European Council Directive (EU2010/63) in the scientific procedures on living animals. All animals received humane care.

AVAILABILITY OF DATA

The data will be made available upon request.

CONFLICTS OF INTEREST

The authors declare that they have no known competing interests.

FUNDING

The study was funded by the authors

AUTHORS' CONTRIBUTIONS

Conceptualisation: OS
Conceptualisation: OS

Literature search: OSI, ATW

Study design: OSI, TWA, OOA, ORI, AOS

Data collection: OSI, ORI

Data analysis- OSI, TWA, ORI

Corrections and final write-up: OSI, TWA, OOA, ORI, AOS

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