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ORIGINAL ARTICLE

Time-dependent effects of topical mucopolysaccharide polysulfate and silver sulfadiazine on wound healing in a rat excisional wound model

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ABSTRACT

Objectives: This study aims to evaluate the effects of topical creams containing mucopolysaccharide polysulfate (MPS) on wound healing using an experimental wound model.

Materials and methods: Standard full-thickness skin defects were created in 32 Wistar rats and monitored for 14 days under four different topical treatment protocols: Control (Group 1, n = 8), Hirudoid (Group 2, n = 8), Silverdin (Group 3, n = 8), and Hirudoid + Silverdin (Group 4, n = 8). Wound closure rates were quantified through photographic analysis, while histological and immunohistochemical healing characteristics were assessed using the Structure, Presence of Cells, Organization, and Tissue Integration (SPOT) score, additional histological indices, and the transforming growth factor-beta 1 (TGF-β1) H-score. Intergroup differences were analyzed using appropriate non-parametric statistical tests.

Results: On Day 3, wound closure rates were higher in the Hirudoid, Silverdin, and Hirudoid + Silverdin groups compared to the control group ($p < 0.01$). By Day 14, this difference sustained only in the Silverdin and Hirudoid + Silverdin groups, indicating that the early advantage observed with Hirudoid alone was not maintained over time ($p < 0.01$). No significant differences in the general histological parameters were observed among the groups ($p > 0.05$). However, the TGF-β1 H-score was lower in the Silverdin group than in the control and Hirudoid groups ($p < 0.01$).

Conclusion: Our study results suggest that MPS may accelerate early wound closure, but does not significantly improve wound closure in later stages, and its combination with silver sulfadiazine offers no additional benefit compared to silver sulfadiazine alone.

Keywords: Excisional wound model, mucopolysaccharide polysulfate, silver sulfadiazine, time-dependent analysis, histology.

The skin serves as a vital barrier between the body and the external environment, playing a key role in maintaining thermal and water balance and preventing microbial invasion.[1] Disruption of skin integrity due to injury presents a significant clinical challenge for orthopedic and other healthcare professionals. Wound healing is a complex process consisting of four overlapping phases: hemostasis, inflammation, proliferation, and remodeling.[2] This process is influenced by multiple local, regional, and systemic factors, as well as nutritional status, comorbidities, radiotherapy exposure, and smoking.[3] Consequently, numerous studies have focused on improving wound healing by targeting these factors.

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Topical creams containing mucopolysaccharide polysulfate (MPS) are widely used in clinical practice for trauma-related conditions, hematoma

management, and vascular disorders owing to their antithrombotic and fibrinolytic properties. Hirudoid® (Santa Farma, İstanbul, Türkiye), a commonly available preparation, contains MPS, a heparinoid derived from sulfated chondroitin sulfate. Previous studies have shown that MPS-based formulations support microvascular stability, preserve barrier function, and enhance skin perfusion through nitric oxide production.[4,5]

Considering its effects on hematoma resolution and microvascular circulation, MPS may positively influence different stages of wound healing and contribute to the overall healing process. However, although previous studies have demonstrated that MPS creams increase cutaneous blood flow and improve microvascular circulation, their effects on wound healing have not been evaluated in an experimental excisional wound model, nor has their combined use with silver sulfadiazine been investigated.

In the present study, we hypothesized that topical application of MPS might accelerate wound healing in open skin injuries. We, therefore, aimed to evaluate the effects of topical MPS on wound healing in terms of wound closure and histological characteristics using an experimental excisional wound model.

MATERIALS AND METHODS

Study design and study subjects

The animals used in this study were obtained from the Düzce University, Experimental Animal Research and Application Center. A total of 32 male Wistar rats (two or three months old, 230 ± 30 g) were housed under optimal laboratory conditions at 23°C, 60 ± 5% humidity, and a 12:12-h light-dark cycle, with *ad libitum* access to food and water.

The study was conducted in accordance with laboratory animal care guidelines and was approved by the Düzce University Faculty of Medicine Local Ethics Committee for Animal Experiments (Date: 20.11.2024, Approval No: 2024-11-04).

Surgical procedure

Following transfer to the laboratory, all 32 rats received intraperitoneal anesthesia with a ketamine/xylazine combination (90/10 mg/kg) (Ketamine, Eczacıbaşı, İstanbul, Türkiye/Xylazine, Bayer Türk Kimya San. Ltd. Şti, İstanbul, Türkiye). The dorsal region of each rat was shaved and prepared with 10% povidone-iodine solution (Batticon, ADEKA İlaç Sanayi ve Ticaret A.Ş., Samsun, Türkiye). Subsequently, a standardized full-thickness open wound with a diameter of 2 cm was created by excising the skin. This size was selected to create a sufficiently large and reproducible wound area for reliable macroscopic and histological assessment, consistent with previously described experimental wound models.[6,7]

Experimental groups, treatments, and administration

Following the surgical procedure, the rats were randomly allocated into four groups, each consisting of eight animals:

Group 1 (Control) received no pharmacological treatment. A wound dressing composed of oxidized cellulose moistened with 2 mL of sterile saline was applied to the lesion and replaced every two days.

Group 2 (MPS, Hirudoid) received a topical cream containing MPS at a concentration of 445 mg/100 g (Hirudoid; Santa Farma, İlaç San. A.Ş, İstanbul, Türkiye), which was applied once daily to fully cover the wound defect for 14 consecutive days.

Group 3 (silver sulfadiazine; Silverdin®, Deva Holding A.Ş., İstanbul, Türkiye) received a topical cream containing silver sulfadiazine at a concentration of 10 mg/g, which was applied once daily to completely cover the wound defect for 14 days.

Group 4 (Hirudoid + Silverdin) received a 1:1 mixture of Hirudoid and Silverdin creams, which was applied once daily to completely cover the wound defect for 14 days.

Throughout the study period, the rats were provided with unlimited tap water and standard rodent chow. They were monitored in temperature-controlled cages (23 to 25 °C) under a 12/12-h light-dark cycle. No antibiotics were administered before or after surgery.

Clinical observation and histopathology

Photographs were taken on postoperative Days 0, 3, 7, 10, and 14 to document wound contraction and granulation tissue formation. To ensure standardized measurement, all wounds were photographed from a fixed height with a ruler placed adjacent to the lesion (Figure 1).

The wound areas were calculated individually using the ImageJ image analysis software.[6] The percentage of wound closure was determined using the following formula:

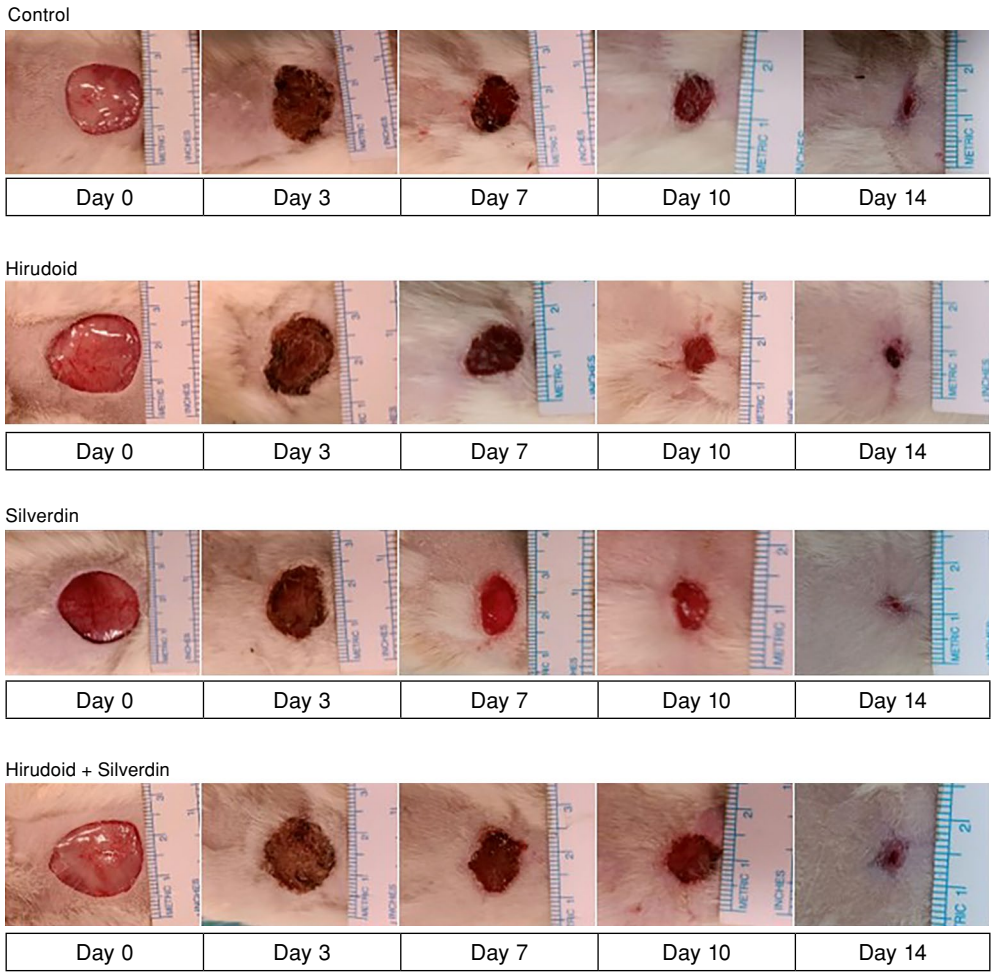


FIGURE 1. Photographs of the wounds taken on Days 0, 3, 7, 10, and 14.

Wound closure (%) = [(original wound area - current wound area)/original wound area] × 100.[6]

For histological examination, all rats were sacrificed on Day 14, and tissue samples were excised with at least a 2-mm margin of healthy tissue surrounding the wound. The collected specimens were placed in 10% formaldehyde solution and fixed for 24 to 48 h, processed using routine histological procedures, and embedded in paraffin. Sections of 4 to 5-µm thickness were obtained from each block for microscopic analysis. Two primary histochemical stains were used: Hematoxylin and Eosin (H&E) and Masson's Trichrome (MT).

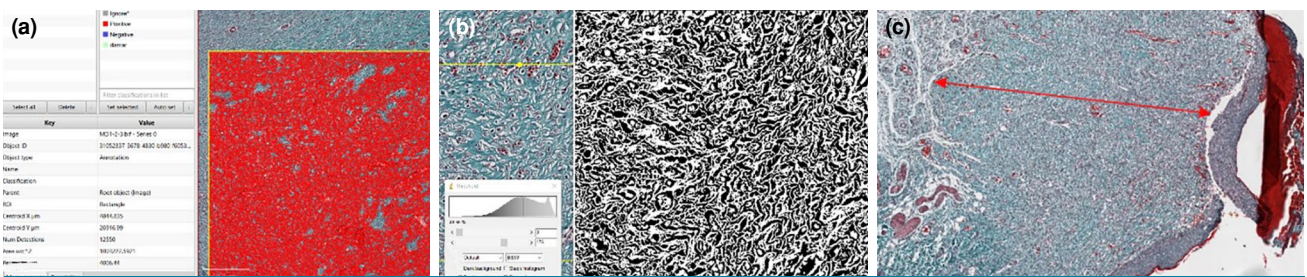


FIGURE 2. (a) Collagen content assessed using threshold function in ImageJ; **(b)** Fibroblast quantification via digital WSI and QuPath software; **(c)** Measurement of scar height (Hypertrophic Index) using Masson's Trichrome-stained digital slides (MT, ×40). WSI, whole-slide imaging.

TABLE I
Histological SPOT score

Parameter	Description	Criteria	Score
Re-epithelization	Percentage of re-epithelialized area relative to total wound area	Complete (95-100%) Partial (< 95%; > 0%) None (0%)	2 1 0
Epidermal thickness index (ETI)	Epidermal thickness at wound site relative to normal skin	Normal (95-105%) Hypertrophy (> 105%) Hypoplasia (< 95%)	2 1 0
Keratinization	Visual assessment, scored only if re-epithelialization is complete	Yes: Loosely attached/lost layers or thick parakeratotic stratum corneum No: None	2 0
Granulation tissue	Visual + quantitative (µm) assessment	Intact dermis: No granular infiltrates Thick GT: > 100 µm Thin GT: < 100 µm	2 1 0
Remodeling	Visual inspection	Complete: All of (1) adipose, (2) appendages, (3) panniculus regeneration Partial: Any of (1) collagen, (2) adipose None: No remodeling	 1 0
Scar elevation index (SEI)	Dermal thickness in wound site *vs.* normal skin	Normal (95-105%) Hypertrophied (> 105%) Hypoplasia (< 95%)	2 1 0

SPOT, structure, presence of cells, organization, and tissue integration.

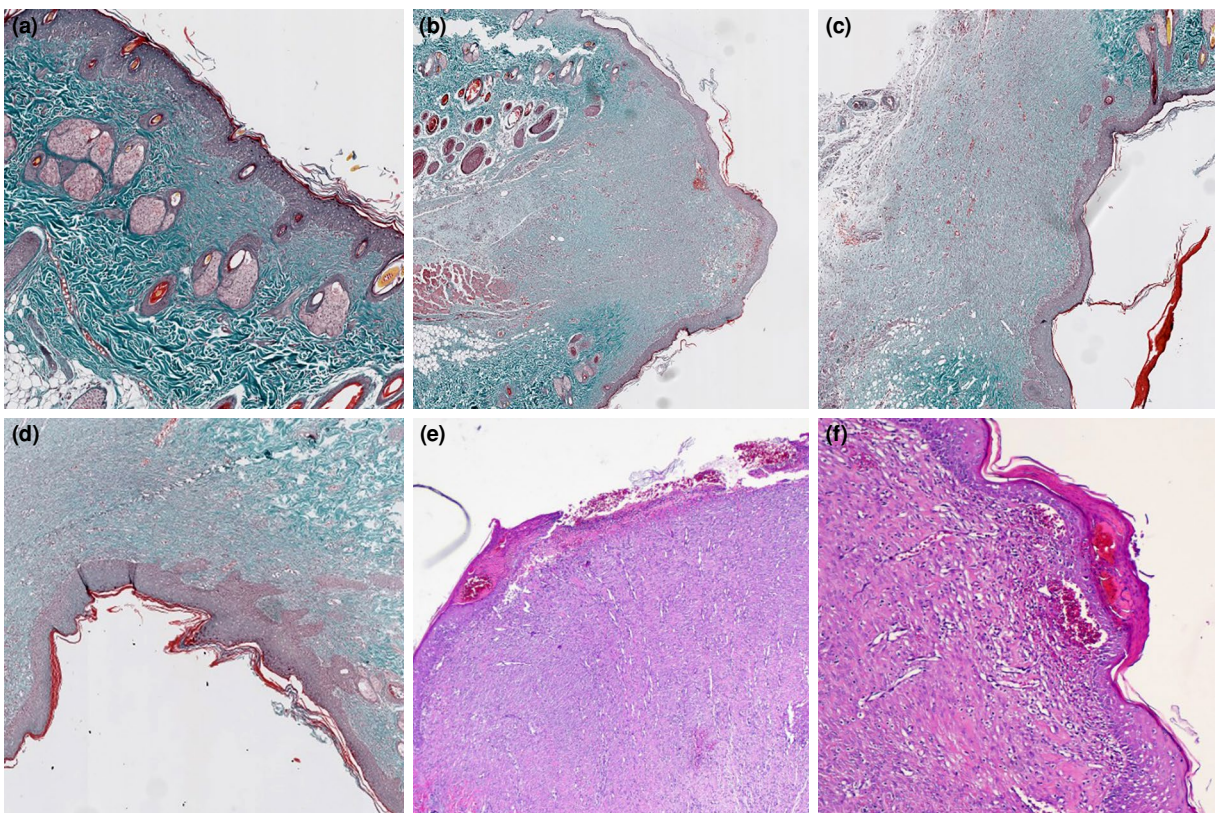


FIGURE 3. (a) Normal skin with preserved adnexal structures (MT, ×40); **(b)** Hypertrophic scar with complete re-epithelialization and slightly thickened epidermis (MT, ×10); **(c)** Hypoplastic scar with complete surface re-epithelialization (MT, ×10); **(d)** Thickened epidermis over healed scar (MT, ×10); **(e)** Incomplete epithelium with mild thickening over hypertrophic tissue (H&E, ×40); (f) Parakeratotic hyperkeratosis in completely re-epithelialized wound (H&E, ×100).
MT, Masson's trichrome; H&E, hematoxylin and eosin.

Histological evaluations were performed using both light microscopy and high-resolution whole-slide imaging (WSI). All histological and morphometric assessments were performed by an experienced pathologist who was blinded to the experimental groups to minimize observer bias. Slides were digitized with a WSI scanner, enabling precise quantitative assessment (Figure 2).

The wound healing process was evaluated using the Structure, Presence of Cells, Organization, and Tissue Integration (SPOT) scoring system developed by van de Vyver et al.[8] (Table I). This system divides wound repair into six components, each scored on a scale of 0-2 (except for keratinization, which does not include the score of 1). The total score ranges from 0 to 12, with higher scores indicating better healing outcomes. The H&E-stained slides served as the basis for scoring, while MT staining was additionally used to assess features of granulation tissue (Figure 3).

In addition to the SPOT system, previously described histological indices known as the Hypertrophic Index (HI) and Collagen Index (CI) were used.[9] Fibroblast density was also measured. All MT-stained sections were digitized to improve objectivity (Figure 2).

Hypertrophic index was measured on whole slide images using the QuPath version 0.5.0 software (Queen's University Belfast, UK). The vertical height of the scar tissue, defined as the distance between the deepest and most superficial points, was recorded (Figure 2).[10]

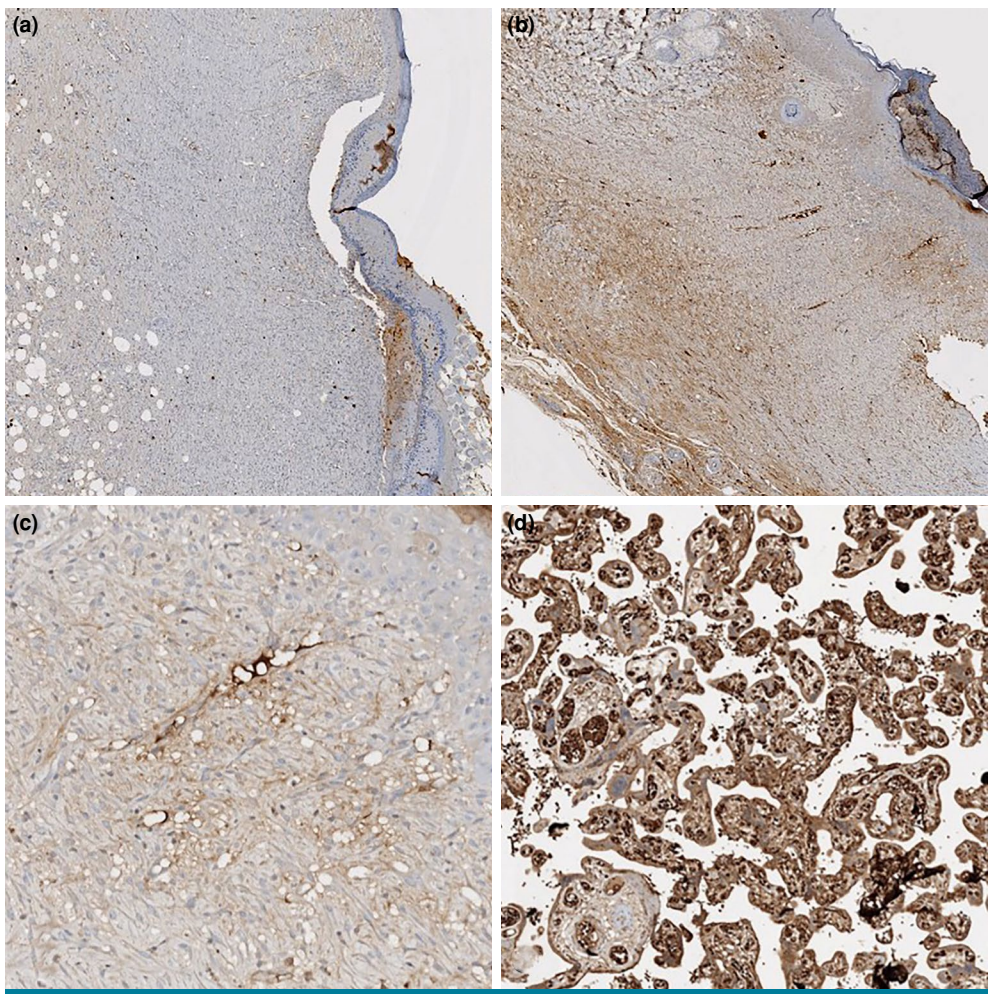


FIGURE 4. (a) TGF-β1 negativity in scar tissue (×40); **(b)** Weak but diffuse TGF-β1 staining in low magnification WSI (×40); **(c)** Similar diffuse staining in high magnification WSI (×100); **(d)** Positive TGF-β1 staining in placental control section (×100).
TGF-β1, transforming growth factor-beta 1; WSI, whole-slide imaging.

TABLE II
Comparison of wound closure percentages (%) among the Control, Hirudoid, Silverdin, and Hirudoid + Silverdin Groups on Days 3, 7, 10, and 14

	Control			Hirudoid			Silverdin			Hirudoid + Silverdin			
	Mean ± SD	Median	IQR	Mean ± SD	Median	IQR	Mean ± SD	Median	IQR	Mean ± SD	Median	IQR	*p*
Closure % day 3	32.83 ± 6.48	34.52	25.63-38.91	45.76 ± 2.84	45.93	43.24-48.41	44.64 ± 6.64	44.81	41.40-50.74	44.01 ± 4.61	44.13	41.20-48.54	**0.002***
Closure % day 7	68.42 ± 2.42	68.60	65.84-70.92	70.03 ± 1.62	69.97	68.26-71.71	71.17 ± 2.42	70.85	69.35-72.31	70.56 ± 2.22	70.33	68.91-71.91	0.238*
Closure % day 10	84.95 ± 2.86	85.59	82.82-87.60	87.85 ± 1.23	87.51	86.98-89.18	88.44 ± 2.41	89.19	85.76-90.20	87.72 ± 3.51	87.71	85.11-91.47	0.103*
Closure % day 14	93.67 ± 1.23	93.63	92.98-94.58	95.12 ± 0.93	94.74	94.40-96.12	96.24 ± 1.46	96.79	94.61-97.20	96.69 ± 1.47	97.09	95.49-97.87	**0.005***
p value		**< 0.001†**			**< 0.001†**			**< 0.001†**			**< 0.001†**		

SD, standard deviation; IQR, inter quartile range; * Kruskal Wallis Variance Analysis; † Friedman Variance Analysis

Collagen Index was calculated using the ImageJ software (Fiji, National Institute of Health, MD, USA). The most densely stained region of the scar on MT-stained slides was thresholded (Image > Adjust > Threshold) to determine the percentage of dark pixels representing collagen content (Figure 2).[11]

Fibroblast density was assessed by selecting a 1 mm² area within the scar tissue in QuPath, and fibroblasts were automatically counted using the Cell Detection function (Figure 2).

Immunohistochemical analysis

Transforming growth factor-beta 1 (TGF-β1) was selected, as it has been shown to play a central role in wound healing by regulating fibroblast proliferation, extracellular matrix deposition, and tissue remodeling.[12] To evaluate the molecular involvement in wound healing, immunohistochemical staining for TGF-β1 (Santa Cruz Biotechnology, dilution 1:100) was performed on paraffin-embedded tissue sections (Figure 4). Although the overall staining intensity was weak, some specimens showed more widespread positivity. Objective quantification was carried out using the H-score method as described by McCarty et al[13] and applied in previous studies.[7] The H-score was calculated using the following formula:

H-score = 1 × (% weak) + 2 × (% moderate) + 3 × (% strong), yielding values ranging from 0 to 300.[7]

Statistical analysis

Statistical analysis was performed using the IBM SPSS version 20.0 software (IBM Corp., Armonk, NY, USA). Continuous variables were presented in mean ± standard deviation (SD) or median and interquartile range (IQR), while categorical variables were presented in number and frequency. Differences in continuous variables among the Control, Hirudoid, Silverdin, and Hirudoid + Silverdin

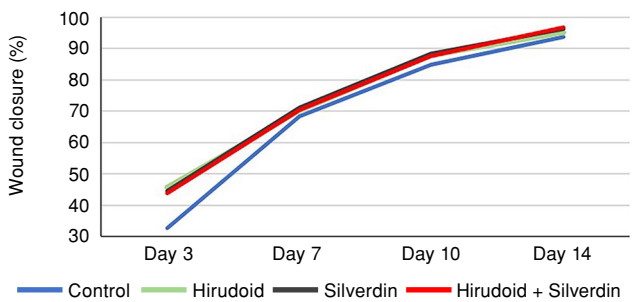


FIGURE 5. Wound closure (%) values among the Control, Hirudoid, Silverdin, and Hirudoid + Silverdin groups on Days 3, 7, 10, and 14.

groups were evaluated using the Kruskal-Wallis variance analysis. Post-hoc pairwise comparisons to determine the source of the differences were performed with Dunn's Multiple Comparisons test, and adjusted p-values were reported. Intragroup differences in wound contraction values on Days 3, 7, 10, and 14 were analyzed using the Friedman's variance analysis. A p value of < 0.05 was considered statistically significant.

RESULTS

A significant difference was identified in wound contraction (%) values on Day 3 among the Control, Hirudoid, Silverdin, and Hirudoid + Silverdin groups ($p < 0.01$) (Table II, Figure 5). Post-hoc analysis demonstrated that the Day 3 wound closure (%) values of the rats in the Control group were lower than those of the rats in the Hirudoid, Silverdin, and Hirudoid + Silverdin groups ($p < 0.05$), (Table III). No significant differences were observed among the Hirudoid, Silverdin, and Hirudoid + Silverdin groups regarding the Day 3 wound closure (%) values ($p = 1$) (Table III).

A significant difference was observed in the 14th-day wound closure (%) values among the Control, Hirudoid, Silverdin, and Hirudoid + Silverdin groups ($p < 0.01$) (Table II, Figure 5). Posthoc analysis demonstrated that the 14th-day wound closure (%) values of the rats in the Control group were lower compared to those in the Silverdin and Hirudoid + Silverdin groups ($p < 0.05$) (Table III). In contrast, no significant difference was observed between the Control and Hirudoid groups on Day 14 ($p = 0.772$).

No significant differences were found in the 7th- and 10th-day wound closure (%) values among the Control, Hirudoid, Silverdin, and Hirudoid + Silverdin groups ($p > 0.05$). Significant differences

TABLE III
Comparison of the 3rd- and 14th-day wound closure (%) values among the groups

Dunn's multiple comparisons test	Day 3	Day 14
Control - Hirudoid	**0.004***	0.772
Control - Silverdin	**0.011***	**0.036***
Control - Hirudoid + Silverdin	**0.026***	**0.006****
Hirudoid - Silverdin	1.000	1.000
Hirudoid - Hirudoid + Silverdin	1.000	0.445
Silverdin - Hirudoid + Silverdin	1.000	1.000

* $p < 0.05$; ** $p < 0.01$.

TABLE IV
Comparison of histological parameters among the Control, Hirudoid, Silverdin, and Hirudoid + Silverdin groups

	Control			Hirudoid			Silverdin			Hirudoid + Silverdin			
	Mean ± SD	Median	IQR	Mean ± SD	Median	IQR	Mean ± SD	Median	IQR	Mean ± SD	Median	IQR	p
Histological SPOT score	5.63 ± 1.65	5.5	4-7	6.00 ± 1.69	6.0	4-7	6.38 ± 1.68	5.5	5-8	6.25 ± 1.28	6	5-7	0.844*
Hypertrophic Index (HI)	1.73 ± 0.26	1.79	1.42-1.97	1.51 ± 0.14	1.55	1.46-1.59	1.63 ± 0.11	1.65	1.56-1.74	1.63 ± 0.19	1.59	1.46-1.83	0.263*
Collagen Index (CI)	49.80 ± 4.98	50.53	48.68-53.08	50.61 ± 4.09	50.15	47.95-54.94	48.33 ± 5.36	47.73	44.49-51.74	50.64 ± 3.58	51.40	47.75-51.81	0.684*
Fibroblast density	7116.50 ± 1427.50	7380	5675-8435	8707.75 ± 2202.18	8804	6646-10350	8124.25 ± 1579.74	8271	6626-9740	8901.75 ± 2279.77	9350	6671-10490	0.246*
Immunohistochemical TGF-β1 H-score	48.72 ± 14.33	50.9	34.7-59.3	57.67 ± 20.93	59.4	44.3-78.2	21.55 ± 9.97	21.0	15.9-26.4	40.57 ± 13.12	38.3	28.9-49.8	0.002*

SD, standard deviation; IQR, inter quartile range; TGF-β1, transforming growth factor-beta 1; * Kruskal-Wallis Variance Analysis.

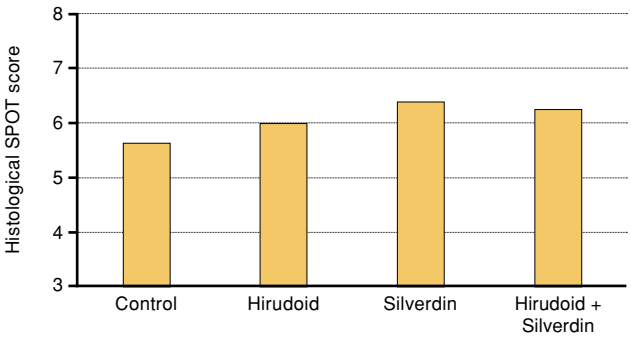


FIGURE 6. Graph of the histological SPOT scores of the groups.
SPOT, structure, presence of cells, organization, and tissue integration.

TABLE V
Comparison of TGF-β1 H-scores among the groups

Dunn's multiple comparisons test	TGF-β1 H-scores
Control - Hirudoid	1.000
Control - Silverdin	**0.014***
Control - Hirudoid + Silverdin	1.000
Hirudoid - Silverdin	**0.002****
Hirudoid - Hirudoid + Silverdin	1.000
Silverdin - Hirudoid + Silverdin	0.131

TGF-β1, transforming growth factor-beta 1; * $p < 0.05$; ** $p < 0.01$.

in wound closure were observed on Day 3, whereas group differences diminished at subsequent time points, except for the Silverdin and Silverdin + Hirudoid groups on Day 14.

No significant differences were observed in the histological SPOT scores of rats in the Control, Hirudoid, Silverdin, and Hirudoid + Silverdin groups ($p > 0.05$) (Table IV, Figure 6). Although no statistically significant differences were detected, treated groups tended to show higher histological scores compared to the control group.

No significant differences were observed among the Control, Hirudoid, Silverdin, and Hirudoid + Silverdin groups in terms of HI, CI, or fibroblast density values ($p > 0.05$; Table IV).

A significant difference was observed in the immunohistochemical TGF-β1 H-scores of rats in the Control, Hirudoid, Silverdin, and Hirudoid + Silverdin groups ($p < 0.01$) (Table IV). Post-hoc analysis showed that the TGF-β1 H-scores of the rats in the Silverdin group were lower than those of the rats in both the Control and Hirudoid groups ($p < 0.05$) (Table V). No significant differences were found among the remaining group comparisons ($p > 0.05$).

DISCUSSION

In the present study, we evaluated the effects of topical MPS on wound healing in terms of wound closure and histological characteristics using an experimental excisional wound model. Wound size reduction showed a time-dependent treatment effect, with all treatment groups demonstrating improved closure compared to the control group in the early healing phase (Day 3). Although MPS, silver sulfadiazine, and combination therapy maintained favorable trends on Days 7 and 10, these differences were not statistically significant. By Day 14, wound closure remained lower in the control group than in the silver sulfadiazine and combination groups, while no difference was observed between the MPS and control groups, suggesting that MPS was mainly effective in the early phase of healing. Histological and immunohistochemical analyses revealed no significant differences among groups, except for reduced TGF-β1 expression in the silver sulfadiazine group, indicating that MPS did not substantially affect proliferative activity or enhance the effects of combination therapy.

In their study, Kurachi et al.[5] investigated the effects of topical MPS on skin perfusion and reported that MPS increased nitric oxide production and enhanced local skin blood flow. Although improved perfusion may theoretically support wound healing, the findings of the present study demonstrated only a transient improvement in wound size reduction on Day 3, while no sustained benefit was observed at later time points. Therefore, our results suggest that the potential effect of MPS may be limited to the early phase of wound healing rather than producing a consistent healing advantage. In a clinical study, Zhang et al.[14] evaluated the effects of MPS creams on postoperative scar formation and reported a preventive effect on scar hyperplasia. However, scar formation was not directly assessed in the present study; therefore, no conclusions regarding the effects of MPS on scar remodeling can be drawn based on our findings.

A study similar to ours in the literature was a clinical trial conducted on burn wounds.[15] This study evaluated the clinical effects of combining MPS cream with silver sulfadiazine cream in patients with deep partial-thickness burns. A total of 128 hospitalized patients with deep partial-thickness burns were randomly assigned

to a control group or a combination therapy group. Patients in the control group were treated with MPS cream, while those in the combination therapy group received both MPS cream and silver sulfadiazine cream. The results showed that pain scores on Days 7 and 14 after treatment differed significantly between the two groups. The combination therapy group exhibited faster wound healing on Days 14 and 28 and achieved earlier wound closure. However, bacterial culture results did not show a significant difference between the groups. According to these findings, the combination of MPS and silver sulfadiazine appeared to be more effective in reducing pain and accelerating wound healing in partial-thickness burns. In contrast, our findings showed that while combination therapy did not provide an additional benefit over silver sulfadiazine alone, it demonstrated superior late-stage wound closure compared to MPS monotherapy. This discrepancy may be related to several differences between the two studies. Our study was conducted in an animal model, and results in humans may differ. Additionally, burn wounds and full-thickness excisional wounds involve different healing mechanisms. Pain scores also could not be evaluated in an animal model. Nevertheless, based on previous findings, the anti-inflammatory effects of MPS creams suggest that such clinical benefits may still be expected in practice.[15,16]

Experimental and clinical studies have demonstrated that MPS creams exert beneficial effects on cutaneous tissues. MPS has been shown to promote microvascular stabilization and barrier integrity in dermal endothelial cells, suggesting a potential role in wound healing.[4] In clinical settings, MPS creams have been associated with improved healing of pressure ulcers and enhanced skin flap viability.[16,17] Collectively, these findings suggest that MPS creams may influence several biological processes related to skin repair. However, the findings of the present study indicate that these effects may be limited and may not necessarily translate into a sustained improvement in wound healing outcomes. In an animal study evaluating the antithrombotic effects of MPS creams, topically applied MPS was shown to significantly prolong the time to thrombus formation and to facilitate earlier reperfusion of an occluded vessel.[18] This mechanism may also contribute to the increased local perfusion observed following topical application, which could help explain the more rapid wound closure observed on Day 3 in our study. Another study focused on an additional property of MPS creams beyond their heparinoid activity, specifically their effects on skin hydration and elasticity.[19] According to the study, the chemical structure of MPS allowed extensive hydrogen bonding with adjacent water molecules, resulting in effective hydration of surrounding tissues. Furthermore, by stimulating endogenous hyaluronate synthesis, MPS increased the water binding capacity and viscoelasticity of the skin. Clinical evaluation in human subjects demonstrated that MPS based creams increased skin hydration compared to placebo. This hydrating effect may have contributed to the early improvements in wound healing observed in our study.

The heparinoid properties of MPS creams raise concerns regarding potential systemic adverse effects, such as bleeding or hematoma formation. A study addressing this issue reported that topical application of a three percent MPS ointment at a dose corresponding to 15 mg MPS per kg (0.5 g/kg ointment) in a group of four monkeys did not produce any alterations in coagulation parameters, including activated partial thromboplastin time, thrombin time, or platelet count.[20] However, this study evaluated the effects of MPS applied to intact skin. Additional research is required to determine absorption dynamics and systemic implications when MPS is applied to open wounds, as in our study.

Although MPS has been studied in relation to skin hydration, microvascular stabilization, and scar formation, data from open wound models remain limited. The present study addresses this gap by evaluating the effects of MPS in an experimental excisional wound model, including time-dependent healing outcomes and its combination with silver sulfadiazine. However, further clinical studies are required to determine the relevance of these findings to human wound healing.

Our study has several limitations that should be acknowledged. As an animal study, inherent differences in wound healing biology and tissue repair mechanisms between rodents and humans may limit the direct translation of the findings to clinical settings. In addition, histological evaluation was performed only on specimens collected on Day 14, which restricts assessment of the early inflammatory and proliferative phases of wound healing. Biomechanical evaluation of the healed tissue, such as tensile strength testing, was not performed; therefore, functional integrity of

the wound could not be assessed. Furthermore, although MPS has known heparinoid and antithrombotic properties, coagulation parameters such as prothrombin time and activated partial thromboplastin time were unable to be evaluated, limiting systemic safety assessment. Finally, the control group received isotonic saline only, and a vehicle-only cream control was not included. This limits the ability to distinguish the pharmacological effects of MPS from the potential contribution of the topical cream base itself.

In conclusion, our study results suggest that MPS-containing creams may have limited effects during the early phase of wound healing, particularly by enhancing wound size reduction on Day 3. Silver sulfadiazine appears to be associated with reduced TGF-β1 expression, indicating a potential influence on the biological healing response, while the combination of MPS and silver sulfadiazine does not offer a clear advantage over monotherapy. Further well-designed experimental and clinical studies are warranted to validate these findings and clarify their translational relevance.

Data Sharing Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

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