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Leech Saliva Extract Enhances Incisional Skin Wound Healing in Rats by Improving Neovascularisation and Tissue Regeneration

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Abstract

Medical leech therapy has been used for centuries due to its therapeutic effects. This therapy is mainly due to two main mechanisms: sucking and removing blood by leeches and secretion of leech saliva containing bioactive substances. This study investigated the effects of leech saliva extract (LSE) and medical leech therapy (MLT) on wound healing in rats with an incisional skin-wound model. The rats were divided into three groups: the control group (group I), the medical leech therapy group (group II), and the leech saliva extract group (group III). The wound healing process was observed and photographed morphologically on days zero and seven. After seven days, the rats were euthanised for detailed histological and biochemical examination. The histopathological findings revealed that group III indicated an accelerated healing process compared to other groups. In Group III, significant improvements in epithelial regeneration, granulation tissue thickness, fibroblast proliferation, neovascularisation, and reduced inflammation were observed. Vascular Endothelial Growth Factor (VEGF) (+) cell percentages were highest in Group III (75.60 ± 0.67), followed by Group II (67.00 ± 1.15), both significantly higher than the control group (46.16 ± 1.01) ($p = 0.001$). LSE demonstrates superior wound healing effects, likely by promoting epithelial and granulation tissue development and enhancing VEGF-driven neovascularisation. These findings underscore LSE's potential as a therapeutic agent for improving skin wound healing, with effects similar to or greater than medicinal leech therapy.

Keywords: Leech therapy, Hirudinea, VEGF, Wound healing, Skin repair, Rats, Complementary medicine

1. Introduction

A wound is a physical injury resulting in a breaking or tearing in the skin's surface [1]. Skin has many functions, such as protection against pathogens, prevention of dehydration, a vital role in thermoregulation, hormone production, and homeostasis [2]. It is essential to know the types of wounds because the treatment and care of each type differ. Several types of wounds include surgical wounds, pressure ulcers, abrasions, lacerations, and burns.
Wounds can also be divided into two categories: partial and full thickness. In full-thickness

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wounds, the deeper layers of the skin, and even the fatty tissue are affected. Therefore, blood circulation is impaired, forming scars when they heal [3].

Wound healing is a complex process consisting of stages that cannot be separated by sharp lines, including haemostasis, inflammation, proliferation, and remodelling phases, cannot separate. Each phase is important for the healthy and smooth progress of wound healing and ensuring correct tissue integrity [1]. Leech therapy has been documented to enhance wound healing by promoting blood flow to the wound area [4]. In a healthy individual, the haemostasis phase begins immediately when the wound forms and bleeding starts. Platelets play a crucial role in this phase by adhering to the exposed sub-endothelial matrix, aggregating, and forming a primary plug to prevent bleeding. Coagulation cascades further stabilise this process, leading to fibrin clot formation and ensuring adequate haemostasis [5, 6]. This phase is followed by the inflammation phase, which is the phase in which immune responses occur in the wound area and starts at almost the same time as the haemostasis phase [7]. Following these precursor responses, the proliferative phase begins, characterised by mitogenicity and migration of keratinocytes, fibroblasts, and endothelial cells. These cellular processes are crucial for promoting re-epithelialisation and tissue healing. Finally, in the reshaping phase, the scarce collagen in the wound area is broken down by proteolytic enzymes, and the wound tissue is rearranged. Thus, the aim is to return the wound tissue to its former healthy and durable state [8].

Delayed or impaired wound healing may occur for many reasons, including local and systemic factors. Local factors pertain to conditions directly affecting the wound site, such as vascular insufficiency, poor oxygenation, and local infection. On the other hand, conditions such as diabetes mellitus, advancing age, increased stress levels, certain medications, obesity, alcohol consumption, smoking, and nutritional deficiencies are systemic factors [9].

Improper wound healing is associated with significant morbidity or mortality and is a significant economic burden [10]. Therefore, various methods are being investigated worldwide to correct and accelerate wound healing. Traditional and complementary treatment methods are at the forefront [11]. Researchers have used various adjunctive therapies for wound healing, such as maggot therapy, apitherapy, and hirudotherapy [12]. Hirudotherapy, known as leech therapy, utilises the therapeutic effects of bioactive substances found in the saliva of leeches especially medical leeches such as *Hirudo medicinalis* [13]. Several supplementary approaches have been suggested to aid in wound healing, including methods like hyperbaric oxygen therapy, ozone therapy, and the utilisation of advanced wound care products. In 2004, the Food and Drug Administration (FDA) approved leeches as medical devices for medicinal purposes [14]. Thus, medical leeches are used for therapeutic purposes, especially in plastic-reconstructive surgery, in the survival of graft tissue by removing venous congestion [15]. Also, surgeons use medicinal leeches worldwide to increase flap survival. Additionally, there are new applications in many medical fields, including surgery and reconstructive procedures related to vascular disorders [14].

Leech therapy's efficacy can be attributed to two fundamental mechanisms. Firstly, the leech initiates a blood-sucking process by piercing the host's skin, resulting in a therapeutic effect by removing blood. Subsequently, the secretion of leech saliva into the wound plays a pivotal role. However, it is important to emphasise that the primary therapeutic benefits of medicinal leeches are derived from the bioactive substances present in leech saliva. Leech saliva contains over 100 bioactive compounds in its salivary glands, which have various effects, including anticoagulant, analgesic, anti-inflammatory, and antimicrobial properties.

Hirudin is an active substance obtained from the salivary glands of leeches and is the most commonly known [16, 17].

Although the effectiveness of leech saliva is known in the scientific literature, no studies have been conducted so far to evaluate the effects of leech saliva extract (LSE) application and Medicinal Leech Therapy (MLT) on skin wound healing parameters. This study aimed to investigate the impact of LSE and MLT on wound healing in rats with an incisional skin-wound model.

2. Material and methods

2.1. Animals

All experiments were conducted at Gazi University's Laboratory Animal Breeding and Experimental Research Centre (GUDAM, Ankara). The Gazi University Animal Research Ethics Committee approved the experimental procedure with code G.Ü. ET –23.026. In practice, 18 female rats weighing the Wistar albino species (250±50 g) were examined as experimental animals. Ambient conditions during the experiment: It was kept constant at 22±4°C (air-conditioned room) and 47±5% humidity under a 12-hour light/dark cycle. During the experiment, under *ad libitum* conditions, tap water was used as drinking water and standard pellet as feed. These conditions were maintained until the end of the experimental procedures.

2.2. Experimental procedures

Eighteen animals were randomly divided into three experimental groups containing six animals each. The rats used in this study were distributed randomly and anonymously to groups by a researcher outside the study to ensure the study's objectivity. The research team was not involved in the grouping process to avoid bias. This method aims to increase homogeneity between groups and the reliability of the results. The animals were grouped as follows: Group I, the control group; Group II, rats treated with MLT; and Group III, treated with crude LSE. The same interventions, including MLT or LSE administration, were replicated in incisional areas for all groups after a 48-hour interval. According to the experimental plan, the designated area was photographed on postoperative days zero and seven to document any visible alterations. Ultimately, the rats were euthanised on the seventh postoperative day under deep anaesthesia through intracardiac blood collection [18]. Following euthanasia, blood samples were carefully collected, and dorsal skin tissue samples were excised for histopathological evaluation around the suture line. Subsequently, all tissue and blood samples obtained from the experimental animals were dispatched for thorough biochemical and histological examination.

2.3. Incisional wound model

Anaesthesia was induced using an intraperitoneal injection of ketamine (80 mg/kg) and xylazine (10 mg/kg). Anaesthesia was confirmed by the absence of a paw withdrawal reflex before the surgical procedure commenced [19]. Following anaesthesia, the surgical site was prepared by shaving. A vertical 2 cm full-thickness incision was made on the skin along the dorsal midline, extending from the medial to the posterior region. Subsequently, all incisions were then closed to their original positions with simple sutures.

2.4. Obtaining leech saliva extract

The Mediterranean medicinal leech, *Hirudo verbana* Carena, 1820 (Clitellata, Hirudinea, Hirudo), was used in this study [20]. The leeches were sourced from an approved sterile leech farm in Isparta, Turkiye. The species and origin of the leeches were confirmed using a stereo zoom microscope (Euromex NZ.1903-S, Germany) equipped with a camera to examine their

morphological characteristics. Species identification was based on established morphological criteria [21]. The technique that enables obtaining medicinal leech saliva without sacrificing the leeches and is widely used in the scientific literature [22] was performed as described in previous studies, and the medicinal leech secretion that we used in this study was obtained [23].

In this study, the total protein concentration of LSE was determined as 50 µg/mL using the Bradford method (Bradford Protein Assay Kit, ABP Biosciences, USA). The study was conducted at this dose. Bradford's method utilises Coomassie Brilliant Blue G-250 dye binding to an unknown protein. When a protein forms a complex with the acidic Coomassie dye reagent, the dye undergoes a colour change from brown to blue, with the intensity of the blue colour directly proportional to the protein concentration in the sample. Protein concentrations are then determined by comparing the colour intensity of the sample to that of a standard curve, which is typically generated using a series of known concentrations of bovine serum albumin (BSA) dilutions [24]. The protein can be detected spectrophotometrically at 595 nm. This kit is designed to quantitate 1 to 1500 µg/mL.

2.5. Application of the medicinal leeches and LSE

In group I (control), rats only underwent an incisional wound operation. No additional treatments were administered to this group beyond the incisional wound surgery.

In group II (MLT), small medicinal leeches (with an average weight of approximately 0.5 gm) were applied to the wound site after the incisional wound surgery. The medicinal leeches were allowed to feed until they completed their blood consumption, typically averaging about 0.5 mL of blood. The weights of the leeches were measured both before and after their application to monitor the amount of blood they consumed during feeding. After feeding, the medicinal leeches were euthanised by immersion in 90% ethanol.

In group III (LSE), 0.5 mL of LSE containing 50 µg/ mL total protein was injected subcutaneously into the wound site after the incisional wound surgery.

2. 6. Histopathological procedures

The histopathological studies were conducted at Gazi University, Faculty of Medicine Department of Histology and Embryology Laboratories. Tissue collection was performed immediately after euthanasia on the seventh day following treatment. Skin samples were excised from the incision sites and fixed in 10% neutral formaldehyde solution for 72 hours. After fixation, the samples were washed under tape water and dehydrated using a graded series of descending alcohol solutions. Subsequently, tissues were cleared with xylene and embedded in molten paraffin. Thin sections (4–5 µm) were prepared from the paraffin-embedded tissue blocks. An expert histologist examined the histological images. These sections were stained using Haematoxylin-Eosin (H&E) and Masson's trichrome methods for histopathological evaluation. Histomorphological changes were examined under a computer-aided light microscopy system (Leica DM4000, Germany), and imaging and analysis were performed using Leica QWin version 3.0 software (Leica Microsystems, Switzerland). Histopathological evaluations and scoring were conducted using standard procedures to assess parameters such as fibroblast proliferation, inflammatory cell infiltration, and tissue regeneration. Histological structures and processes, such as fibroblast proliferation and inflammatory cell infiltration, were assessed using a semi-quantitative method. Three stained tissue sections from 10 randomly selected fields per rat were examined. Epidermal regeneration and granulation tissue thickness were scored on a scale from 1 to 3, while fibroblast proliferation, angiogenesis, and inflammatory cell presence were evaluated on a 0 to 4 scale [25].

2.7. Immunohistochemical procedures

Immunohistochemistry procedures for Vascular Endothelial Growth Factor (VEGF) were performed. Antibodies used for this study were as follows: a rabbit polyclonal anti-VEGF antibody (Cat: 114409 Lot: 05310, Fine Test, Wuhan, China). Thin sections (4-5 microns thick) were submerged in xylene twice for 15 minutes each to clear the sections. Sections were sequentially immersed in 100%, 96%, 90%, 80%, and 70% ethanol for 10 minutes each. After initial preparation, tissue sections were hydrated twice in distilled water for 5 minutes each. The sections were then placed on an immunohistochemistry bar in a humid chamber. The tissue areas were circumscribed with a PAP-Pen to prevent non-specific binding and washed three times with Phosphate Buffer Saline (PBS) for 3 minutes each. Primary antibodies were applied to the sections and incubated overnight at +4°C. After incubation, sections were treated with 3% hydrogen peroxide for 15 minutes to block endogenous peroxidase activity, followed by PBS washing. A biotin-labeled secondary antibody was then applied. The sections were rewashed with PBS three times, for 3 minutes each, before a chromogen solution containing diaminobenzidine (DAB) substrate was added, allowing a visible immune reaction to develop. Mayer's Haematoxylin was used as a counterstain, followed by a final PBS wash. The sections were dehydrated through a graded alcohol series, cleared in xylene, and mounted with coverslips using Stellan. Antibody uptake was evaluated by counting the labelled cells in 10 separate and randomly selected fields on each slide. A scoring system was employed for immunohistochemical evaluation, likely based on the number of labelled cells or the intensity of the observed staining [26]. The level of immunoreactivity with VEGF is obtained in 5 fields per slide. The number of positive cells in the obtained sections was evaluated as a percentage.

2.8. Determination of IGF-1, HIF1α, and TGF-α levels in blood and tissue sample

The removed skin tissues were rinsed in ice-cold phosphate-buffered saline (PBS) to remove excess blood thoroughly and weighed before homogenisation. 100 mg of skin tissues were added in 900 µL lysis buffer (PBS), and the tissue was homogenised by homogeniser on ice. Then, the homogenates were centrifuged at 10,000 x g for 5 minutes at 4˚C. The supernatant was collected, transferred to a new tube, and immediately analysed. The test principle carried out in these kits is Sandwich enzyme immunoassay. The microtiter plate in these kits is coated with antibodies specific for TGFα, IGF-1, and HIF-1-α.

2.9. Statistical analysis

Values are stated as mean ± SEM. Statistical analyses were made using the SigmaPlot (Systat Software Inc., USA) version 11 for Windows. One-way ANOVA was performed to measure the data derived among groups as a statistical significance test. In the analysis of variance, when there was a difference between groups and variances were homogeneous, as a conclusion of the Barlett test, Tukey's multiple comparisons test was implemented for post hoc comparison of groups. Moreover, Statistical Package for the Social Sciences for Windows (version 21.0; SPSS Inc., Chicago, IL, the USA) was used for statistical analysis in histopathological data. Values were accepted as significantly different when $p < 0.05$.

3. Results

3.1. Morphological findings

The skin incisions were investigated on the seventh postoperative day for changes in the wound healing process. The results showed that hair growth near the wound area and wound closure occurred more in groups II and III than in the control group. However, this rate was even higher in group III, Figure -1.

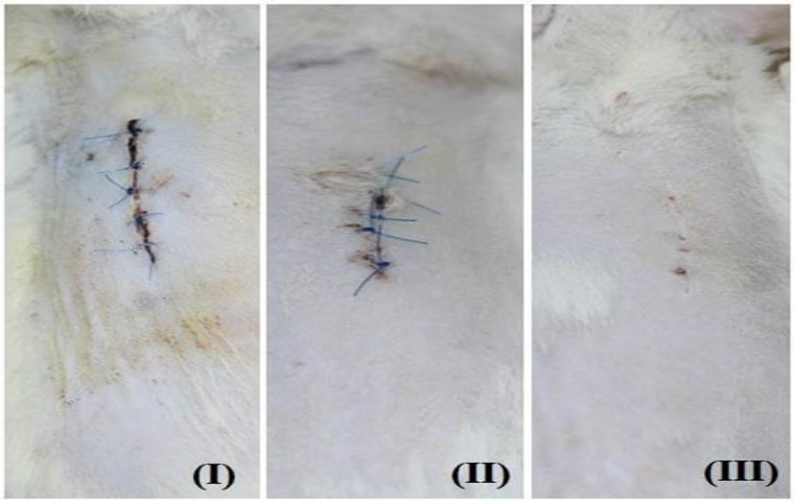

Figure 1: Digital photography of wound appearance changes in selected objects of groups I, II, and III

3.2. Histopathological and immunohistochemical findings

Tissue samples were analysed for epithelial regeneration, granulation tissue thickness, fibroblast proliferation, neovascularisation, presence of inflammatory cells, and VEGF (+) cells (%) parameters to evaluate wound healing, Figure -2. The histopathological findings revealed that group III indicated an accelerated healing process compared to other groups. In group III, collagen fibre loss was also monitored at the lowest level. It was observed that collagen production was more disorganised in group I compared to group III. Epithelial regeneration was detected more clearly in group III than in groups II and I. In group III, vascularisation was monitored at a more advanced level, compatible with epithelial regeneration (Table 1).

3.2.1. Epithelial regeneration

A statistically significant difference was observed between the experimental groups regarding epithelial regeneration scores ($p = 0.025$), indicating that the treatments had varying effects on the healing process. The epithelial regeneration score in Group III (3.80 ± 0.20) was found to be significantly higher than in Group I (2.83 ± 0.16) ($p = 0.018$). No significant difference was detected between other groups.

3.2.2. Granulation tissue thickness

A statistically significant difference was observed between the experimental groups regarding granulation tissue thickness score ($p = 0.035$). The granulation tissue thickness score (3.60 ± 0.24) in Group III was found to be significantly higher than that of Group I (2.66 ± 0.21) ($p = 0.028$). No significant difference was detected between other groups.

3.2.3. Fibroblast proliferation

A statistically significant difference was observed between the experimental groups regarding fibroblast proliferation score ($p = 0.016$). The fibroblast proliferation score (3.40 ± 0.24) in the group III group was found to be significantly higher than the group I (2.50 ± 0.22) ($p = 0.013$). No significant difference was detected between other groups.

3.2.4. Neovascularization

A statistically significant difference was observed between the experimental groups regarding neovascularisation score ($p = 0.032$). The neovascularisation score in group III

(3.60 ± 0.24) was found to be significantly higher than in group I (2.66 ± 0.21) (p = 0.042). No significant difference was detected between other groups.

3.2.5. presence of inflammatory cells

A statistically significant difference was found between the experimental groups in terms of inflammatory cell presence scores (p = 0.015), suggesting that the treatments influenced the level of inflammation during the healing process. The groups were compared individually to find the source of the significant difference. The inflammatory cell presence score in group III (1.40 ± 0.24) was found to be significantly lower than in group I (2.33 ± 0.21) (p = 0.014). No significant difference was detected between other groups.

Table 1: Scoring of skin healing for different treated groups

Histopathological Evaluation	Groups	Mean ± SEM	p-value
Epithelial Regeneration	Group I Group II Group III	2.83 ± 0.163,2 3.50 ± 0.22 3,80 ± 0,20	**0,025**
Granulation Tissue Thickness	Group I Group II Group III	2,66 ± 0,21 3,33 ± 0,21 3,60 ± 0,24	**0,035**
Fibroblast Proliferation	Group I Group II Group III	2,50 ± 0,22 3,16 ± 0,16 3,40 ± 0,24	**0,016**
Neovascularisation	Group I Group II Group III	2,66 ± 0,21 3,50 ± 0,22 3,60 ± 0,24	**0,032**
Inflamatuar Cell Presence	Group I Group II Group III	2,33 ± 0,21 1,66 ± 0,21 1,40 ± 0,24	**0,015**
VEGF (+) Cell (%)	Group I Group II Group III	46,16 ± 1,01 67,00 ± 1,15 75,60 ± 0,67	**0,0001**

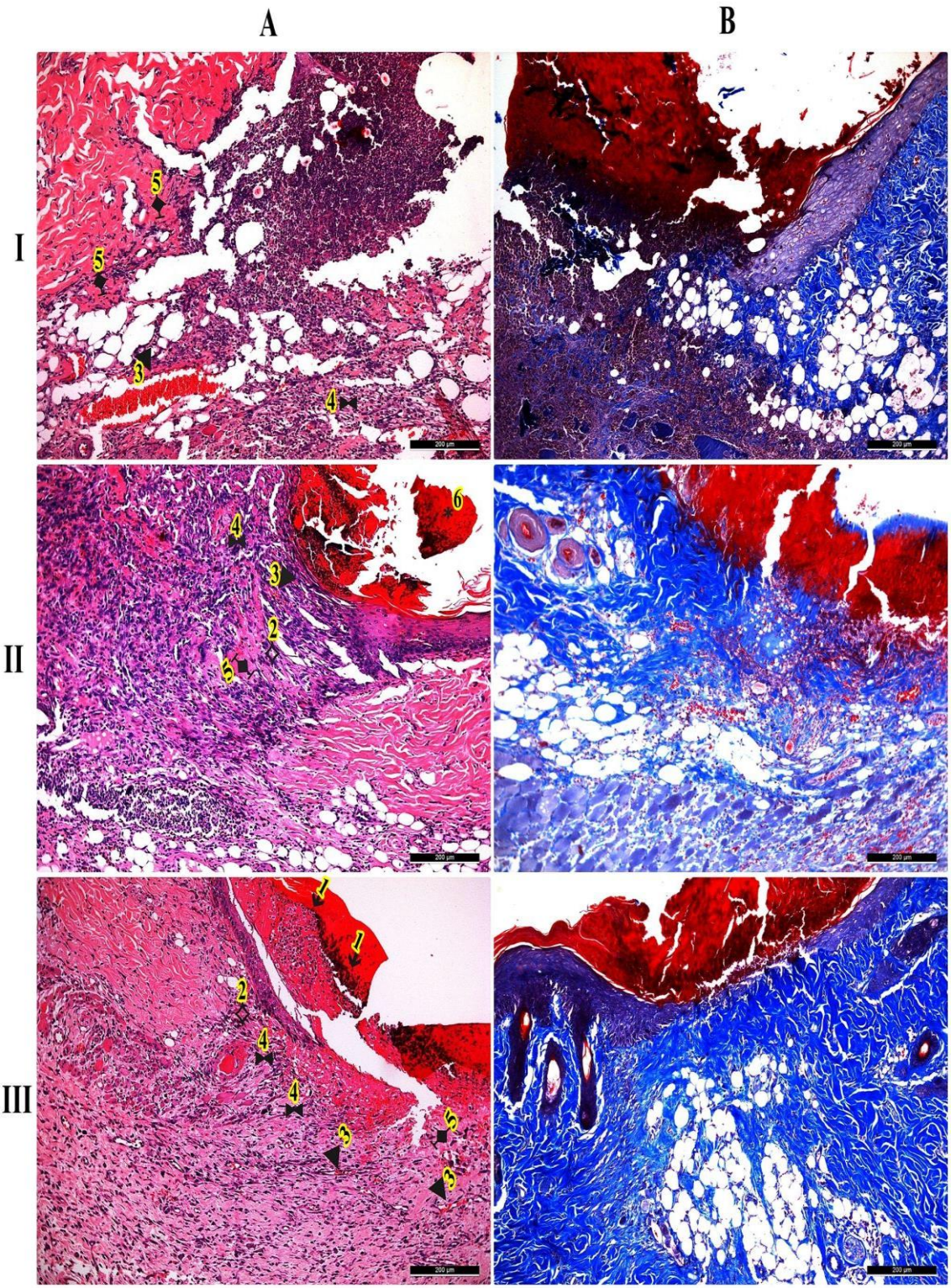

Figure 2: Histological examination A Haematoxylin & Eosin (200 µm), and B: Masson's Trichrome (200 µm). I: Control group; II: MLT group; III: LSE group. Epidermal regeneration: 1 ➔, Fibroblast proliferation: 2 ✧, Neovascularisation: 3 ►, presence of inflammatory cells: 4 ⋈, disorganised collagen production: 5 ⇟, Callus: 6 *

3.2.6. VEGF (+) cell percentage (%)

There was a statistically significant difference between the experimental groups regarding VEGF (+) Cell Percentage (%). VEGF (+) Cell Percentage (%) scores in group II (67,00 ± 1,15) and group III (75,60 ± 0,67) were found to be significantly higher than the group I (46.16 ± 1.01) (p = 0.001). VEGF (+) Cell (%) findings were observed at the highest level in group III, followed by group II (Figure 3).

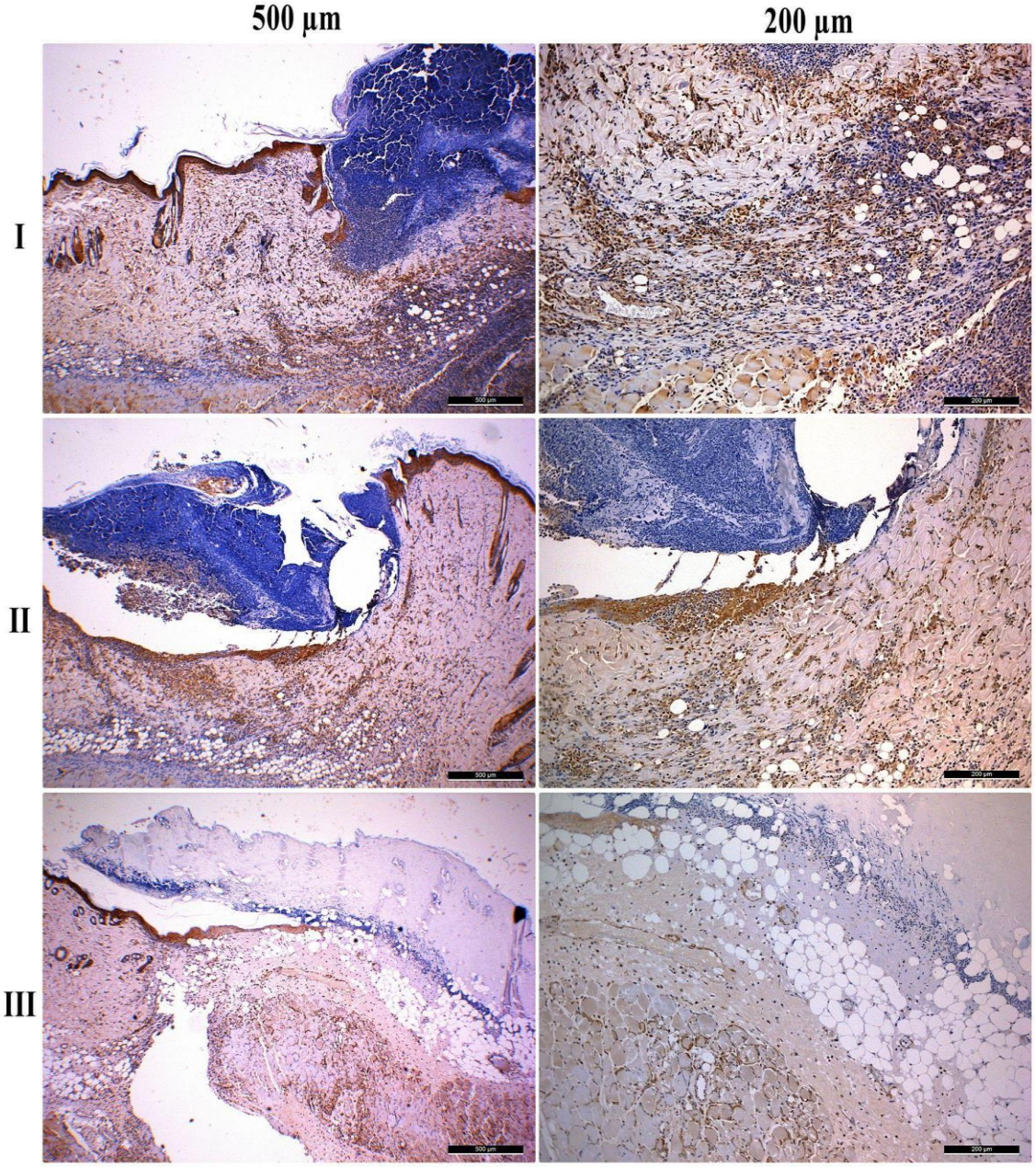

Figure 3: Histological examination for immunohistochemistry (VEGF). I: Control group; II: MLT group; III: LSE group.

3.3. Biochemical findings

There was no statistically significant difference in serum and tissue samples between the experimental groups in terms of HIF-1-α (ng/mL), TGF-α (pg/mL), and IGF-1 (ng/mL) concentrations (p > 0.05).

4. Discussion

Medicinal leech therapy has gained global attention due to the complex composition of leech saliva, which contains over 100 bioactive substances [27]. Among these, hirudin, a potent anticoagulant, prevents blood clotting, allowing prolonged feeding [28, 29]. The saliva also contains anti-inflammatory and analgesic enzymes that reduce pain and inflammation. Leech therapy has shown promise in promoting wound healing, supported by scientific research [14]. Hirudin enhances blood circulation to the wound bed, facilitating the delivery of oxygen and nutrients essential for tissue repair. The enzymatic profile, including proteases and hyaluronidase, may aid tissue regeneration by breaking down extracellular matrix components and promoting cellular turnover [30, 31].

Additionally, leech saliva components' anti-inflammatory and antioxidant properties help mitigate inflammation and oxidative stress, creating a favourable environment for wound repair [32]. However, due to the individualised nature of health conditions, it is crucial to consult healthcare professionals before considering leech therapy to ensure personalised treatment plans and optimal outcomes. Previous studies have demonstrated the potential of MLT in enhancing wound healing, relieving venous congestion, and improving flap survival in flaps [4, 13, 33].

Considering that the wound healing process consists of a complex and dynamic process, we chose seven days for our study because our study aimed to evaluate the effects of the treatments on these stages, explicitly focusing on inflammation, proliferation, and early remodelling phases. In the first stage of our study, the skin incision was photographed on the seventh postoperative day and evaluated morphologically for wound healing. Consistent with the findings of Darestani *et al.*, [34] on the effectiveness of MLT in incisional wounds, our results showed that both MLT and LSE positively affected the healing of incisional wounds; along with that, our study showed that LSE had a more significant effect on wound healing than MLT. These positive effects of medicinal leech saliva on wound healing can be attributed to the bioactive substances known to increase blood flow, such as hirudin, destabilase, and acetylcholine, found in medicinal leech saliva. Therefore, it is reasonable to assume that these substances increase blood flow in the treated areas, increase vascularity, and ultimately improve wound healing [13].

In the second phase of our study, we performed histopathological examinations on skin tissue taken from incision sites via haematoxylin and eosin and Masson's Trichrome staining methods. Therefore, we evaluated various parameters related to wound healing, including tissue regeneration, granulation tissue thickness, fibroblast proliferation, neovascularisation, and the presence of inflammatory cells. We observed a significant difference between group III and the control group regarding epithelial regeneration, granulation tissue thickness, and fibroblast proliferation scores among the experimental groups. These results underline the positive effects of LSE treatment on epithelial regeneration, which is important for the re-establishment of the skin barrier, and fibroblast proliferation, which is an important element in the migration of cells to the wound area. Neovascularisation scores were also notably higher in group III, further emphasising the significant contribution of LSE to this crucial aspect of vascularisation. The lowest level for loss of collagen fibre in group III made it possible for this application to be practical in wound healing through collagen fibre synthesis. Collagen synthesis is performed by fibroblasts in connective tissue. In this respect, the high value of fibroblast proliferation detected in group III is compatible with the loss of low collagen fibre obtained in the same group. Accordingly, the application can be beneficial through fibroblast activation. However, the need for specific studies of these pathways continues.

Moreover, the low count of inflammatory cell scores in group III suggests that LSE applications have an anti-inflammatory effect, which can further support wound healing. Additional studies conducted by Darestani [34], Zakian [35], and Mousavi [36] demonstrated the effectiveness of medicinal leech therapy in promoting wound healing and corroborated our findings. Although these studies were carried out in different wound models and with different medicinal leech types, they show that MLT has a high success rate in wound healing, which is consistent with our results.

We also evaluated VEGF-positive cells using the immunohistochemical method. VEGF is pivotal in wound healing, particularly in regulating angiogenesis [34]. Our results showed a significant increase in VEGF (+) Cell Percentage (%) in MLT and LSE groups compared to the control group. These findings highlight the potential of both MLT and LSE to stimulate angiogenesis, a critical process in wound healing. This finding is in line with a study by Yingxin *et al.*, who highlighted the effect of hirudin, a natural anticoagulant substance found in medicinal leeches, on VEGF gene expression [30] Therefore, the significant increase in VEGF (+) Cell Percentage (%) observed in our study can be attributed to the presence of hirudin in medicinal leech saliva, which is known to increase angiogenesis. Although other anticoagulant molecules, such as heparin, are available, hirudin is the most potent, natural, and specific thrombin inhibitor that works without requiring a cofactor [13]. Our findings from another *in vitro* study showed that LSE increased VEGF mRNA gene expression in healthy cell lines [23].

In the final stage of our study, we evaluated the HIF-1-α, TGF-α, and IGF-1 protein concentrations in serum samples and tissue homogenates taken from skin incisions using the ELISA method. We found no significant difference between the experimental groups regarding these parameters. We attribute this to the fact that we chose only the seventh postoperative day as the sample collection day. It is known that under normal conditions, HIF-1-α and TGF-α are synthesised in the early stages of wound healing, while IGF-1 is synthesised in the later stages. Therefore, our sampling period may not have captured substantial differences in these factors [37-39]

5. Conclusion

This study demonstrates that MLT and LSE treatments positively affect incisional wound healing. These effects are evidenced by improved epithelial regeneration, granulation tissue formation, increased fibroblast proliferation, increased neovascularisation, decreased inflammatory cell presence, and an increase in VEGF (+) cell percentage. In particular, it has been determined that LSE can be used as a potential agent to promote wound healing and support the formation of new blood vessels.

However, the study has a limitation in that the long-term effects of LSE cannot be assessed due to its short-term experimental design. Future studies are recommended to include longer experimental periods, different wound models, and extended observation periods. Additionally, a more detailed examination of the molecular mechanisms and biochemical pathways supporting the effects of LSE is necessary. These findings suggest that LSE can be developed as a promising therapeutic agent for wound healing and may improve patient outcomes.

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Conflict of Interest

The authors declare that no conflicts of interest exist.

Additional Information

Some parts of this study were presented at the 13th International Hippocratic Congress of Medicine and Health Sciences, Ankara, December 2023.

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