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MORPHOFUNCTIONAL CHARACTERISTICS OF INCISED SKIN WOUNDS IN RATS AFTER PHARMACOLOGICAL CORRECTION WITH A WATER-SALT EXTRACT OF THE MEDICINAL LEECH *HIRUDO VERBANA*

Actuality. *Wound healing of the skin is an important and complex process that consists of a cascade of dynamic and highly regulated cellular, humoral, and molecular mechanisms. It begins immediately after injury and can last for years, often requiring pharmacological correction. One such means of correction may be a water-salt extract of the medicinal leech Hirudo verbana, due to its wide range of therapeutic effects.*

The purpose of the work. *Is to reveal the morphofunctional characteristics of incised skin wounds in rats after pharmacological correction with a water-salt extract of the medicinal leech Hirudo verbana.*

Materials and methods. *Ninety male white laboratory rats weighing 245–260 g were used in the experiment. Circular full-thickness skin wounds measuring 1.5 cm in diameter were created using a standardized template. In the control group, wound healing occurred spontaneously without any therapeutic intervention. In the first experimental group, moistened compresses with a water-salt extract of the medicinal leech (Hirudo verbana) were applied to the wound site on days 1, 2, 3, 7, 10, and 14. In the second experimental group, "Wundahyl" ointment was applied on the same days. The concentration of the water-salt extract was 0.022 mg/mL. Tissue samples from the wound bed and the adjacent intact area (within 1 cm from the wound edge) were collected on the day of application, as well as on days 3, 7, and 30 post-injury.*

Research results. *Under the influence of the water-salt extract of the medicinal leech, the number of newly formed active hair follicles and blood vessels significantly increased at early stages of the study compared to the "Wundahyl"-treated group. By day 3, the first experimental group showed intensive granulation tissue formation and a noticeable reduction in the inflammatory response. Numerous active and developing hair follicles at various stages of morphogenesis, along with new blood vessel formation, were observed. This trend persisted on days 7 and 30 in both experimental groups. In the control group, mostly mature (old) hair follicles were detected, some exhibiting morphological signs of apoptosis.*

Conclusions. *A marked reduction in inflammation by day 3, along with intensive granulation tissue formation, epithelialization, and an increased number of newly formed hair follicles and blood vessels at all time points in the group treated with the leech extract, underscores its ability to accelerate regenerative processes compared to both the control group and the reference preparation.*

Key words: *cutaneous wound healing, stages of reparative regeneration, medicinal leech, Hirudo verbana.*

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МОРФОФУНКЦІОНАЛЬНА ХАРАКТЕРИСТИКА РІЗАНОЇ РАНИ ШКІРИ ЩУРІВ ПІСЛЯ ФАРМАКОЛОГІЧНОЇ КОРЕКЦІЇ ВОДНО-СОЛЬОВИМ ЕКСТРАКТОМ МЕДИЧНОЇ П'ЯВКИ *HIRUDO VERBANA*

Актуальність. *Загоєння ран шкіри є важливим і складним процесом, який складається з каскаду динамічних і високорегульованих реакцій клітинних, гуморальних і молекулярних механізмів, що починається безпосередньо після поранення й може тривати роками та потребувати фармакологічної корекції, чим може слугувати водно-сольовий екстракт медичної п'явки Hirudo verbana за рахунок прояву широкого спектру терапевтичної дії.*

Мета дослідження *– дослідити морфофункціональну характеристику різаної рани шкіри щурів після фармакологічної корекції водно-сольовим екстрактом медичної п'явки Hirudo verbana.*

Матеріал і методи. *Використано 90 білих самців лабораторних щурів вагою 245–260 г. У тварин вирізали круглі ділянки шкіри розміром 1,5 см за допомогою шаблону. У контрольній групі загоєння рани відбувалося спонтанно без будь-якої корекції; у першій дослідній групі на 1, 2, 3, 7, 10 та 14 добу на місце ураження наносили примочки, змочені водно-сольовим екстрактом медичної п'явки, у другій дослідній групі наносили мазь «Вундехіл». Концентрація водно-сольового екстракту становила 0,022 мг/мл. Зразки з ложа рани й сусідньої інтактної групи (до 1 см за краєм рани) збирали в день нанесення, на 3, 7 і 30 дні після травми.*

Результати дослідження. *Під впливом водно-сольового екстракту медичної п'явки кількість новоутворених активних волосяних фолікулів і кровоносних судин значно збільшується на початкових термінах дослідження порівняно з маззю «Вундехіл». До 3-го дня в першій дослідній групі спостерігається інтенсивне формування грануляційної тканини й помітне зменшення запаленої реакції, реєструється значна кількість активних і молодих фолікулів на різних стадіях і наявність утворення судин. Ця динаміка продовжується й на наступних термінах – 7 й 30 добу, в обох дослідних групах. У контрольній групі переважають старі волосяні фолікули, частина з яких має морфологічні ознаки апоптозу.*

Висновок. *Значне зменшення запальної реакції вже на 3 добу досліду, інтенсивне формування грануляційної тканини й епітелізація, збільшення кількості новоутворених волосяних фолікулів і судин на всіх термінах досліду під впливом водно-сольового екстракту підкреслює пришвидшені регенеративні процеси порівняно з контрольною групою та референт-препаратом.*

Ключові слова: *загоєння шкірних ран, етапи репаративної регенерації, медична п'явка, Hirudo verbana.*

Introduction. The skin is the largest organ of the body and performs a wide range of functions. It serves as a vital sensory organ, participates in gas and heat exchange, excretes metabolic waste products, water vapor, and bactericidal substances, and is involved in the synthesis of vitamin D (Mansfield et al., 2020; Jiao et al., 2024; Makyeyeva et al., 2025). The skin also plays a key role in the immune system, protecting the body from harmful environmental factors (Mansfield et al., 2020; Makyeyeva et al., 2025). When a wound occurs, both local and systemic reactions are triggered. Systemic responses include increased basal metabolism and catabolism under the influence of the sympathetic nervous system and hormonal regulation. Local responses are directed toward wound healing and follow a well-defined pattern (Mansfield et al., 2020).

The healing of an acute skin wound involves four main, sequential, and regulated phases: hemostasis, inflammation, proliferation, and remodeling (Mansfield et al., 2020; Peña et al., 2024; Makyeyeva et al., 2025). The first phase begins immediately after injury. Hemostasis provides an immediate response to trauma and functions to prevent blood loss at the wound site. The next phase, inflammation, begins in its early stage with the activation of the complement system and the classical molecular cascade, leading to infiltration of the wound by polymorphonuclear leukocytes (Makyeyeva et al., 2024; Peña et al., 2024). In the later stages of inflammation, the number of these cells decreases, and monocytes migrate into the wound, where they differentiate into macrophages (Makyeyeva et al., 2024; Alghazal et al., 2024). Lymphocytes also infiltrate the wound environment during the late inflammatory phase, influencing fibroblast proliferation and collagen biosynthesis. The absence of neutrophils and a decrease in macrophage numbers indicate the resolution of inflammation and the onset of the proliferative phase. During the third phase of wound healing, cell proliferation dominates, aiming to restore the vascular system and fill the wound defect with granulation tissue. In the subsequent remodeling phase, scar tissue is formed (Broughton et al., 2006; Gonzalez et al., 2016; Jiao et al., 2024;). For physiologically normal wound healing, additional support is often required, as wounds frequently become chronic, leading to complications involving various organs and systems (Sun et al., 2022; Rippon et al., 2022; Makyeyeva et al., 2024). As a result, increasing attention has been paid to substances of endogenous or natural origin – including plant– and animal-based compounds – that can be used to stimulate and normalize reparative regeneration (Saleh et al., 2022; Makyeyeva et al., 2024). One of these approaches is hirudotherapy, which involves the therapeutic use of medicinal leeches (ML) (Aminov et al., 2017; Sharma et al., 2020; Aminov et al., 2022; Aminov et al., 2023; Chhayani et al., 2023). This method is considered one of the most effective and natural ways to support wound healing mechanisms, as MLs contain more than 100 biologically active substances (BAS) with a wide range of therapeutic effects (Balasooriya et al., 2021; Aminov et al., 2023; Chhayani et al., 2023). Due to these properties, leeches can be used to treat chronic non-healing wounds such as diabetic foot ulcers, pressure sores, and venous leg ulcers, as demonstrated in experimental studies (Balasooriya et al., 2021; Aminov et al., 2023; Chhayani et al., 2023). It has been shown that species such as *Hirudo orientalis* and *Hirudo medicinalis* can accelerate the healing of primary incised skin wounds in rats (Amani et al., 2021; Zakian et al., 2022). Our previous studies have confirmed the positive physiological and reparative regeneration of the thymus and spleen in rats treated with MLs or their water-salt extract (Aminov et al., 2017; Aminov et al., 2022; Aminov et al., 2023). MLs are also widely used to address venous congestion problems. For example, they help maintain venous drainage and revascularization of reimplanted tissue. In surgical reimplantation – even in cases of gangrene – leeches can reduce pain and necrosis (Sharma et al., 2020; Chhayani et al., 2023). Moreover, the combination of Pongamia pinnata bark and hirudotherapy has been proven to accelerate the healing of large ulcerative wounds and reduce pain (Balasooriya et al., 2021). Given the wide therapeutic effects of medicinal leeches, it has become relevant to investigate the morphofunctional characteristics of incised skin wounds in rats following pharmacological correction with a water-salt extract of the ML *Hirudo verbana*.

Materials and methods. The study was conducted on 90 male white laboratory rats weighing 245–260 g. The experimental animals were housed under standard sanitary and hygienic conditions and fed a balanced compound feed. After the induction of experimental incised wounds, the animals were housed individually in separate cages with weekly bedding changes under aseptic conditions. During the observation period, no signs of contaminating bacterial infection were detected in any of the animal groups. Throughout the study, the rats were kept in a vivarium under controlled conditions: temperature 20–25°C, relative humidity not exceeding 55%, and a natural day-night light cycle. All experimental procedures were performed in accordance with the “International Guidelines for Biomedical Research Involving Animals” and the national “Joint Ethical Principles for Animal Experiments” (Ukraine, 2001), as well as the EU Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes, as well as the protocol from the bioethics commission at the Faculty of Biology of Zaporizhzhia National University for the planned research (protocol No. 1). The ML were maintained using the modern jar method. They were bred at the educational-scientific-research laboratory of cellular and organismal biotechnology at Zaporizhzhia National University (TU U 05.0-02125243-002:2009 “Medicinal Leech”, sanitary-epidemiological opinion of the Ministry of Health of Ukraine № 05.03.02–06/49982, dated 12.08.2009).

The wound model was created as follows: under ketamine anesthesia (40 mg/kg body weight), and after thorough shaving of the interscapular region, surgical procedures were performed under aseptic and antiseptic conditions. The animals were then randomly divided into three groups of 30 animals each. Circular skin areas measuring 1.5 cm in diameter were excised using surgical scissors and a standard template (Makyeyeva et al., 2024, 2025). In the control group, wound healing occurred spontaneously without any pharmacological intervention. In the first experimental group, on days 1, 2, 3, 7, 10, and 14, compresses soaked in a water-salt extract of the medicinal leech (*Hirudo verbana*) were applied to the site of injury. In the second experimental group, a reference preparation “Wundahyl” ointment (produced by “EIM”, Ukraine) – was applied on the same days.

Preparation of the water-salt extract:

A total of 35 medicinal leeches (Hirudo verbana), weighing 0.8–1.1 g each, were selected. After being gently dried on sterile filter paper, the leeches were fragmented and ground in crushed sterile glass with physiological saline to extract tissue components. The homogenate was filtered, washed with saline, dried again, and re-ground. The resulting material was mixed with physiological saline at a mass ratio of 1:10. The mixture was extracted in a refrigerator, then centrifuged, and the supernatant was passed through sterile Millipore bacteriological filters. The protein concentration in the final extract was determined using a colorimetric method with pyrogallol red and molybdate on a Beckman Coulter AU480 biochemical analyzer (Aminov et al., 2018).

The concentration of the extract was 0.022 mg/mL. Tissue samples from the wound bed and the adjacent intact area (within 1 cm from the wound edge) were collected on the day of application, and on days 3, 7, and 30 after injury. Skin samples were fixed in a 10% neutral formalin solution in dark glass containers and stored at room temperature for 3 days prior to histological analysis. Standard histological procedures were followed:

tissues were embedded in paraffin blocks, and serial sections (5 μm thick) were cut using a Thermo Scientific HM 325 microtome (Thermo Scientific, Massachusetts, USA). The sections were stained with hematoxylin and eosin. Microphotographs were taken using a PrimoStar iLED microscope and an Axio Cam ERc5s camera (ZEISS, Germany), followed by analysis using ZEISS ZEN 3.5 microscopy software (Blue Edition).

Statistical comparison of the results was performed between two samples for each measured indicator. Initially, comparisons were made between the intact group and the control group at each time point of wound healing, and between the intact group and the experimental group at each time point. Subsequently, the control and experimental groups were compared at each time point. Statistical analysis was performed using parametric methods (Student's t-test with Bonferroni correction), after verifying the normal distribution of the samples with a one-sample Kolmogorov-Smirnov test. Data were processed using IBM SPSS Statistics 21.0 software (USA). Differences were considered statistically significant at $p < 0.05$. The results of the morphological analysis of rat skin during wound healing were expressed as mean±standard deviation (mean±SD).

Results. As a result of the study, the epidermal thickness in the intact group was 14.05±2.43 μm (table), with indistinct boundaries between the epidermal layers, and their thickness corresponding to physiological norms (fig. 1a) (Makyeyeva et al., 2025 Mansfield et al., 2020; Jiao et al., 2024). The dermis occupied the major portion of the skin (cross-sectional thickness – 217.76±11.23 μm). The thickness of the subcutaneous tissue was 99.43±8.44 μm (table), primarily dispersed between the dermis and underlying muscle tissue. In the control group on day 3, the epidermal thickness in the periwound area increased significantly to 40.83 ±5.45 μm (table 1), which was statistically significantly higher than in both the intact and experimental groups ($p \leq 0.05$). The wound was covered by a scab, and inflammatory reactions were observed along the wound margins (fig. 1b). Leukocyte infiltration was evident in the dermis. The thickness of subcutaneous tissue increased by 3.77%, while the area of hair follicles decreased by 20.77%, and the area of sebaceous glands decreased by 34.17% compared to the intact group (table) ($p \leq 0.05$). Most of the hair follicles were mature, and some showed morphological signs of apoptosis. By day 7 in the control group, the wound remained covered by a scab. In contrast, in the first experimental group by day 3, granulation tissue had already formed in the wound bed, accompanied by active angiogenesis, reduced inflammation, and an almost complete absence of leukocyte infiltration at the wound site (figs. 1c, d). In comparison with the second experimental group treated with the reference preparation "Wundahyl" (fig. 1e, j, m), where intensive development of granulation tissue was observed only starting from day 7.

A large number of active and young newly formed hair follicles at various developmental stages were noted. No desquamation of the stratum corneum was observed, and only a small number of leukocytes were present in the papillary layer. The epidermal thickness in the periwound area was 16.80±2.33 μm (table), which did not differ significantly from the intact group, but was significantly lower than in the control group ($p \leq 0.05$). Also at this period, the control group showed signs of granulation tissue growth, fibroblast proliferation, collagenogenesis, and angiogenesis, along with the onset of re-epithelialization and marked inflammation (fig. 1g). A thin scab was present at the wound site, and numerous leukocytes were detected in the papillary layer (fig. 1g). Some residual inflammation and purulent inclusions were still observed beneath the epidermis. Upon scab separation, newly forming epithelium was observed. Epidermal thickness began to decrease, reaching 21.24±2.21 μm, which was still significantly higher than in both the intact and experimental groups (table). The thickness of the subcutaneous tissue decreased by 10.50%, while the area of hair follicles increased by 36.32% compared to the intact group ($p \leq 0.05$). Only a small number of hair follicles were present, many of which showed morphological signs of apoptosis. On day 7 in the first experimental group, there was a trend toward an increase in dermal thickness to 200.08±11.13 μm compared to the control group, along with a 34.43% increase in the area of hair follicles and a 14.24% decrease in the area of sebaceous glands compared to the intact group ($p \leq 0.05$) (table 1). The thickness of the subcutaneous tissue also increased by 19.36% compared to the intact group and by 27.83% compared to the control group (table) ($p \leq 0.05$). A substantial number of newly formed young hair follicles were observed, distributed across all skin layers. Intensive granulation tissue development and subsequent epithelialization were evident (figs. 1 h, i). Inflammatory cells were virtually absent in the dermis, and the tissue layers appeared more mature, with a high density of blood vessels and active hair follicles (figs. 1 h, i). The thickness of the epidermis began to decrease and did not differ statistically from that of freshly excised (intact) skin (table). On day 30, complete wound epithelialization was observed in both the control and experimental groups, with some areas showing scarring (figs. 1k, l, m). In the experimental groups, a large number of hair follicles were present (figs. 1 l, m). The wound area was fully covered with newly grown hair, and scarring was barely visible, in contrast to the control group where hair regrowth was minimal. In the the first experimental group, the thickness of the subcutaneous tissue as well as the area of hair follicles and sebaceous glands were significantly greater compared to both the control, intact and second experimental groups (table). A considerable number of hair follicles were observed across all skin layers in the first experimental group. Notably, hair follicles were also detected within the scar tissue of the experimental group. When comparing the efficacy of the leech-derived extract with the reference drug "Wundahyl", both agents demonstrated enhanced reparative properties relative to the control and intact groups (table). However, in the first experimental group treated with the medicinal leech extract, granulation tissue formation was already evident at early stages-from day 3-accompanied by active angiogenesis and reduced inflammation. In contrast, in the second experimental group, granulation tissue development was only observed starting from day 7. At later stages, the effects of both treatments were nearly equivalent when compared with the intact and control groups (table).

It is also noteworthy that under the influence of the reference drug, compared to the leech extract, the number and area of hair follicles were, on average, 28.87% lower, sebaceous glands were reduced by an average of 20.44%, and the thickness of the hypodermal layer was reduced by an average of 21.92% (table, figs. 1l, m).

Table

Morphological changes in an excisional wound (mean±SD)

Indicator	Intact (n=60)	Control group (n=30)			The first experimental group (n=30) water-salt extract of the medicinal leech			The second experimental group (n=30) ointment «Wundahyl»		
		Day 3 (n=10)	Day 7 (n=10)	Day 30 (n=10)	Day 3 (n=10)	Day 7 (n=10)	Day 30 (n=10)	Day 3 (n=10)	Day 7 (n=10)	Day 30 (n=10)
Thickness epidermis, μm	14.05±2.43	40.83±5.45*	21.24±2.21*	19.14±2.88	16.80±2.33**	15.59±2.01**	16.21±2.11	26.55±2.93*	19.41±2.77	17.68±2.23
Thickness dermis, μm	217.76±11.23	176.8±8.77*	187.1±11.01*	277.25±16.45*	187.10±12.76*	200.08 ±11.13	228.50±16.97**	180.8±7.66*	199.1±10.32	240.25±15.33**
Thickness subcutaneous tissue , μm	99.43±8.44	103.33±9.11	88.99±7.43	105.33±17.31	114.80±17.89	123.30±13.84*,**	155.45±11.76*,**	98.33±8.66	94.99±7.66	110.33±14.32
Hair follicle area, μm^2	296.00±17.77	234.51±12.17*	464.8±19.51*	292.70±17.69	330.79±17.66**	451.40±19.33*	539.50±23.24*, **	255.51±10.22*	440.8±17.44*	300.71±17.33
Sebaceous gland area, μm^2	490.50±20.63	322.91±18.31*	453.24±17.44	410.57±20.75*	605.47±24.27*,**	420.67±18.12*	630.63±25.33*,**	390.77±16.22*,**	410.13±15.32*	490.66±20.66**

* – Differences the control and experimental groups on days 3, 7, and 30 compared to the intact group, (p≤0.05)

**– Differences between the experimental and control groups on days 3, 7, and 30, (p≤0.05)

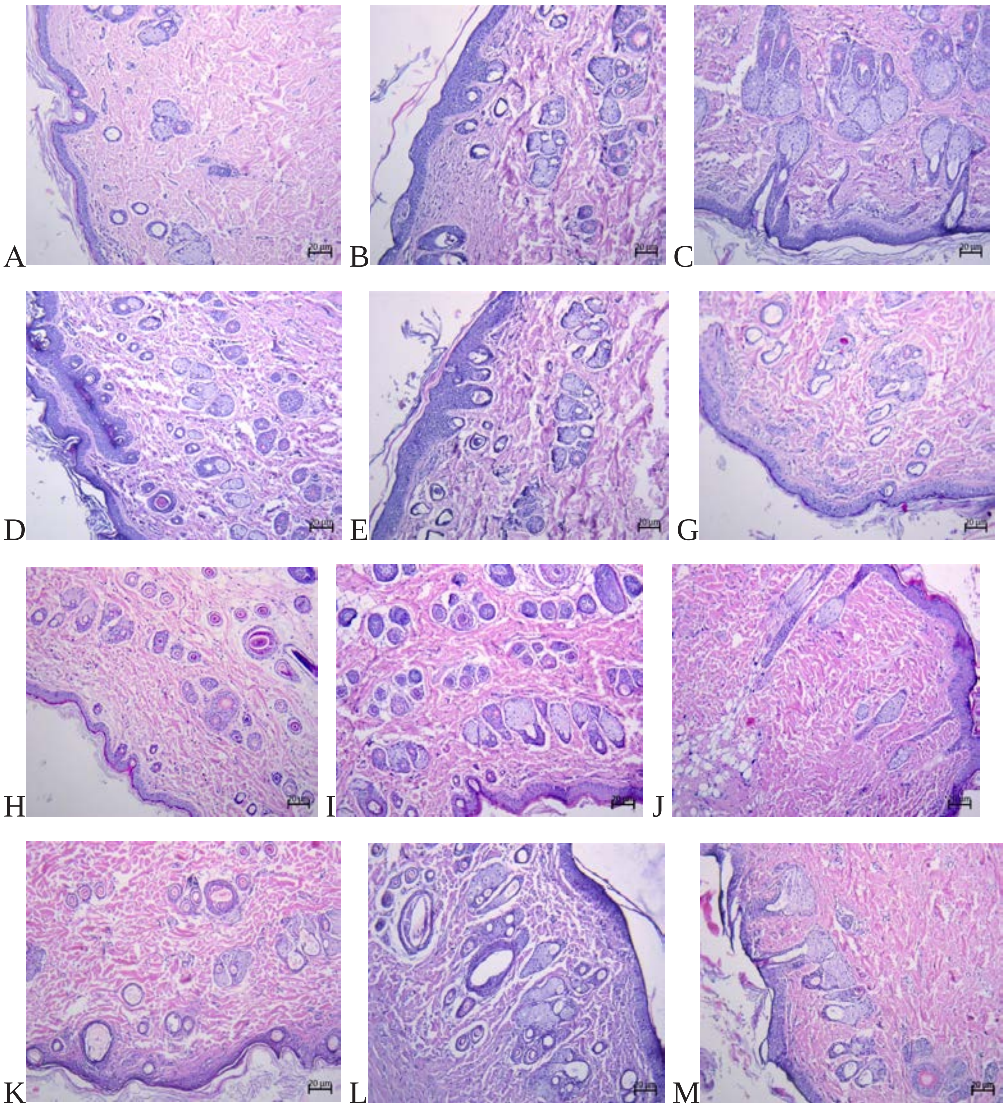

Fig. 1. Microphotographs of a skin flap excised in periwound and wound area (A–M) in control and experimental groups of rats at different periods of healing:
A – Day of wounding, intact group
B – Day 3 of wound healing, control group
C, D – Day 3 of wound healing, the first experimental group (water-salt extract of the medicinal leech)
D E – Day 3 of wound healing, the second experimental group (ointment E “Wundahyl”)
F G – Day 7 of wound healing, control group
G H, I – Day 7 of wound healing, the first experimental group (water-salt extract of the medicinal leech)
J – Day 7 of wound healing, the second experimental group (ointment H “Wundahyl”)
I K – Day 30 of wound healing, control group
J L – Day 30 of wound healing, the first experimental group (water-salt extract of the medicinal leech)
M – Day 30 of wound healing, the second experimental group (ointment K “Wundahyl”)

Discussion. Skin wound healing is a crucial and complex process consisting of a cascade of dynamic and tightly regulated cellular, humoral, and molecular mechanisms that begins immediately after injury and may last for years. This process is regulated through sequential but overlapping phases (Mansfield et al., 2020; Jiao et al., 2024). Typically, it proceeds through three overlapping stages: hemostasis/inflammation, proliferation/differentiation, and remodeling (Broughton et al., 2006; Gonzalez et al., 2016; Jiao et al., 2024). One of the factors that can accelerate wound healing is tissue perfusion, and BAS found in leeches contribute to this process by enhancing perfusion. In addition, ML release various anticoagulants into the wound, such as hirudin and factor Xa inhibitors, which prevent scab formation and thereby accelerate skin wound healing (Balasooriya et al., 2021; Aminov et al., 2023; Chhayani et al., 2023).

These findings are consistent with our own results, where we observed intensive granulation tissue formation in the experimental group as early as day 3, while the control group showed comparable granulation only by day 7. Furthermore, in the experimental group, a significant number of newly formed young hair follicles at various developmental stages were present at each observation time point. These follicles were located across all examined skin layers. This observed effect may be attributed to the action of leech-derived peptidases, which can influence the functional activity of various cell types, including endothelial cells, lymphocytes, platelets, and macrophages – thus promoting granulation tissue formation. Moreover, the water-salt extract may stimulate the production of cytokines, their receptors, and growth factors. During the wound healing process, growth factors play a critical role in the migration of neutrophilic granulocytes, monocytes, and fibroblasts to the site of inflammation. They also stimulate cell proliferation and collagen synthesis, enhance angiogenesis, and are involved in the morphogenesis of hair follicles. Additionally, the presence of anticoagulant substances such as kallikrein inhibitors, apyrase, and hirudin is essential in regulating blood coagulation mechanisms. The presence of eglin C also contributes to the reduction of free oxygen radicals in neutrophils, preventing inflammation and tissue destruction (Amani et al., 2021; Zakian et al., 2022). This anti-inflammatory effect is clearly observed in the experimental group by day 3, where inflammation and leukocyte presence are virtually absent. Rapid regrowth of new hair at the site of the excised flap, similar to what was observed in our experimental group, can be attributed to improved blood supply to the hair follicles as a result of enhanced local circulation and the formation of new, active follicular structures (Balasooriya et al., 2021; Chhayani et al., 2023). This effect may also be linked to the release of vasodilators, such as histamine-like substances, acetylcholine, and carboxypeptidase A inhibitors, which are capable of increasing local blood flow, reducing edema, and preventing venous congestion. These actions help restore proper tissue perfusion, supplying oxygen and nutrients essential for regeneration to the surrounding skin and hair follicles. Numerous studies have confirmed the presence of anti-inflammatory and vasodilatory agents in leech saliva (Amani et al., 2021; Zakian et al., 2022), which enhance microcirculation in tissues, support thrombolytic and immunostimulatory activities, and improve tissue nutrition and immune function (Balasooriya et al., 2021; Amani et al., 2021; Zakian et al., 2022; Aminov et al., 2023; Chhayani et al., 2023). Our results support these findings. As early as day 3, we observed a near absence of leukocytes and a marked reduction in inflammation in the experimental group, in contrast to the control group, where inflammation persisted through day 7. This suggests an accelerated transition from the inflammatory to the proliferative phase of wound healing, supported by the intense granulation tissue formation seen in the experimental group on both day 3 and day 7. Notably, by day 7, the subcutaneous tissue as well as regions containing hair follicles and sebaceous glands had increased significantly – especially in the experimental group – which may reflect enhanced proliferative activity induced by the water-salt extract of *Hirudo verbana* (Balasooriya et al., 2021; Zakian et al., 2022; Chhayani et al., 2023). Beginning on day 7 and continuing throughout subsequent time points, we also observed a significant increase in the number of blood vessels in the experimental group compared to controls. Similar effects have been reported by other authors. For instance, in the treatment of diabetic foot ulcers, hirudotherapy was associated with successful wound healing due to enhanced vascularization and reduced tissue stagnation (Balasooriya et al., 2021; Chhayani et al., 2023). It should also be noted that the water-salt extract of Hirudo verbana induced accelerated regenerative processes compared to the reference preparation “Wundahyl”. Specifically, the extract promoted faster reparative skin regeneration at the early stages of wound healing, which subsequently leveled out at the later stages in both treatment groups.

Conclusions. Complex of bioactive compounds present in the organism of the medicinal leeches significantly increase the number of newly formed active hair follicles and blood vessels, directly indicating the regenerative potential of these substances. By day 3, the first experimental group exhibited intense granulation tissue formation and a marked reduction in inflammation compared to both the control, intact and second experimental groups. From day 7 onward, an increase in the papillary dermal layer – responsible for nourishing the epidermis – was observed.

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