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Original article

EXCISIONAL WOUND MORPHOLOGICAL CHARACTERISTICS UNDER THE INFLUENCE OF MEDICINAL LEECH BIOLOGICALLY ACTIVE SUBSTANCES

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Background. The stages of wound healing following surgery are generally consistent, but immune responses and increased inflammation can delay the normal healing process. As a result, additional support is crucial. Perfusion plays an important role in accelerating skin wound healing, and the saliva of medicinal leeches has been shown to enhance this process.
Objective. To evaluate the effect of the medicinal leech *Hirudo verbana* on the morphological changes in an excisional wound.
Methods. An excisional wound was created in the interscapular region of animals in both the control and experimental groups. In the experimental groups, one medicinal leech was applied on days 1, 3, 7, and 14. Tissue samples from the wound edge were collected immediately after leech application, and then at days 3, 7, 14, and 30 during the healing process. These samples were processed using standard histological techniques.
Results. In the experimental group, intensive formation of granulation tissue was observed as early as day 3, compared to the control group. An increase in the thickness of the papillary layer, which was vascularized and supported epidermal nutrition, was noted in the experimental group at almost all time points. This may have contributed to enhanced proliferation processes, an increase in the number of hair follicles and sebaceous glands, and a reduction in scar size. On day 3, the number of leukocytes decreased, signaling a reduction in inflammation compared to the control group. By day 7, a significant reduction in subcutaneous tissue and the areas of hair follicles and sebaceous glands was observed, suggesting an increase in basal metabolic activity.
Conclusions. The complex of biologically active substances from the medicinal leech *Hirudo verbana* positively affects the processes of reparative regeneration in excisional wounds, accelerating all stages of wound healing. It significantly increases the number of newly formed hair follicles and blood vessels, directly indicating the regenerative properties of these substances.

Keywords: wound healing; periwound; epidermis; dermis; immune system.

Introduction

The skin is the largest organ of the human and animal body, serving as a barrier against the external environment and its harmful factors, while also playing a critical role in the immune system. A wound is a physical injury that results in a rupture or disruption of the skin's integrity. Wound healing is a physiological process necessary for maintaining the integrity and function of the skin or tissues by repairing defects that may occur due to accidents, injuries, illnesses, or surgeries [1–12].

The healing process begins immediately after an injury and involves a complex, well-organized interaction between different tissue types and cells. Normal healing of skin wounds consists of three interconnected stages: the inflammatory, proliferative, and remodeling phases [11, 13–16]. The inflammatory stage is characterized by the involvement of leukocytes, such as neutrophils and macrophages, at the wound site. During the proliferative phase, the migration and proliferation of keratinocytes, fibroblasts, and endothelial cells lead to re-epithelialization and granulation tissue formation. In the remodeling phase, excess collagen at the wound site is broken down by various proteolytic enzymes, completing tissue repair [11, 14, 17, 18].

Although the stages of wound healing following surgery are similar to those of other wounds, immune responses and increased inflammation in the body can delay the normal healing process. Therefore, additional support is crucial [18–23]. Furthermore, perfusion is a key factor in accelerating skin wound healing, and the saliva of medicinal leeches (ML) has been shown to enhance perfusion. Hirudotherapy the use of ML is one of the most effective and natural methods for supporting wound healing, as ML contain more than 100 biologically active substances (BAS) with a wide range of therapeutic effects [17, 24–32]. Due to these

properties, ML are beneficial for post-traumatic wounds, such as those occurring during replantation of amputated tissues or plastic surgery [17, 18, 30–33]. They can also be used to treat chronic non-healing wounds, such as diabetic foot ulcers, pressure sores, and venous leg ulcers, as demonstrated experimentally [31–34].

Studies in rats and mice have shown that ML can reduce necrosis by improving blood flow in the bite area and enhance the wound healing process. Species such as *Hirudo orientalis* and *Hirudo medicinalis* have been shown to accelerate the healing of primary incised skin wounds in rats within 24 hours after surgery [17, 18, 35, 36]. In a mouse model, the use of ML was found to reduce necrosis and increase the survival rate of heart flaps to an average of 88%, compared to the control group [17]. Additionally, our previous studies and those of other researchers have demonstrated the effectiveness of hirudotherapy in restoring organs, both under normal conditions and after traumatic injuries. For example, in the early stages of human ear wound healing, the use of ML has been shown to promote signs of adequate revascularization, and at later stages, complete revascularization is observed [17, 18]. Our earlier findings also revealed positive physiological and reparative regeneration of the thymus and spleen in rats treated with ML or their water-salt extract [24–29]. ML are also widely used to address venous congestion; for example, they can support venous drainage and revascularization of a reimplanted lip.

As a result, lip reimplantation proceeds without complications [37]. In cases of nasal fractures, the flap used remains infection-free and viable throughout treatment. In surgical reimplantation of fingers, even in cases of gangrene, pain and necrosis are reduced [38]. In a case of a persistent, non-healing, large ulcer on the lower leg, caused by scratching after a mosquito bite, hirudotherapy promoted the rapid appearance of healthy granulation tissue, leading to fast wound healing [17]. The combination of *Pongamia pinnata bark* and hirudotherapy has been shown to accelerate the healing of large ulcerative wounds and reduce pain [39]. In necrotizing ulcers associated with systemic scleroderma, leech therapy resulted in quick and complication-free wound healing [17, 40]. In diabetic ulcers, the use of leeches has been observed to increase vascularization and reduce wound congestion, leading to the disappearance of necrotic tissue and rapid wound healing [34, 41].

Given the broad therapeutic effects of ML, it is hypothesized that ML may accelerate wound healing by secondary intention. Therefore, it became relevant to study the effect of *Hirudo verbana* on the morphological changes in an incised wound.

Materials and Methods

The primary method for studying histological samples, including skin, remains light microscopy, which is widely utilized in both clinical and experimental settings. Assessing the morphological structure of the skin is essential for obtaining objective data regarding its condition under normal, experimental, and pathological conditions. Histological analysis allows for the detection of morphological changes in organs, tissues, and cells in response to various influences. It provides a detailed comparison of morphological alterations alongside clinical, biochemical, and pathophysiological findings, making it a valuable method for evaluating new drugs. In our studies, we selected *Hirudo verbana*, a species of medicinal leech, due to its wide range of therapeutic effects, which have been substantiated by numerous scientific publications [30–41].

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 No. 05.03.02-06/49982, dated 12.08.2009).

For our experiment, we used a model of excisional skin wounds, where round sections of skin with a diameter of 1.5 cm were excised using a template. This model of secondary tension is widely adopted by researchers to study the effects of drugs, pathologies, treatments, and other factors [3–5, 42, 43]. This model allows for a more precise and effective evaluation of various effects, including the regenerative properties of therapeutic agents. The primary aim of our study was to investigate the effect of *Hirudo verbana* on morphological changes in an incised wound in a laboratory rat model, with the goal of confirming the hypothesis that ML possess reparative regenerative properties.

The study was conducted on 60 male white laboratory rats weighing between 245–260 g. The experimental animals were housed under standard sanitary conditions. Following the creation of an incised experimental wound, the animals were placed in separate cages with weekly bedding replacements under aseptic conditions. During the observation period, no signs of bacterial contamination were observed in any of the animal groups. The animals

were kept in a vivarium maintained at a temperature of 20–25 °C, with humidity not exceeding 55%, under a natural "day-night" cycle. They were individually housed in plastic cages and fed a balanced diet. Experimental procedures were carried out in accordance with the "International Guidelines for Medicinal and Biological Research Using Animals" and the national "Joint Ethical Principles of Experiments on Animals" (Ukraine, 2001), in compliance with Council Regulation (EU) 2010/63/EU of the European Parliament and 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 wound model was created as follows: under ketamine anesthesia (40 mg/kg body weight), after hair removal from the back of the animals, aseptic and antiseptic procedures were strictly followed. Prior to the experiment, the animals were randomly divided into two groups of 30 animals each: Group 1 (control) where wound healing occurred spontaneously without treatment, and Group 2 (experimental), where one medicinal leech (*Hirudo verbana*) weighing 0.6-0.7 g was applied near the affected area on days 1, 3, 7, and 14. Circular areas of skin with a diameter of 1.5 cm were excised using a template and surgical scissors. Excised material from both groups at the day of wounding ($n = 60$) was assigned to the intact group for comparison.

Treatment began 24 hours after the wound was created. Samples from the wound bed and adjacent intact group (up to 1 cm beyond the wound edge) were collected on days 3, 7, 14, and 30 after the injury. The periwound area (up to 5 mm beyond the wound edge) was also analyzed. The time points were chosen in accordance with the modern periodization of wound healing, where days 1 and 3 correspond to the inflammatory phase, days 7–14 to the proliferative phase, and day 30 to the remodeling phase [42].

Skin samples were fixed in a 10% neutral formalin solution in dark glass containers and stored at room temperature for 3 days before histological analysis. The skin was then processed using standard histological techniques, 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. Photomicrographs were captured using a PrimoStar iLED microscope and an Axio Cam ERc5s camera (ZEISS, Germany), with subsequent analysis using the ZEISS ZEN 3.5 microscopy software (blue edition).

Statistical comparison of results was performed between two samples of each studied indicator. First, the analysis was performed between the intact group and control at each time point of healing and beteween the intact group and experimental group at each time point of healing. Later, the control and experimental groups were compared at each time point of healing.

Statistical analysis of the obtained data was performed using parametric methods (Student's t-test with Bonferroni correction) after verifying the samples for normal distribution with the one-sample Kolmogorov–Smirnov test. IBM SPSS Statistics 21.0 (USA) was used for statistical processing. Differences were considered statistically significant at $p < 0.05$. The results of the study of morphological features of rat skin during wound healing were expressed as the arithmetic mean and standard deviation (mean ± SD).

Results

As a result of the study, the thickness of the epidermis in the intact group was 14.82 ± 2.12 (the Table), the boundaries of the epidermal layers were indistinct, their thickness corresponded to physiological norms (the Figure, a) [2, 8, 11, 23]. The dermis occupied the main part (thickness in cross section – 174.22 ± 9.11 μm) of the skin. The thickness of the subcutaneous tissue was 111.61 ± 13.89 μm (the Table).

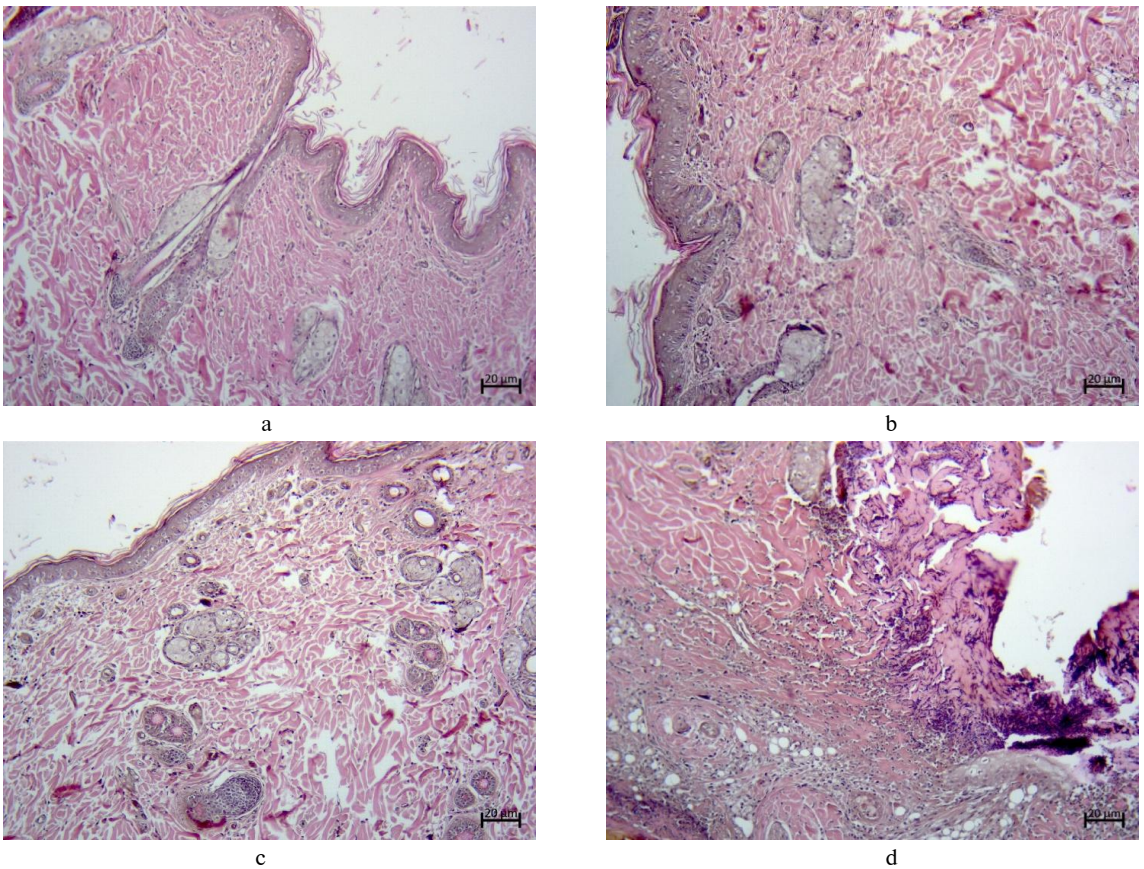
It contained a large number of adipocytes. In the control group, by day 3, the wound was covered with a scab, with areas showing purulent foci. Demarcation inflammation was observed at the edges of the wound, accompanied by leukocyte infiltration and the formation of a leukocyte shaft, which extended across all layers of the dermis (the Figure, b). The epithelial structures at the wound edges exhibited vacuolization and desquamation of cells down to the basal layer, with a small number of hair follicles in the catagen phase. The thickness of the epidermis in the periwound area was 31.11 ± 4.67 μm (the Table), which was statistically significantly greater than that of the intact group ($p \leq 0.05$).

Infiltration of the dermis by leukocytes and increased vascularization were observed. Subcutaneous tissue thickness increased by 23.08%, while the area of hair follicles decreased by 40.08%, and the area of sebaceous glands reduced by 50.40% compared to the intact group (the Table) ($p \leq 0.05$).

Table: Morphological changes in an excisional wound (mean ± SD)

Indicator	Intact (s = 60)	Control (s = 30)				Experiment (s = 30)			
		Day 3 (n = 8)	Day 7 (n = 8)	Day 14 (n = 7)	Day 30 (n = 7)	Day 3 (n = 8)	Day 7 (n = 8)	Day 14 (n = 7)	Day 30 (n = 7)
Thickness epidermis, μm	14.82 ± 2.12	31.11 ± 4.67*	37.76 ± 4.87*	30.78 ± 5.11*	14.1 ± 2.11	21.44 ± 2.23*,**	18.57 ± 2.12**	15.77 ± 2.45**	13.96 ± 2.33
Thickness dermis, μm	174.22 ± 9.11	155.3 ± 15.44	180.7 ± 10.12	176.5 ± 8.76	173.37 ± 9.34	183.00 ± 9.97**	172.,11 ± 1 0.11	182.81 ± 9.88	209.51 ± 13.01*,**
Thickness subcutaneous tissue, μm	111.61 ± 13.89	145.10 ± 13.32*	76.51 ± 9.77*	81.30 ± 10.89*	105.33 ± 7.66	151.09 ± 14.92*	40.21 ± 5.91*,**	188.44 ± 17.21*,**	253.00 ± 18.67*,**
Hair follicle area, μm^2	259.40 ± 14.89	155.42 ± 13.21*	162.32 ± 12.76*	223.23 ± 19.89	230.21 ± 17.76	234.30 ± 18.87**	173.40 ± 11.44*	556.21 ± 23.11*,**	517.92 ± 24.32*, **
Sebaceous gland area, μm^2	430.63 ± 19.94	213.56 ± 16.44*	253.84 ± 15.65*	313.82 ± 16.38*	405.11 ± 21.44	636.11 ± 25.33*,**	185.44 ± 16.44*,**	430.62 ± 18.35**	506.11 ± 20.14*,**

Notes. * – differences the control and experimental groups at each time point of wound healing (days 3, 7, 14 and 30) compared to the intact group ($p \leq 0.05$), ** – differences between the experimental and control groups at each time point of wound healing (days 3, 7, 14 and 30) ($p \leq 0.05$).



a b c d

Figure: Microphotographs of a skin flap excised in periwound and wound area (a, o) 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) day 3 of wound healing, experimental group; (d) day 3 of wound healing, experimental group – granulation tissue is formed

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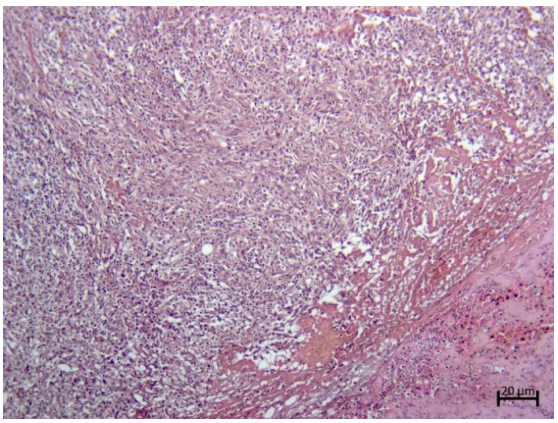

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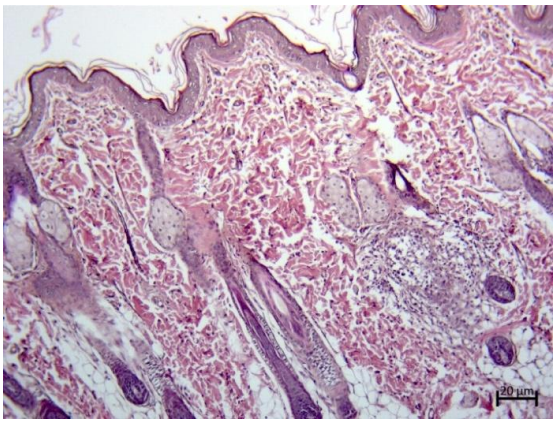

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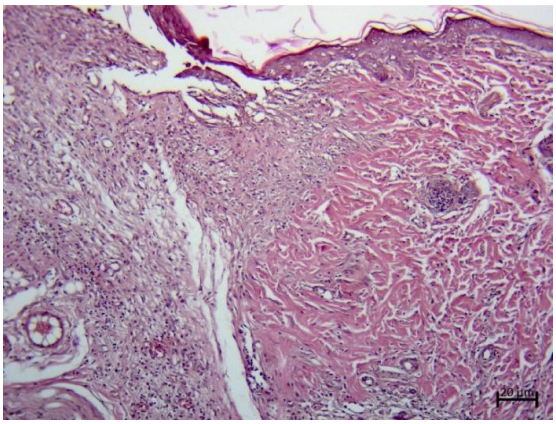

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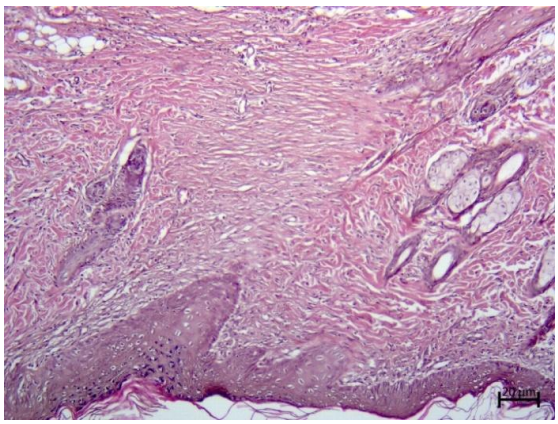

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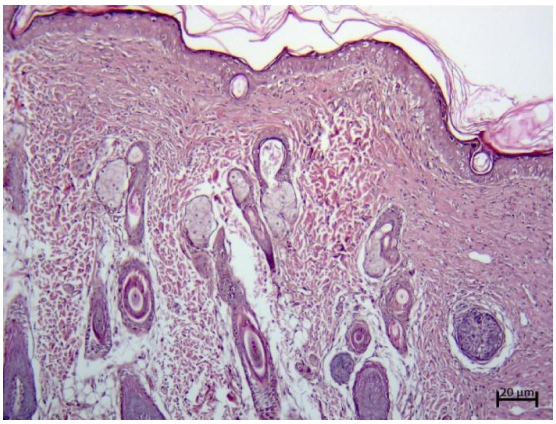

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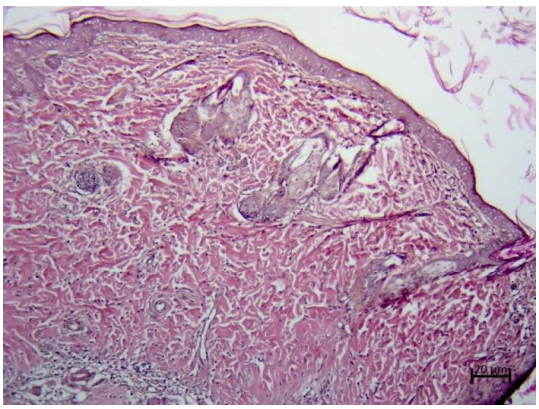

k

Figure [*Continuation*]**:** Microphotographs of a skin flap excised in periwound and wound area (a, o) in control and experimental groups of rats at different periods of healing: (e) day 7 of wound healing, control group; (g) day 7 of wound healing, control group – granulation tissue is formed; (h) day 7 of wound healing, experimental group; (i) day 7 of wound healing, experimental group – granulation tissue; (j) day 14 of wound healing, control group; (k) day 14 of wound healing, experimental group

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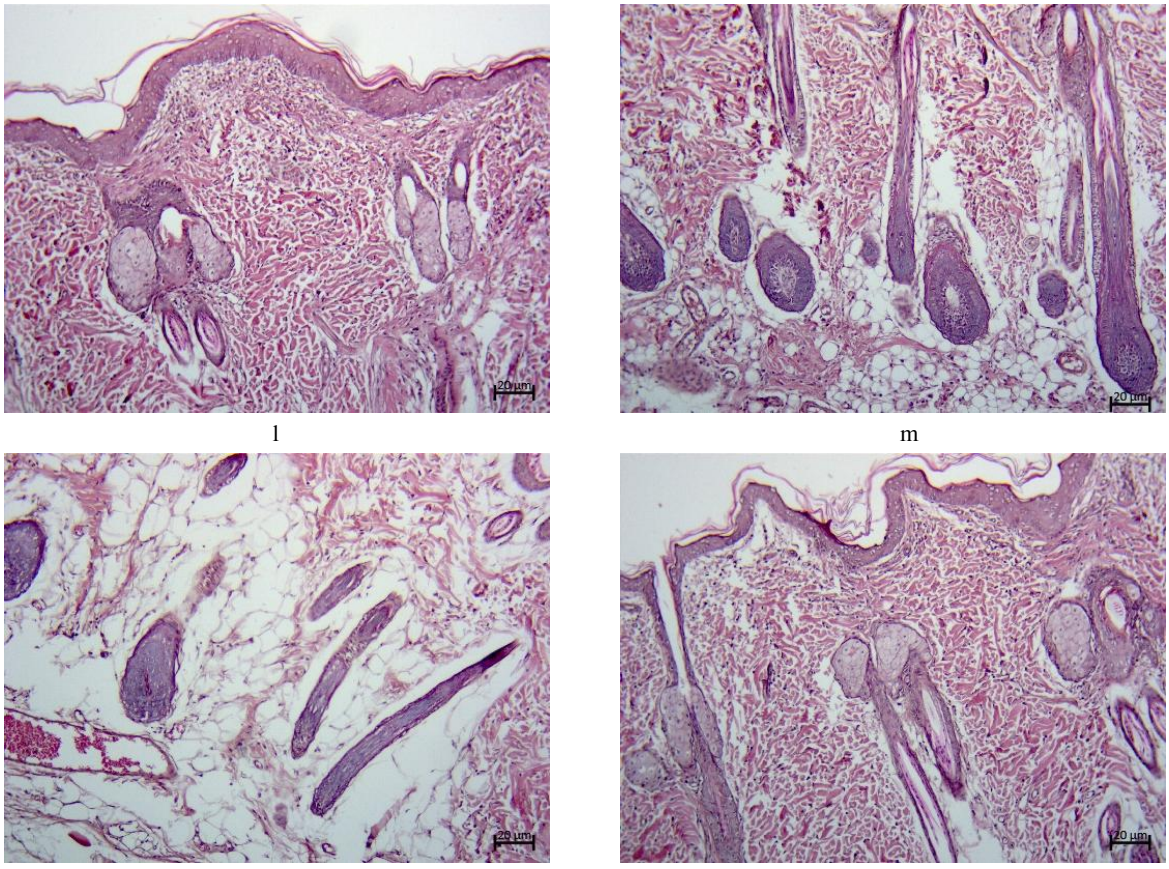

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Figure [*End*]**:** Microphotographs of a skin flap excised in periwound and wound area (a, o) in control and experimental groups of rats at different periods of healing: (l) day 14 of wound healing, experimental group; (m) day 30 of wound healing, control group; (n) day 30 of wound healing, experimental group; (o) day 30 of wound healing, experimental group

In the experimental group, by day 3, granulation tissue had already formed in the wound bed, with activation of angiogenesis, reduced inflammation, and infiltration of the lesion site by leukocytes (the Figure, d). A large number of hair follicles, with 2-3 hair shafts, were noted. No desquamation of the stratum corneum was observed, and only a small number of leukocytes were present in the papillary layer. In the periwound epithelium, there were no clear boundaries with the dermis, and the papillary layer was enlarged (the Figure, c). The thickness of the epidermis in the periwound area was 21.44 ± 2.23 μm (the Table), which was statistically significantly greater than that of the intact group and significantly lower than in the control group ($p \leq 0.05$).

On day 7, the dermis thickness in the experimental group increased to 183.00 ± 9.97 μm, with a 33.67% increase in the area of hair follicles and nearly a threefold increase in sebaceous glands compared to the control group ($p \leq 0.05$). The subcutaneous tissue thickness also increased by 26.13%, and the area of sebaceous glands increased by 32.30% compared to intact group (the Table) ($p \leq 0.05$). In the control group, by day 7, there was intensive growth of granulation tissue, fibroblast proliferation, collagenogenesis, and angiogenesis, along with the onset of re-epithelialization and moderately pronounced inflammation (the Figure, e, g). A thin scab had formed at the wound site, and a large number of leukocytes were present in the papillary layer (the Figure, e). Below the epidermis, slight inflammation and purulent inclusion were still present. As the scab detached, a new epithelium began to form. The thickness of the epidermis reached a maximum value of 37.76 ± 4.87 μm, significantly greater than in intact group (the Table). The thickness of the subcutaneous tissue decreased by 31.45%, and the areas of hair follicles and sebaceous glands decreased by 37.43% and nearly 50%, respectively ($p \leq 0.05$).

In the experimental group on day 7, there was intensive development of granulation tissue and further epithelialization (the Figure, h, i). Fewer inflammatory cells were observed in the dermis, and the layers appeared more mature, with a significant number of blood vessels and active hair follicles present (the Figure, h). The thickness of the epidermis began to decrease but did not statistically differ from the freshly excised skin (the Table). A sharp statistical decrease was noted in the thickness of the subcutaneous tissue (nearly threefold), as well as in the area of hair follicles and sebaceous glands, both of which decreased by nearly 50% compared to the control group (the Table).

By day 14, complete epithelialization of the wound surface and remodeling of the dermal connective tissue were observed in the control group (the Figure, j). Significant flaking of the stratum corneum was noted, with a small number of hair follicles and sebaceous glands. The boundary between the dermis and epidermis was not clearly defined. The epithelium had developed further, with visible boundaries between all layers (the Figure, j). Inflammation was absent, and the wound was covered by a thin epithelium. The thickness of the epidermis began to decrease, while the hypodermal layer and the area of sebaceous glands increased (the Table).

In the experimental group on day 14, no inflammation was present, and numerous well-formed hair follicles were observed (the Figure, k, l). The tissue appeared more mature, with a large number of blood vessels and newly formed hair follicles in the active phase (the Figure, k). Accelerated epithelialization was noted, and scarring had occurred. The thickness of the epidermis did not statistically differ from that of the intact group, but there was an increase in the thickness of the subcutaneous tissue, as well as in the areas of hair follicles and sebaceous glands, compared to the control group.

On day 30, both the control and experimental groups showed complete epithelialization of the wound (the Figure, m, n, o). In the experimental group, the papillary layer of the dermis was enlarged, and a significant number of hair follicles were observed (the Figure, n, o). The wound was fully covered with new hair, and scarring was barely noticeable, compared to the control group, where hair coverage was not restored. In the control group, all measured parameters were within the range of intact group values (the Table). In the experimental group, the thickness of the dermis and subcutaneous tissue, as well as the areas of hair follicles and sebaceous glands, were significantly larger compared to both the control group and intact group (the Table).

Discussion

Wound healing is a complex process involving the interaction of molecular and cellular regulatory mechanisms, both locally and systemically [4–8]. It typically progresses in three overlapping phases: hemostasis/inflammation, proliferation/differentiation, and remodeling [3–7, 14, 15, 17]. One of the factors that can accelerate healing is perfusion, and BAS found in ML contribute to this process through their ability to enhance perfusion. ML secrete various anticoagulants, such as hirudin and factor Xa inhibitors, into the wound, preventing scab formation and thereby accelerating the healing process [17, 24–31].

The complex of BAS in ML can be categorized into several groups: lytic compounds that destroy tissues and microvessels; antihemostatics that block hemostasis mechanisms; blockers of the body's defense reactions that counteract the inflammatory responses to tissue damage; and auxiliary substances [24, 35, 36, 39]. For instance, in a study on rat epigastric flaps with venous impairment, leech treatment before definitive surgical revascularization was shown to partially reduce the degree of flap necrosis due to venous impairment [17, 33, 37]. Other studies have also demonstrated the effectiveness of ML in treating venous congestion in flaps [37]. These findings align with our results, where we observe accelerated reparative regeneration in rat skin. In the experimental group, intensive granulation tissue formation was already evident on day 3, compared to the control group, which only showed similar formation on day 7. By day 7, the experimental group's wound healing was comparable to the control group at day 14.

This accelerated healing may be linked to the presence of peptidases in the ML, which influence the functional activity of various cells, such as endothelial cells, lymphocytes, platelets, and macrophages, thereby promoting granulation tissue formation. Additionally, antihemostatic substances like calins, apyrase, platelet-activating factor antagonists, and hirudin play a role in regulating blood clotting mechanisms. The presence of eglin C also reduces free oxygen radical levels in neutrophils, preventing inflammation and tissue destruction [25, 35].

In our study, the papillary layer was noticeably thicker in the experimental group across all time points. This layer, which is rich in vessels and

responsible for epidermal nutrition, likely facilitated proliferation and increased the number of hair follicles and sebaceous glands, while reducing scarring, as observed in our results. The faster regrowth of new hair may be attributed to improved blood supply to hair follicles due to enhanced circulation in the wound area an effect of ML observed by other researchers [18, 35, 36]. These effects are likely due to the release of vasodilators, such as histamine-like substances, acetylcholine, and carboxypeptidase-A inhibitors, which increase blood flow to the site of the bite and reduce local swelling. Acetylcholine, in particular, can relax endothelial muscles, dilate blood vessels, and promote microcirculation, delivering fresh oxygenated blood to the affected area [39, 40]. This restores normal blood flow and provides tissues and hair follicles around the wound with the oxygen and nutrients required for regeneration.

It is also important to note that similar reparative regenerative abilities, though to a lesser extent and at different observation periods, were observed in a rat skin cut wound model treated with local applications of indigo leaf extract, recombinant leptin, or kasticin. These treatments significantly enhanced the healing process, accelerating wound closure with a noticeable increase in collagen formation, angiogenesis, and epithelialization [44–46].

Numerous studies have demonstrated the presence of anti-inflammatory and vasodilatory agents in leech saliva [17, 31], which improve blood circulation in certain organs, promote thrombolytic, anti-inflammatory, and immunostimulatory activities, and enhance tissue nutrition and immune function [24–32]. For instance, the enzyme hyaluronidase found in leech saliva can degrade tissues containing hyaluronic acid and also acts as an antimicrobial agent. It increases the viscosity of interstitial walls, facilitating the antibiotic action of other substances in the saliva. This enzymatic action supports the infiltration and diffusion of additional leech-derived substances into deep or congested tissues. Other key anti-inflammatory compounds include antistasin, hilantens, guamerin, piguamerin, bdelastasin, bdelins, and eglins [39].

The presence of these substances is corroborated by our results. For example, starting on day 3, we observed a reduction in leukocyte content and a decrease in the inflammatory process compared to the control group, which was no longer present by day 7. This suggests an accelerated transition from the inflammatory phase to the proliferative phase, as evidenced by the early development of granulation tissue in the experimental group. Our findings are consistent with the work of other researchers, who observed similar outcomes under the influence of the plant alkaloid sinomenine, which demonstrated significant anti-inflammatory effects during wound healing in rats [47].

This observed effect may be attributed to the presence of bdelins, ellins, and the multifunctional protein destabilase-lysozyme, which not only has destabilase activity but also exhibits lysozyme and antimicrobial properties. As an antibiotic, destabilase inhibits the growth of many bacteria, fungi, and archaea. Moreover, substances such as chloromycetin, theromycin, thermisin, and destabilase in leech saliva demonstrate strong antimicrobial activity by destroying bacterial cell components. The denatured form of destabilase exhibits a dose-dependent bacteriostatic effect against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* [39, 40].

It is also important to note that on the 7th day, the subcutaneous tissue, as well as the areas of hair follicles and sebaceous glands, are significantly reduced, particularly in the experimental group. This reduction may indicate an increase in basal metabolism and accelerated metabolism during the proliferation phase, which is associated with the appearance of newly formed active hair follicles. This effect has been observed in previous studies, where ML were shown to influence metabolism [17, 25–27]. Our findings are consistent with those of other researchers, such as the study on *P. polyphylla* in rats, which found that it accelerated diabetic wound healing and restored the morphological structure of epidermal layers, granulation tissues, hair follicles, fibroblasts, and collagen [38–41].

Furthermore, starting from day 7, we observed a significant increase in the number of blood vessels in the experimental group, a trend that continued through all subsequent time points compared to the control group. Based on these effects, it can be concluded that the enzymes present in the saliva of *Hirudo verbana* have a pronounced impact on damaged tissues, promoting angiogenesis and enhancing blood flow to the affected areas. The vasodilatory substances in leech saliva also contribute to alleviating venous congestion and accelerating the phases of wound healing [18]. Our results are the first to demonstrate the substantial reparative and regenerative effects of *Hirudo verbana* in wound healing by secondary intention.

Conclusions

It should be noted that under the influence of the complex substances found in ML, the number of newly formed active hair follicles and blood vessels is significantly increased, directly indicating the regenerative properties of these substances. By day 3, intensive granulation tissue formation and a noticeable reduction in inflammation are observed. Starting from day 7, the papillary layer, which is responsible for nourishing the epidermis, begins to increase. By day 14, the epidermis indicators in the experimental group align with those of the intact animals. The significant increase in the number of newly formed hair follicles and vessels further underscores the regenerative effects induced by the substances in ML.

Interests Disclosure

The authors have no conflict of interest to declare.

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МОРФОЛОГІЧНА ХАРАКТЕРИСТИКА РІЗАНОЇ РАНИ ПІД ВПЛИВОМ БІОЛОГІЧНО АКТИВНИХ РЕЧОВИН МЕДИЧНОЇ П’ЯВКИ

Проблематика. Стадії загоєння ран після операції подібні до інших, але імунна відповідь і посилення запалення в організмі можуть призвести до затримки нормального перебігу загоєння, тому додаткова підтримка є дуже важливою. Крім того, перфузія вважається важливим фактором прискорення загоєння ран шкіри, а слина медичних п’явок має цю здатність.
Мета. Оцінка впливу медичної п’явки *Hirudo verbana* на морфологічні зміни в різаній рані.
Методика реалізації. У контрольній та експериментальній групах тварин було нанесено різану рану в міжлопатковій ділянці. В експериментальних групах наносили по одній медичній п’явці на 1, 3, 7 та 14-й дні. Матеріал із крайової зони рани збирали відразу після нанесення, на 3, 7, 14 та 30-й дні загоєння рани та обробляли за стандартними гістологічними методиками.
Результати. В експериментальній групі вже на 3-й день спостерігається інтенсивне утворення грануляційної тканини порівняно з контролем. Збільшення сосочкового шару, який пронизаний судинами та відповідає за живлення епідермісу, спостерігалося в експериментальній групі майже у всі терміни експерименту. Це могло вплинути на процеси проліферації та збільшення кількості волосяних фолікулів, сальних залоз і зменшення розміру рубців. На 3-й день вміст лейкоцитів зменшується і запальний процес починає знижуватися порівняно з контрольною групою. Гіподерма, площа волосяних фолікулів і сальних залоз значно зменшуються на 7-й день, що може свідчити про підвищення основного обміну речовин.
Висновки. Комплекс речовин медичної п’явки *Hirudo verbana* здатний позитивно впливати на процеси репаративної регенерації різаної рани, прискорювати всі стадії загоєння рани. Під впливом комплексу речовин медичної п’явки кількість новоутворених активних волосяних фолікулів і судин значно збільшується, що прямо вказує на регенеративну властивість цих речовин.

Ключові слова: загоєння ран; крайова зона ран; епідерміс; дерма; імунна система.