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Treatment of burns with polyethylene glycol-based preparations: Dynamics of regeneration at the biochemical and histological levels of organization in rats

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In Ukraine, more than 12 thousand children and a large number of adults suffer burn injuries yearly. Almost one in four cases is classified as severe or extremely severe. Currently, the system of medical care for burn survivors is in an extremely critical condition. Ukraine is among the five countries with the highest rate of deaths caused by burns in the world. Before the full-scale war, most burn injuries resulted from domestic incidents: careless handling of kitchen appliances, electrical appliances or pyrotechnic products. In adults, in 80% of casualties, the cause was fire, and among children (70%) – boiling water. After 2022, the situation has changed dramatically. In 2023 alone, more than 35% of the victims sustained burns during military attacks. Mine-explosive injuries are considered particularly severe and are often accompanied by extensive burns of various parts of the body. Such injuries require immediate surgery, professional assistance and a long-term rehabilitation process. In addition, due to regular shelling of critical infrastructure facilities and interruptions in electricity and gas supply, the number of household burns has increased dramatically. The most common causes are explosions of gas cylinders, ignition of generators, fires in homes, and boiling water burns. This article presents the findings of an experimental study of the effectiveness of treatment of burn injuries using anti-burn agents. In laboratory rats, thermal skin injuries with an area of up to 10% of the total body surface were induced, which corresponded to II–III degree burns. During the experiment, the therapeutic effect of the preparation under study, which was developed based on polyethylene glycol, was compared with the officially registered medical product – Pantestin ointment. Particular attention was paid to the analysis of pathophysiological and pathomorphological changes accompanying the course of burn injury. The most pronounced therapeutic effect was recorded with the use of the experimental ointment based on polyethylene glycol. On the fifth day of the experiment, the survival rate of animals in the group receiving this product was 100%, while for the group treated with Pantestin, the rate was 60%, and among untreated control animals, the mortality rate reached 40%.

Keywords: metabolism; pathomorphology; biochemistry, burn stress; rats, polyethylene glycol.

Introduction

Skin integrity is critical for the normal functioning of the body (Wang & Zhang, 2019; Garcia & Torres, 2020). Skin is a barrier protecting the internal environment from fluid loss, infection and the effect of external factors. It is involved in the regulation of body temperature, metabolism (in particular, vitamin D synthesis), and also provides neurosensory perception and immune protection. Skin is a complex structure originating from the ectodermal and mesodermal germ layers and consists of two main layers – the epidermis and the dermis (Lopez & Hernandez, 2021; Borovuk & Zazharska, 2022; Chen & Zhao, 2023). The epidermis is an outer non-vascular layer formed by multiple layers of epithelial cells. Its thickness varies depending on the anatomical area of the body. The dermis (corium) is located under the epidermis and is represented by connective tissue, where collagen predominates. It contains a branched microvascular network, including arterioles, venules and capillaries. There is a close functional and structural connection between the epidermis and the dermis, which ensures the integrity of the skin as an organ. In the dermis, skin appendages are localized – sudoriferous and sebaceous glands, as well as hair follicles. The epithelial cells lining these structures enable the dermis to regenerate the damaged epidermis, in particular in case of thermal injuries (Kim & Park, 2018; Sklyarov et al., 2020).

Skin also contains numerous nerve endings playing a key role in the perception of pain, temperature, touch and other sensations. It is the large number of receptors that causes severe pain in burns. Dermis connective tissue acts as the support framework of the skin, ensuring its elasticity and strength (Singh & Kumar, 2021). In thermal injuries, the structure of the skin is disrupted, which leads to serious systemic changes: significant loss of water and electrolytes, thermoregulation disorders and increased susceptibility to infections. This type of damage poses not only local but also general risks, affecting the homeostasis of the whole body (Tkachenko et al., 2016; Singh & Patel, 2019; Kim & Lee, 2023).

Burns are one of the most common and detrimental forms of injury. Patients with severe thermal injuries require immediate specialized care to minimize morbidity and mortality. Scientists are considering novel biomaterials used to treat burn injuries, including hydrogels, nanomaterials, and biocompatible bandages (Evans & Nguyen, 2019). The authors discuss the advantages and limitations, as well as the prospects for their clinical use to stimulate regeneration and prevent infections. Zhao & Chen (2020) reviewed preclinical studies of the use of mesenchymal stem cells (MSCs) and their secretion in the treatment of burn wounds. The authors describe the mechanisms of action of MSCs – stimulation of angiogenesis, anti-inflammatory activity, maintenance of cellular homeostasis, as well as their potential for clinical application. Miller & Johnson (2021) show that hypoxia can both stimulate angiogenesis and cause chronic inflammation inhibiting the regeneration and healing of burn wounds. Zhang & Li (2019) provide a detailed description of macrophages (M_1 and M_2) polarization and their effect on the balance between inflammation and recovery, which is important for the development of targeted therapy for

burn injuries. Bi & Sun (2020) believe that targeted therapy to regulate miR-27b may be an effective way to accelerate skin regeneration after thermal injuries.

Development of new methods for modeдling burn injuries of skin in laboratory animals is an important area of contemporary biomedical research. This allows a more profound study of the peculiarities of the processes of damaged tissue restoration and contributes to the development and implementation of more effective approaches to the treatment of burn injuries. Studies of the pathogenesis of burn injury, of wound healing mechanisms and preclinical evaluation of new therapeutic agents cannot be fully implemented at the level of cell cultures alone. This is due to the fact that thermal injuries cause systemic metabolic and immune changes affecting the entire body, and not just a local skin area. In addition, a serious complication caused by deep or large-scale burns is the burn disease, which significantly increases the risk of death. It occurs due to the destruction of a significant amount of tissues and is accompanied by impaired hemodynamics, microcirculation disorders, primary hypovolemia, systemic inflammatory response and excessive release of cytokines. It is worth noticing the use of plant extracts in wound healing, the study of the microbial landscape (Kolchyk et al., 2024; Melnychuk et al., 2024; Zazharskyi et al., 2024).

The objective of this article is to characterize the pathogenesis of burn injury in rats, as well as to describe cellular and tissue repair and restorative processes in the affected area based on histological changes and metabolic transformations discovered according to the results of blood screening.

Materials and methods

Modeөling of burn wound infection in laboratory animals. The scientific research was carried out in the educational and scientific laboratory of the Department of Infectious Animal Diseases of the Dnipro State Agrarian and Economic University and in the Research Centre for Biosafety and Environmental Control of Resources of the agro-industrial complex "Biosafety-Centre". During the experiments, compliance with the requirements of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986), the General Ethical Principles of Performing Research in Animals approved by the National Congress on Bioethics (Kyiv, 2001), the provisions of the Law of Ukraine "On the Protection of Animals from Cruelty" (2006), as well as the provisions of the European Union Directive 2010/63/EU dated September 22, 2010, was ensured.

The research program was approved by the Local Ethics Committee of the Faculty of Veterinary Medicine of the Dnipro State Agrarian and Economic University (Minutes No. 2 dated March 12, 2023).

The study used an experimental medicinal product, the activity of which was compared with the official Pantestin ointment produced by Darnytsia Pharmaceutical Company (Ukraine), which is one of the most common drugs to treat burn injuries. Pantestin ingredients are as follows: dexpanthenol (D-panthenol) 50 mg, miramistin 5 mg; excipients: propylene glycol, polyethylene glycol (macrogol 400), poloxamer, cetyl alcohol, stearyl alcohol, purified water.

The product under study had the following composition: ionol – 25.0 g/L, dimethyl sulfoxide – 37.5 g/L, polyethylene glycol PEG 400 – 230.0 g/L, PEG 1500 – 540.0 g/L in the form of a soluble drug, with gel-like consistency.

The animals were quarantined for 14 days (according to the sanitary rules "Structure and Maintenance of Experimental Biological Clinics", Order of the Ministry of Health of Ukraine No. 755 dated August 12, 1997) in compliance with the standard water and food diet, with free access to water and food and taking into account the maintenance standards (addendum dated December 04, 1997 to the Order of the Ministry of Health of Ukraine No.163 of March 10, 1996 "On the Daily Feeding Standards for Laboratory Animals and Producers"). The study used a model of contact thermal burn as modified by Minukhin et al. The depilated and trimmed area of the skin on the sides of the animal body, previously anesthetized through local infiltration anaesthesia with 0.5% Novocain solution, received contact exposure with a device for simulating burn injury. The diameter of the heating plate was 25 mm, the exposure duration was 8 seconds. As a result, rats suffered a 3rd-degree thermal injury confirmed by the results of histological analysis of the affected skin areas. The area of the burn wound was calculated using the Meeh-Rubner formula; it was about 10% of the total body surface of animals, on average – 79.1 ± 2.6 mm².

To determine the dynamics of the mass of rats in experimental models, mathematical calculations of linear dynamics were used, taking into account the rate of increase or loss of mass over time.

The study was conducted on 40 rats - randomized common Wistar males (5 weeks old) with an average body weight of 56.8 ± 4.6 g. The animals with body weight of 210–230 g were delivered from the central vivarium of the Dnipropetrovsk Regional State Laboratory of the State Service of Ukraine on Food Safety and Consumer Protection. The animals were divided into four groups (10 rats in a group):

Group I – experimental (burn and treatment with the experimental ointment);

Group II – experimental (burn and treatment with Pantestin ointment);

Group III – control (intact animals – clinically healthy, without burns and treatment, K+);

Group IV – control (untreated burn, K-).

The experimental and control treatment was applied to the wound during the period of 21 days.

Monitoring the effectiveness of treatment – weighing, studying the dynamics of changes in morphological, biochemical, and histological parameters of the rats' bodies after burns was carried dynamically: before the burn, as well as on the 3rd, 7th, 14th and 21st days after the start of the experiment. To assess the condition of the wound surface, the periods of wound cleansing from purulent necrotic masses, the time of granulations and complete epithelization of the wound surface were studied.

Morphological and biochemical blood tests. The biochemical parameters of blood were studied using Microlab-200 photometers (International Microlab, Shenzhen, China, 2021) and Vitalab Eclipse photometers (Merck, Netherlands, 2011) with the relevant software after the reaction was registered with the appropriate diagnostic test kits by Lachema (Erba Lachema, Karásek, Czech Republic, 2021).

Screening of metabolic changes in the physiological state of the macroorganism of the experimental rats exposed to extreme stress, and in the control was carried out using standardized biochemical methods both manually and using appropriate tools: total protein was measured by the biuret test, the protein fractions of blood – by reaction with bromocresol green (albumin fraction), the content of globulins and the protein coefficient by evaluation, the content of aspartate and alanine aminotransferase – by the Reitman-Frankel method, the content of creatinine – by Popper's method, and the content of uric acid – by means of Folin-Ciocâlteu reagent. The number of erythrocytes and leukocytes in the blood was measured by assessing the blood corpuscles in the grid of Goryaev's chamber. The hemoglobin concentration was evaluated using the hemoglobin-cyanide method. Leukogram was assessed based on blood smears prepared by the Pappenheim method. In serum samples, the following was assessed: urea – enzymatically using urease with the Berthelot's reaction, glucose – enzymatically using glucose oxidase, and then the Trinder reaction, total calcium – by reaction with Arsenazo III, inorganic phosphorus – by reaction with ammonium molybdate, creatinine – kinetically by the rate of increase in color intensity in reaction with picric acid, uric acid – by the urease test. The study was carried out using a Miura-200 automatic biochemical analyser (I.S.E. Srl, Milan, Italy, 2020). When measuring biochemical parameters, ready-to-use reagents manufactured by Dialab (Wiener Neudorf, Austria, 2020), Spinreakt (Girona, Spain, 2019) and Cormay (Lublin, Poland, 2020) were used. Using the Mindray BS-230Vet automatic analyzer (Mindray, China, 2016), the activity of aminotransferases (alanine aminotransferase, ALT, and aspartate aminotransferase, AST) in serum was also measured by the kinetic method based on the Warburg optical test (using Spinreakt reagents (Girona, Spain)), as well as alkaline phosphatase measured by 4-nitrophenol formation rate (Cormay, Warsaw, Poland). The content of lipoproteins was studied by the Burshtein-Samail turbidimetric me-

thod, measuring the solution absorption on the Ulab 102 spectrophotometer (Himlaborreaktyv LLC, Brovary, Ukraine, 2016).

Planimetric method. The wound surface was measured using the planimetry method taking into account the total defect area (mm²). The rate of wound healing was assessed using the average rate of wound surface reduction (mm² per day) and wound area reduction (in percent per day) with the L. M. Popova test, which is based on the measurement of wound area over time (Cherniakova, 2017). A sterile sheet of cellophane was applied to the wound, on which the contours of the wound were drawn with a marker. Then cellophane with the obtained outline was placed on graph paper and the wound area was measured by counting the number of square millimetres inside the contour.

Histological studies. The material of the study was the burn-injured skin sections of rats studied over time (before burn and after burn – after day one, 3, 7, 14, 21), preserved in 10% aqueous formalin solution for 24 hours. To obtain histological preparations, organs were embedded with paraffin according to generally accepted methods (Liu & Xu, 2011). Histological sections for review specimens with a thickness of 7–10 μm were made from paraffin blocks on a MS-2 sledge microtome (Medlife, Kharkiv, Ukraine, 2002), followed by their staining with hematoxylin and eosin according to generally accepted methods (Liu & Xu, 2011). Microscopic examinations of histological specimens were performed using a MICROmed XS-3330 light microscope (Ningbo Shengheng Optics & Electronics Co, Langxiang, Yuyao, China, 2020). Histopreparations and their individual sections were photographed with a MICROmed MDC-500 camera (Ningbo Shengheng Optics &Electronics Co, Langxiang Yuyao, China, 2019).

Statistical analysis of results. The results of the study were analysed using BioStat LE (AnalystSoft Inc., Walnut, California, USA, 2019) and MedCalc (MedCalc Software Ltd, Ostend, Belgium, 2016) computer programs. Statistical analysis was carried out using standard methods of variational statistics. Samples were compared using analysis of variance (ANOVA) and Tukey HSD (different letters indicate significant differences). The data in the tables are presented as a mean value ± standard deviation (x ± SD).

Results

Morphological and biochemical blood tests. The burn procedure was quite difficult to endure for the animals: it caused a severe psychophysiological trauma and an extreme pathological effect on the macroorganism of rats. Animals were whining for a long period of time, trembling, rejected food, were in a state of agitation and stupor for the first day. Immediately after the burn, the rats of the first and second groups were treated with ointment: the animals of the first group were given the ointment based on the experimental drug, and the animals of the second group were given Pantestin ointment. The animals of the fourth group received no treatment (K-). At the burn site, the skin in the contact area was initially red. Then, during the first hour, swelling was added to the redness. During the experiment, there were no dead rats in the first group, while in the second and fourth groups there were four and six, respectively (Table 1). The causes of death were associated with post-traumatic shock and burn intoxication. On day two after the burn, survival rate of rats in group I (100%) was higher than that in groups II (90%) and IV (80%). On day 5 of observations and by the end of the experiment, the survival rate in groups II and IV was 60% and 40% respectively.

The changes in the body weight of animals during the observation period indicate the inhibited growth in rats due to the effects of burn stress (Table 2). After 3 days, the body weight decreased by 8.5% 27.2% and 29.0% in rats of all study groups as compared to the weight before burns, while the weight of intact animals increased by 2.1%. However on the seventh day of observation, the body weight of rats in group I reached the values of clinically healthy animals, while in groups II and IV the rats' body weight decreased by 15.5% and 39.5% ($P < 0.05$) as compared to the intact animals (K+). After 7 days, the weight of rats in group II was less than that in group III (K+) by 5.8%, in group II – by 12.7% ($P < 0.05$). From the 7th to the 21st day of the experiment, there was an increase in the body weight of group I animals treated with the experimental drug (from 65.6 to 91.5 g), while in group II animals this value grew by 9.6%.

Morphological and biochemical parameters of blood in rats during the process of burn treatment with the experimental drug are shown in Table 3. There was an increase in protein concentration in group I after the use of the experimental drug on the 3rd and 7th day of observation (active phase) by 9.8% ($P < 0.05$) and 7.7% due to globulins by 46.7% ($P < 0.01$) and 42.9% ($P < 0.05$) as compared to the animals before burns, which may indicate an acute phase of inflammation, an immune response to γ-globulin in this period. On the 21st day of the experiment, the protein concentration in the group of rats treated with the preparation under study remained within the physiological range, exceeding the value in clinical control animals (K+) by 1.2 g/L. On the 3rd and the 7th days of the experiment, a decrease in the level of calcium by 90.0% ($P < 0.01$) was also registered, which indicated a Ca/P electrolyte imbalance and was characteristic of a burn syndrome. At the time of recovery (the 21st day of the experiment), the urea concentration in the blood plasma of group I rats increased by 6.5 and 19.8% ($P < 0.05$) as compared to the values on the 3rd and 7th days after burn.

Table 1
Survival rate during the observation period (n = 10)

Group of animals	Observation period, days/survival, %					
	1	3	5	7	14	21
I (experimental drug)	10/100	10/100	10/100	10/100	10/100	10/100
II (Pantestin)	10/100	9/90	6/60	6/60	6/60	6/60
III (K+)	10/100	10/100	10/100	10/100	10/100	10/100
IV (K–)	10/100	8/80	4/40	4/40	4/40	4/40

Table 2
Dynamics of rat body weight during observation period (x ± SD, n = 10)

Group of animals	Observation period, days/g					
	before burn	3	5	7	14	21
I (experimental drug)	56.8 ± 4.1^{b}	52.0 ± 3.4^{b}	54.5 ± 4.0^{b}	65.6 ± 5.8^{bc}	76.0 ± 6.2^{cd}	91.5 ± 7.1^{de}
II (Pantestin)	56.7 ± 3.2^{b}	41.3 ± 2.6^{a}	41.7 ± 3.8^{a}	56.5 ± 5.3^{b}	71.1 ± 6.5^{cd}	82.7 ± 6.4^{de}
III (K+)	56.9 ± 3.3^{b}	58.1 ± 4.5^{b}	59.0 ± 5.2^{b}	66.9 ± 4.1^{c}	76.4 ± 4.7^{d}	91.6 ± 6.3^{e}
IV (K–)	56.8 ± 3.1^{b}	40.3 ± 2.7^{a}	39.5 ± 2.6^{a}	40.5 ± 3.7^{a}	48.0 ± 4.2^{ab}	57.1 ± 5.4^{b}

Notes: ^{a}, ^{b}, ^{c} – results of Tukey HSD test different letters indicate significant differences ($P < 0.05$).

Since alanine aminotransferase is an important enzyme involved in the amino acid metabolism, its insufficient content may indicate poor function of the liver and tissue damage. On the 3rd day of the experiment, an increase in the level of AST by 33.8% ($P < 0.05$) and a decrease in ALT by 33.0% ($P < 0.05$) were noted in the blood of rats of the experimental drug group, and on the 7th day of the experiment, on the contrary, a decrease in the level of AST and an increase in ALT by 8.4% and 186.7% ($P < 0.01$) was recorded.

Blood glucose is a key indicator required for providing energy to the body, and its low levels may indicate intoxication in the animal body as a result of a burn. In animals of the experimental treatment group I, on the 7th day of the experiment, the glucose level dropped

by 32.9% compared to the day 3 ($P < 0.05$), and on the 21st day it reached the level of clinically healthy animals – 3.61 mmol/L.

Gamma glutamyl transferase (GGT) is an enzyme localized in liver, kidney, and pancreatic cells and plays an important role in amino acid metabolism. On the 3rd day of the experiment, the level of GGT in group I rats rose by 47.8% as compared to the level before burns ($P < 0.01$). During this period, burns can cause systemic stress and disorders of the hepatobiliary system.

Calcium is a vital element for the formation of bones, muscle function (including smooth and skeletal), the nervous system, as well as for the normal functioning of the immune system. Phosphorus is involved in the accumulation and release of energy in cells, it is a component of DNA and RNA, which is important for growth, cell division and storage of genetic information, and is also necessary for the normal functioning of the heart and kidneys and the transmission of nerve impulses. A drop in calcium levels on the 3rd and 7th days of the experiment in group I animals by 90.0% with an increase in the level of phosphorus by 17.4 % and 150.4% ($P < 0.01$) is characteristic of a burn syndrome.

Hemoglobin is a protein in erythrocytes that transports oxygen to the tissues and organs of animals. Erythrocytes contain hemoglobin, which binds oxygen, transports it to organs and tissues, removes carbon dioxide, and also reflects the efficiency of their synthesis in the bone marrow. On the 7th day of the experiment in rats treated with the preparation under study, the level of hemoglobin decreased by 24.7% ($P < 0.05$) due to red blood cells by 36.3% ($P < 0.01$), followed by normalization and posthemorrhagic adaptation.

In the animals of the experimental group I, on the 7th day of the experiment, the level of leukocytes dropped by 26.6% ($P < 0.05$) due to segmented neutrophils by 25.2% ($P < 0.05$) with a rise in lymphocytes level by 38.2% ($P < 0.05$), which may indicate immunosuppression and suppression of neutrophils activity during this period. Activation of the hematopoietic system, aimed at enhancing haematopoiesis and increasing resistance, is an adaptive reaction of the body, which ensures the protective function of the blood and the immune status of animals. Application of the preparation under study contributes to the stimulation of the functional activity of the immune system and increases animals' resistance to stress caused by thermal damage.

Table 3

Morphological and biochemical changes in blood of rats after burn with the use of experimental drug (x ± SD; n = 10 for t = 3 days; n = 3 for t = 14–21 days)

Parameters	Observation period, days				
	before burn	3	7	14	21
Total protein, g/L	53.1 ± 4.3 ^{ab}	58.3 ± 5.2 ^{b}	57.2 ± 4.8 ^{b}	47.4 ± 3.6 ^{a}	54.3 ± 4.2 ^{ab}
Albumins, g/L	30.3 ± 2.6 ^{a}	27.4 ± 2.4 ^{a}	28.1 ± 3.1 ^{a}	28.2 ± 2.7 ^{a}	31.1 ± 2.9 ^{a}
Globulins, g/L	21.2 ± 1.8 ^{ab}	31.1 ± 2.5 ^{c}	30.3 ± 2.2 ^{c}	19.4 ± 1.6 ^{a}	24.2 ± 2.3 ^{b}
Protein coefficient, times	1.42 ± 0.11 ^{c}	0.93 ± 0.08 ^{a}	0.92 ± 0.06 ^{a}	1.41 ± 0.22 ^{bc}	1.24 ± 0.13 ^{b}
Urea, mmol/L	9.04 ± 0.53 ^{c}	7.66 ± 0.42 ^{b}	6.81 ± 0.38 ^{a}	6.77 ± 0.34 ^{a}	8.16 ± 0.42 ^{b}
Urea nitrogen, mg/L	17.81 ± 1.02 ^{c}	14.63 ± 1.24 ^{ab}	12.92 ± 0.91 ^{a}	12.74 ± 0.83 ^{a}	15.52 ± 1.31 ^{b}
Creatinine, µmol/L	48.3 ± 5.7 ^{c}	10.9 ± 1.3 ^{a}	20.4 ± 2.2 ^{c}	11.2 ± 1.5 ^{a}	78.1 ± 6.4 ^{d}
Aspartate aminotransferase (AST), U/L	180 ± 12 ^{a}	241 ± 16 ^{b}	195 ± 11 ^{a}	231 ± 14 ^{b}	229 ± 15 ^{b}
Alanine aminotransferase (ALT), U/L	82.4 ± 7.6 ^{b}	55.2 ± 4.2 ^{a}	236.2 ± 12.3 ^{c}	63.3 ± 4.4 ^{ab}	74.6 ± 6.2 ^{b}
AST/ALT, ratio	2.28 ± 0.24 ^{b}	4.31 ± 0.33 ^{e}	0.88 ± 0.16 ^{a}	3.74 ± 0.21 ^{d}	3.14 ± 0.28 ^{c}
Alkaline phosphatase, U/L	392 ± 27 ^{c}	418 ± 30 ^{c}	282 ± 23 ^{b}	210 ± 21 ^{a}	401 ± 26 ^{c}
Amylase, U/L	421 ± 33 ^{a}	634 ± 45 ^{b}	587 ± 39 ^{b}	888 ± 64 ^{c}	991 ± 72 ^{c}
Total bilirubin, µmol/L	4.02 ± 0.34 ^{a}	4.23 ± 0.31 ^{ab}	4.22 ± 0.27 ^{ab}	5.34 ± 0.32 ^{c}	4.73 ± 0.24 ^{b}
Glucose, mmol/L	3.97 ± 0.63 ^{bc}	4.26 ± 0.42 ^{c}	2.86 ± 0.11 ^{a}	3.13 ± 0.25 ^{ab}	3.61 ± 0.32 ^{b}
Ca, mmol/L	2.21 ± 0.04 ^{c}	0.22 ± 0.03 ^{a}	0.22 ± 0.04 ^{a}	2.01 ± 0.23 ^{b}	2.12 ± 0.16 ^{bc}
P, mmol/L	2.76 ± 0.42 ^{a}	3.24 ± 0.38 ^{a}	6.91 ± 0.53 ^{b}	2.86 ± 0.17 ^{a}	2.74 ± 0.22 ^{a}
Ca/R, ratio	0.81 ± 0.01 ^{b}	0.11 ± 0.02 ^{a}	0.12 ± 0.01 ^{a}	0.72 ± 0.14 ^{b}	0.74 ± 0.18 ^{b}
Cholesterol, mmol/L	2.30 ± 0.13 ^{d}	2.62 ± 0.14 ^{e}	1.24 ± 0.12 ^{b}	0.93 ± 0.11 ^{a}	1.72 ± 0.13 ^{c}
Gamma-glutamyl transpeptidase (GGT), U/L	4.08 ± 0.22 ^{a}	6.03 ± 0.31 ^{c}	5.16 ± 0.24 ^{b}	4.05 ± 0.25 ^{a}	5.01 ± 0.23 ^{b}
Hemoglobin, g/L	90.4 ± 8.6 ^{b}	90.2 ± 7.3 ^{b}	68.1 ± 5.2 ^{a}	96.2 ± 8.4 ^{b}	126.0 ± 9.3 ^{c}
Haematocrit, %	28.2 ± 2.3 ^{c}	29.1 ± 2.2 ^{c}	17.4 ± 1.8 ^{a}	25.5 ± 1.3 ^{b}	35.2 ± 4.7 ^{d}
Erythrocytes,10^{12}/L	4.91 ± 0.24 ^{c}	5.94 ± 0.36 ^{d}	3.13 ± 0.22 ^{a}	3.62 ± 0.24 ^{b}	5.93 ± 0.74 ^{cd}
Mean corpuscular volume MCV, 10^{-15} L	57.1 ± 4.2 ^{ab}	49.2 ± 3.7 ^{a}	56.1 ± 4.4 ^{ab}	70.8 ± 8.6 ^{a}	59.6 ± 4.2 ^{b}
Mean corpuscular hemoglobin (MCH), 10^{-12} g	21.3 ± 2.6 ^{b}	15.3 ± 2.1 ^{a}	21.9 ± 2.7 ^{b}	26.7 ± 3.3 ^{b}	21.4 ± 2.6 ^{b}
Mean corpuscular hemoglobin concentration (MCHC), %	37.4 ± 4.8 ^{ab}	31.2 ± 3.3 ^{a}	39.1 ± 4.1 ^{b}	37.7 ± 4.2 ^{ab}	36.9 ± 3.5 ^{ab}
Colour index, units	1.02 ± 0.13 ^{b}	0.91 ± 0.15 ^{ab}	0.82 ± 0.04 ^{a}	1.01 ± 0.16 ^{ab}	1.23 ± 0.20 ^{b}
Erythrocyte sedimentation rate (ESR), mm/hour	1.06 ± 0.24 ^{a}	1.03 ± 0.12 ^{a}	1.02 ± 0.13 ^{a}	2.14 ± 0.12 ^{b}	1.03 ± 0.16 ^{a}
Platelets, 10^{9}/L	320 ± 29 ^{ab}	347 ± 32 ^{b}	285 ± 27 ^{a}	312 ± 29 ^{ab}	422 ± 33 ^{c}
Leucocytes, 10^{9}/L	2.14 ± 0.38 ^{ab}	3.31 ± 0.43 ^{c}	1.57 ± 0.22 ^{a}	2.24 ± 0.38 ^{b}	2.26 ± 0.31 ^{b}
Basophils, %	0.0 ± 0.0 ^{a}	0.0 ± 0.0 ^{a}	0.0 ± 0.0 ^{a}	0.0 ± 0.0 ^{a}	0.0 ± 0.0 ^{a}
Eosinophils, %	1.02 ± 0.13 ^{a}	1.01 ± 0.08 ^{a}	1.02 ± 0.06 ^{a}	0.00 ± 0.00 ^{b}	1.03 ± 0.12 ^{a}
Myelocytes, %	0.0 ± 0.0 ^{a}	0.0 ± 0.0 ^{a}	0.0 ± 0.0 ^{a}	0.0 ± 0.0 ^{a}	0.0 ± 0.0 ^{a}
Metamyelocytes, %	0.0 ± 0.0 ^{a}	0.0 ± 0.0 ^{a}	0.0 ± 0.0 ^{a}	0.0 ± 0.0 ^{a}	0.0 ± 0.0 ^{a}
Band neutrophils, %	1.04 ± 0.07 ^{a}	1.02 ± 0.05 ^{a}	1.02 ± 0.03 ^{a}	1.03 ± 0.12 ^{a}	1.02 ± 0.11 ^{a}
Segmented neutrophils, %	16.3 ± 2.2 ^{c}	9.1 ± 1.4 ^{a}	12.2 ± 1.7 ^{b}	23.4 ± 2.3 ^{d}	17.1 ± 1.2 ^{c}
Lymphocytes, %	64.1 ± 5.3 ^{a}	91.4 ± 6.9 ^{c}	88.6 ± 5.1 ^{c}	77.2 ± 5.4 ^{b}	72.3 ± 4.6 ^{ab}
Monocytes, %	0.00 ± 0.00 ^{a}	0.00 ± 0.00 ^{a}	0.00 ± 0.00 ^{a}	0.00 ± 0.00 ^{a}	1.02 ± 0.13 ^{b}

Notes: ^{a}, ^{b}, ^{c}, ^{d} – see Table 2; physiological range – the results of Dang & Rao (2008) studies.

Planimetric characteristics of the wound surface over time. To assess the condition of the wound surface, the timing of wound cleansing from purulent and necrotic masses, the time of granulation and the beginning of marginal epithelization, as well as the timing of complete epithelization of the wound surface were studied. The wound surface was measured using the planimetry method taking into account the total defect area in mm². The wound healing rate was assessed based on the parameters as follows: average wound surface reduction rate in mm²/day, wound area reduction in % per day, healing area in mm. According to the results of the experiment, in the group of animals receiving experimental ointment the wounds were completely cleansed from purulent and necrotic masses 2.3 days earlier than in rats in the Pantestin group. In the animals of the AKM group, a shorter period of granulation tissue formation, marginal and complete epithelization of a burn wound was recorded as compared to the Pantestin group by 2.8, 4.3 and 4.1 ($P < 0.05$) days, respectively, with a decrease in the wound area by 1.8% ($P < 0.05$) per day (Table 4).

The index of L. M. Popova was significantly higher in the experimental group receiving the preparation under study than that in the Pantestin group. In an experimental simulation of infected thermal

burn in rats, it was found that the use of the experimental ointment provides more intensive wound healing compared to the reference product Pantestin ($P < 0.05$). Successive changes in the appearance of burn injuries in the process of treatment with experimental ointment are shown in Figures 1–7. The area of burn wounds in group I rats treated with the experimental ointment was measured. The following values were obtained: 0.66–0.72 cm^2.

Table 4

Planimetric dynamics of burn wounds healing in the animals of different experimental groups (x ± SD)

Planimetric parameters, units of measurement	Experimental groups of animals	
	I (experimental drug)	II (Pantestin)
Complete cleansing of wounds from purulent necrotic masses, day	3.6 ± 0.1***	5.9 ± 0.3
Formation of granulation tissue, day	3.8 ± 0.3***	6.6 ± 0.2
Marginal epithelization of a burn wound, day	6.3 ± 0.1***	10.6 ± 0.4
Complete epithelization of the wound surface, day	16.8 ± 0.2***	20.9 ± 0.3
Reduction of wound area per day (Popova's index), %	4.7 ± 0.1***	2.9 ± 0.2
Average rate of wound surface area reduction, mm^2/day	3.8 ± 0.2***	2.2 ± 0.1
Healing area, mm^2	78.1 ± 0.2*	79.3 ± 0.2

Notes: * – $P < 0.05$, *** – $P < 0.001$.

Fig. 1. Appearance of the burn wound: the first day of the experiment *a* – experimental drug group; *b* – Pantestin; *c* – group K-

On the third day of the experiment, the signs of post-traumatic shock and burn intoxication were recorded, a drying crust appeared on the area of injury, which looked thinner in the animals treated with Pantestin as compared to that in the animals of the experimental group I (Fig. 2).

The results of the fifth day of the experiment indicate the stabilization of the general condition of the experimental animals. Initial shock reactions diminished. The granulation tissue formed 42.4% faster ($P < 0.01$) when the experimental drug was used as compared to the animals of group II and took 3.8 days against 6.6 days, respectively. Appetite recovered, psychophysiological reactions normalized. The prognosis regarding the overall condition and local process is positive (Fig. 3). On the seventh day of the experiment, the condition of group I rats (treatment with experimental drug) was satisfactory, appetite was normal, psycho-emotional reactions were characteristic of animals with healthy physiological status.

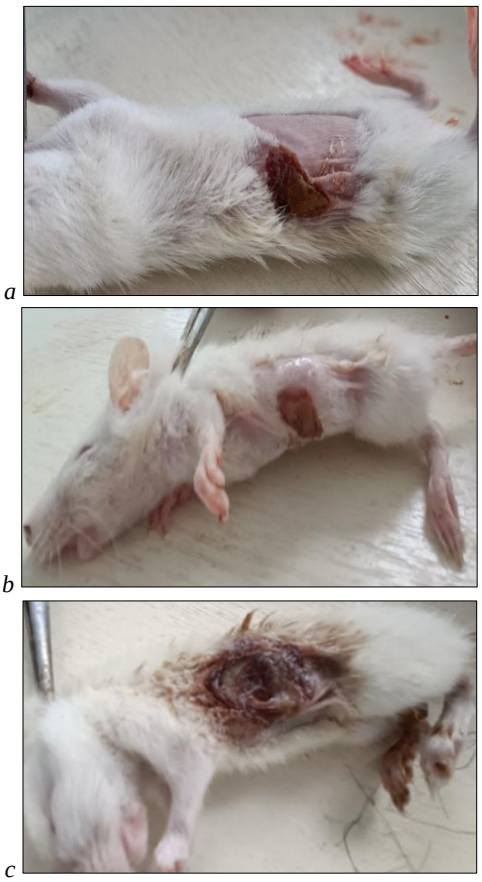

Fig. 2. General view and measurement of the area of burn wounds in rats: *a* – experimental drug group; *b* – Pantestin; *c* – group K-; third day of the experiment

The wound surface reduced and burn wounds healed. The prognosis regarding the overall condition and the local process is positive. Marginal epithelization of a burn wound was 40.6% ($P < 0.01$) faster than in rats of group II and took 6.3 versus 10.6 days. In the rats of the Pantestin group, appetite was normalized, the colour and size of feces was natural, but the fur around the burn was not clean (Fig. 4).

The results of the 14th day of the experiment indicate the satisfactory condition of rats of the experimental group I. Appetite was preserved, psycho-emotional reactions were within normal physiological ranges. The average rate of reduction of the wound surface area was 3.8 mm^2 per day, which was 72.7% faster ($P < 0.01$) than in the animals of group II. In rats of the control group (K-), wound healing was slow, purulent inflammation was observed (Fig. 5).

On day 21 of the study, no abnormal appetite was recorded in the rats of experimental group I treated with the experimental drug. The epithelization of the wound surface was completed on average after 16.8 days. The healed area was 78.1 mm^2 (Fig. 6).

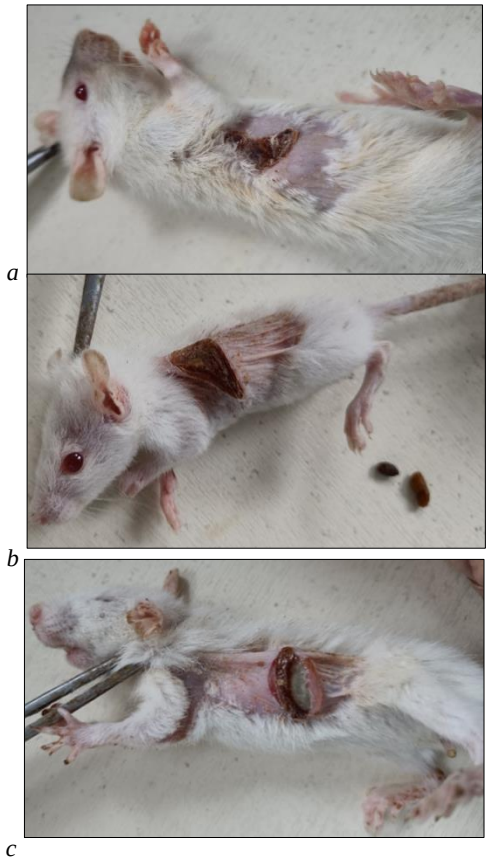

Fig. 3. General view and measurement of the area of burn wounds in rats: *a* – experimental drug group; *b* – Pantestin; *c* – group K-; the fifth day of the experiment

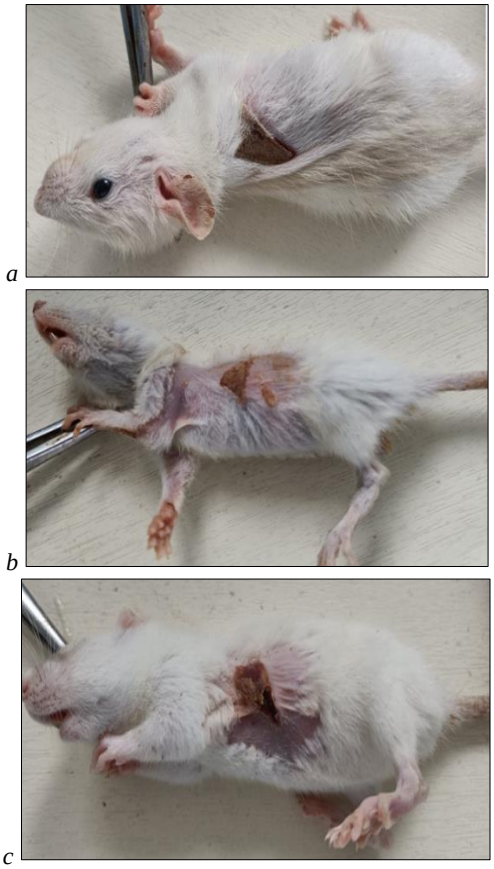

Fig. 4. Measurements of the burn wound area in rats are as follows: *a* – group of experimental drug; *b* – Pantestin; *c* – group K-; the seventh day of the experiment

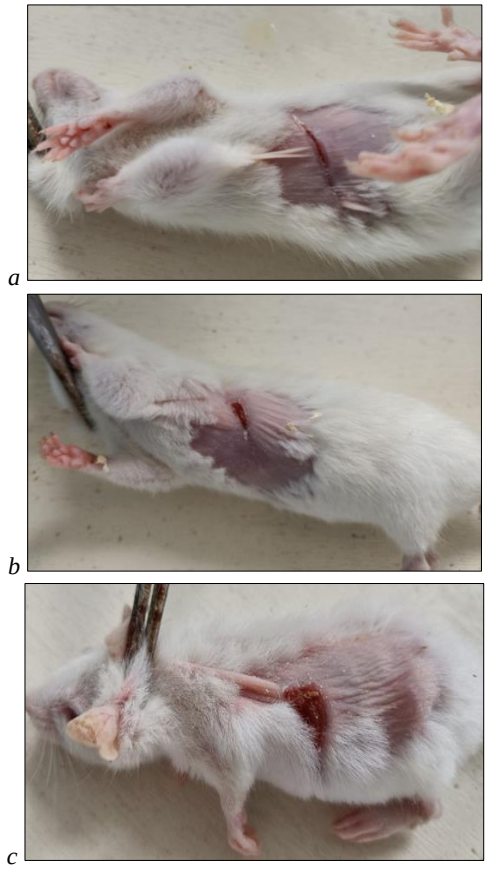

Fig. 5. Measurements of the burn wound area in rats are as follows: *a* – group of experimental drug; *b* – Pantestin; *c* – group K-; the fourteenth day of the experiment

The results of histological examinations of rat skin samples before and after burns. The skin of rats before the burn had a typical histological structure (Fig. 7). The epidermis with a thickness of 10–18 μm consists of a germ layer, represented by basal epidermocytes placed in one row and 2–5 rows of polygonal cells of spiny layer, above which there are several rows of granular layer cells and the keratinised cells of the stratum corneum in the form of loosened keratinised plates. The dermoepidermal junction is indistinct. The dermis under the epidermis, shaped as a wide strip 300–500 μm thick, consists of papillary and reticular layers. In the papillary layer, well-formed bundles of collagen fibers, thin elastic and reticular fibers and dermal cells are tightly packed. Hair follicles are numerous and surrounded by skin glands. The subcutaneous layer is represented by soft connective tissue and subcutaneous muscles.

As a result of thermal burn, pathological changes in the skin of rats were observed, manifested by peeling of the stratum corneum of the epidermis, necrosis of the main layers of the epidermis and dermis in the form of a large number of vesicles of different sizes, the cavity of which was filled with granular and homogeneous content slightly stained with eosin. At the dermoepidermal junction, venous plethora and stasis were observed, manifested by swelling in the dermis. Skin appendages (sudoriferous and sebaceous glands, hair follicles) were noticeably wrinkled, cell nuclei were sharply pycnotic and often necrotized (Fig. 8). On day three after thermal trauma, with the experimental drug application, vascular reactions and proliferation activity of cambial elements increased in the skin of experimental rats, as a result of which a demarcation line was formed. The formed scab of necrotized cells of the epidermis and matrix was gradually softening and detached fragmentarily (Fig. 9).

In rats treated with Pantestin ointment the structures of the epidermis and dermis were represented by a formed thick scab of dead cells. Vascular reactions (hyperemia, stasis, hemorrhages), moderate subdermal edema, intensive infiltration of leukocyte cells were pronounced in the dermis. Skin appendages (hair follicles and glands) were sharply shrivelled (Fig. 10).

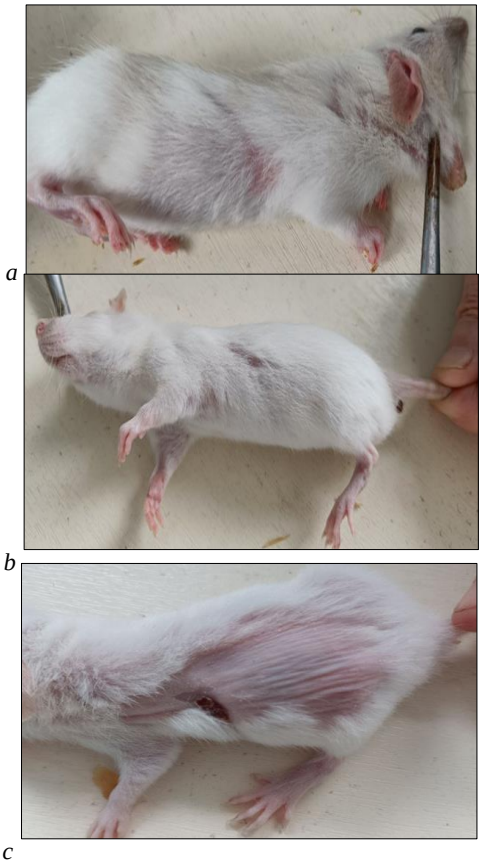

Fig. 6. Measurement of the burn wounds area in rats: *a* – group of experimental drug; *b* – Pantestin; *c* – group K-4; the twenty-first day of the experiment

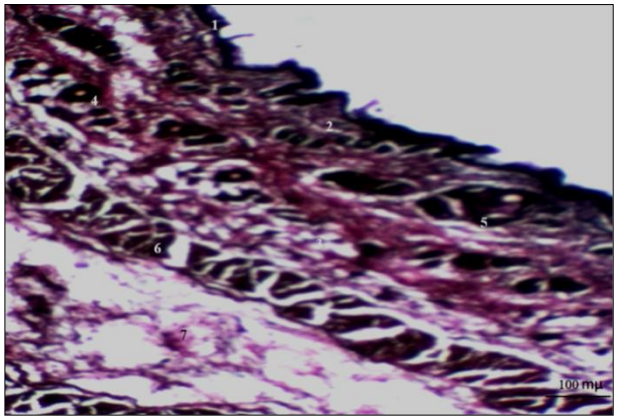

Fig. 7. Microscopic structure of rat skin before burn: *1* – epidermis; *2* – papillary layer of the dermis; *3* – reticular layer of the dermis; *4* – hair follicles; *5* – skin glands; *6* – subcutaneous muscles; *7* – hypodermis; hematoxylin and eosin

In rats without treatment, the surface of the skin injury was covered with voluminous necrotic masses of dead cells of the epidermal and dermal layers and dead leukocytes. Purulent necrotic masses were sometimes surrounded by an indistinct demarcation leukocyte shaft. Vascular reactions were pronounced in the dermis, subdermal edema was recorded, fibrous structures were homogenized, intensively basophilic (Fig. 11).

On the fifth day, in the animals of group I treated with the experimental drug, the regenerative potential of the skin increased, as evidenced by the fragmentary detachment of the scab, in place of which erosive areas of the dermis emerged. In the dermis, there were moderate signs of circulatory disorders (plethora of blood vessels, small focal haemorrhages), edema, as well as increased cellular infiltration of leukocytes, lymphocytes, macrophages and fibroblastic cells (Fig. 12).

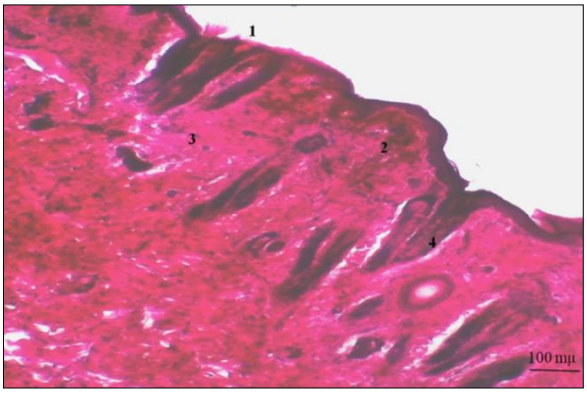

Fig. 8. Microscopic structure of rat skin on the 1st day after burn: *1* – necrosis of the stratum corneum of the epidermis; *2* – partially preserved germinal layer of the epidermis; *3* – disorganization of the dermis (collagen fibers are homogenized with the inclusion of cells, dermal edema); *4* – wrinkled and partially destroyed hair follicles; hematoxylin and eosin

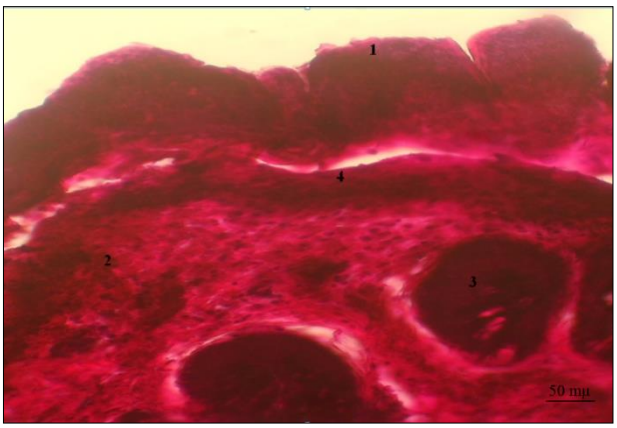

Fig. 9. Microscopic structure of rat skin 3 days after burn with the use of the experimental drug: *1* – scab (necrotic mass of the epidermal layer); *2* – disorganization of the dermis (collagen fibers are homogenized with the inclusion of cells); *3* – partially destroyed hair follicles with sebaceous glands; *4* – formation of a demarcation line; hematoxylin and eosin

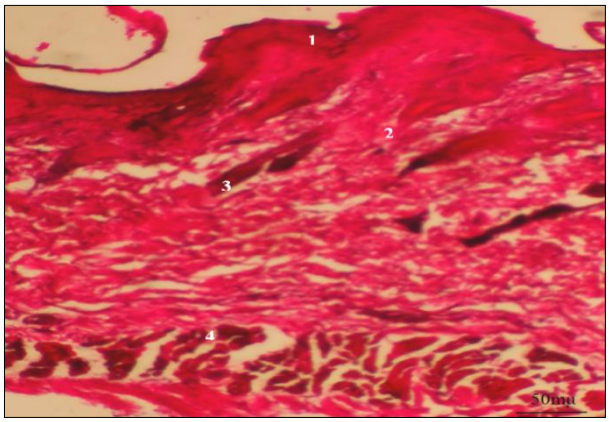

Fig. 10. Microscopic structure of rat skin 3 days after burn with Pantestin ointment applied: *1* – scab (homogeneous necrotic mass of epidermis and papillary dermis); *2* – disorganization of dermis with edema and structureless collagen fibres; *3* – remnants of hair follicles; *4* – subcutaneous muscles; haematoxylin and eosin

In the animals receiving Pantestin ointment treatment of burn wounds, the cells of the epidermal layer were necrotized and formed as a compact tissue of intense basophilic colour with an indistinct structure (scab). Dermal papillae were smoothed and appeared as groups of deformed nuclei of connective tissue cells, connective tissue fibres were

defragmented and intensively basophilic. The vessels of the dermis were unevenly dilated and plethoric (Fig. 13).

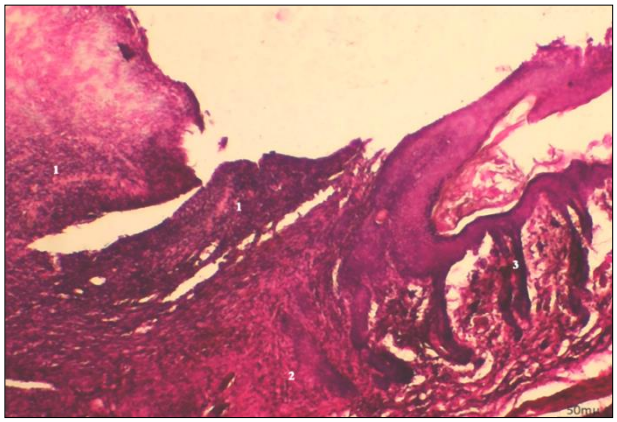

Fig. 11. Microscopic structure of rat skin without treatment on the 3rd day after burn: *1* – scab; *2* – disorganization of dermis; *3* – formation of a demarcation line on preserved skin areas; haematoxylin and eosin

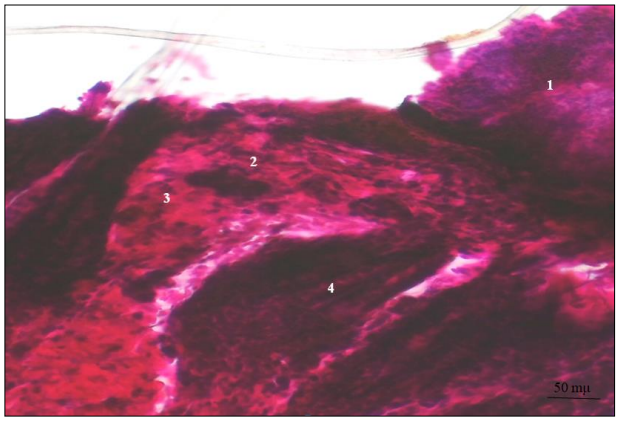

Fig. 12. Microscopic structure of rat skin on the 5th day after burn with the use of the experimental drug: *1* – erosive areas at the site of a partially exfoliated scab; *2* – fragmented collagen fibers of the dermis with cell inclusions; *3* – deformed hair follicles; haematoxylin and eosin

On the 14th day of treatment of damaged skin areas in rats with the use of the experimental drug, acanthotic growth of the epidermis was observed due to the active reproduction of cells of the basal and acanthocyte layers. At the same time, focal polymorphic cellular infiltration and active formation of granulation tissue due to fibrous structures were observed in the dermis (Fig. 14). In the animals receiving treatment of thermal skin burn with Pantestin ointment, the processes of regeneration of skin structures were significantly slowed down. At the edge of the perifocal zone, there was a moderate regenerative activity of the epidermis and dermis elements due to polymorphic cell infiltrateon of leukocytes, lymphocytes, characterized by the presence of few capillary vessels and fibrous structures, as well as macrophages, fibroblastic cells and the formation of granulation tissue. In animals without treatment, regenerative processes were deferred.

On the 21st day of treatment of thermal burn in animals using the experimental drug, a complete restoration of the affected skin areas was observed, characterized by uneven keratinization of the stratum corneum, thickening of the germinal layer due to active proliferation of epidermocytes and their ingrowth into the dermis. The dermis was soft and had an increased content of stromal cells, the grids of elastic and collagen fibres were somewhat sparse, the vessels were plethoric. The formation of new hair follicles was observed (Fig. 15).

In animals receiving Pantestin ointment treatment, the structures of the epidermis were in the stage of regeneration with the restoration of the stratum corneum and an uneven increase in the thickness of the germinal layer of epidermis. The dermal-epidermal junction was distinct. Moderate signs of fibrosis were observed in the granulation tissue of the dermis. The newly created fibers were located chaotically without a clear orientation, hair follicles were actively forming (Fig. 16). In the animals without treatment, in contrast to the experimental groups, the processes of regeneration of damaged skin areas were deferred.

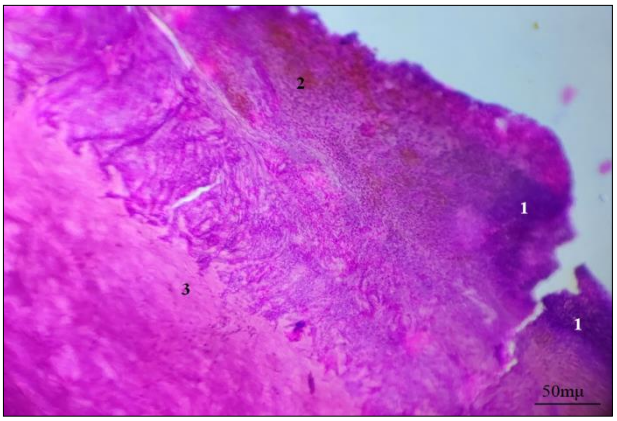

Fig. 13. Microscopic structure of rat skin on the 5th day after burn with Pantestin ointment applied: *1* – scab; *2* – vascular reactions in the dermis; *3* – cell proliferation; haematoxylin and eosin

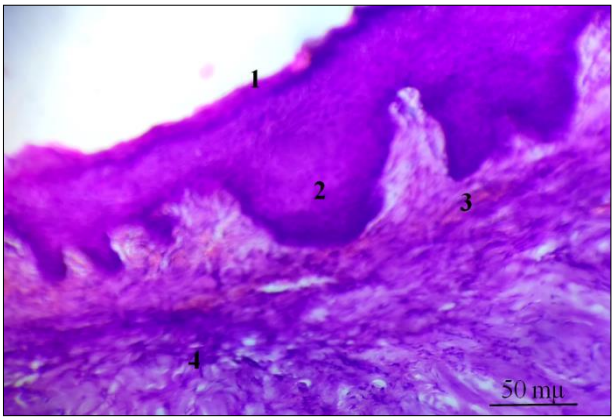

Fig. 14. Microscopic structure of rat skin on the 14th day after burn with the use of the experimental drug: *1* – restoration of the stratum corneum of the epidermis; *2* – acanthotic proliferation of the basal layer of the epidermis; *3* – increased vascularization and cellular infiltration of the dermis; haematoxylin and eosin

Discussion

Burn injury is a major cause of a wound that can result in death or disability, requiring long-term recovery and high public and animal health costs (Roh & Orgill, 2019; Nasrullah et al., 2024). In burn injury, the depth and size of the burn wound, burn area, and age are the most important factors influencing morbidity and mortality (Oranges et al., 2019; Zazharskyi et al., 2024a).

Burn depth is also the most important parameter preconditioning the patient's long-term appearance and functionality (Shakoor et al., 2025). Ji & Xia (2024) note that the American Association has recommended relying on tissue biopsies to determine the severity of a burn wound infection. They classified wound infections as non-invasive and invasive depending on the degree of microbial penetration into the skin and subcutaneous tissues. Similarly, the recommendations of the American Society of Infectious Diseases (Duane & Sanders, 2021) determined the degree of infection of the skin and soft tissues according to the degree of infection penetration into the skin, subcutaneous tissues, muscles and other structures. In another retrospective study, the degree of wound infection was classified into five grades based on tissue biopsy results, patient clinical information, microbial survival status, and levels of invaded tissue as follows: Grade 0, a clean wound with no signs of microorganisms; Grade 1, a wound with microorganisms within the limits of its surface; Grade 2, a wound with microorganisms invading the surface layers of the dermis; Grade 3, a wound with

microorganisms affecting the entire dermal layer; and Grade 4, a wound with microorganisms invading subcutaneous tissues (Church et al., 2006; Chen & Zhang, 2021).

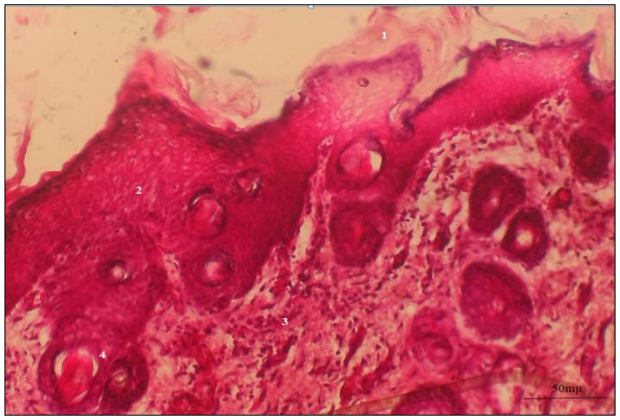

Fig. 15. Microscopic structure of rat skin on the 21st day after burn with the use of the experimental drug: *1* – restored stratum corneum of the epidermis; *2* – thickened germinal layer of the epidermis; *3* – dermis; *4* – hair follicles; hematoxylin and eosin

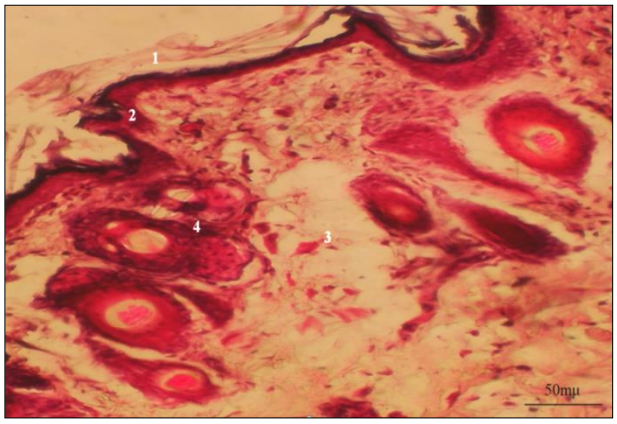

Fig. 16. Microscopic structure of rat skin on the 21st day after burn with the use of Pantestin: *1* – restoration of the stratum corneum of the epidermis; *2* – uneven thickening of the germinal layer of the epidermis; *3* – thinned dermis due to the thinning of fibres; *4* – dermal appendages; haematoxylin and eosin

Conditions such as immunosuppression, large burn area, and malnutrition provide a favourable environment for microorganisms, and unfortunately, infections are common and are among the key causes of morbidity in patients with burns (Kassich et al., 2019; Zazharskyi et al., 2023). Although mortality is declining due to new approaches to burn wound treatment, secondary infections and prolonged recovery period can still cause mortality. Growth factors play a crucial role in the process of normal healing of wounds as well as in its disruption. Growth factors such as insulin-like growth factor-1 (IGF-1) and platelet-derived endothelial cell growth factor (PDGF) inhibit apoptosis pathways that allow rapid cell renewal and thus catalyse physiological wound healing at different stages. The direct or indirect effect of growth hormone on wound healing is also thought to be related to IGF-1 expression (Ozkan et al., 2022). The study by Unal (2018) demonstrated that the use of platelet-rich plasma (PRP) provided rapid recovery of the extracellular matrix and its components in deep second-degree burns in horses, and also noted that two applications of PRP therapy accelerated the formation of the extracellular matrix during the first half of wound healing (Maciel et al., 2012; Kao et al., 2021; Liu et al., 2022). In addition, Ozcelik et al. (2016) reported that the use of PRP with xenogeneic acellular dermal matrix to treat the second-degree deep burns reduced infection and accelerated wound healing.

Pan et al. (2020) found through immunohistochemical studies that on day 7, there was a significant increase in the number of blood vessels in superficial partial burn wounds compared to deep partial burn wounds. Through the analysis of the findings of the experiment it was discovered that the application of the original anti-burn drug under study contributed to the early healing of wounds as compared to the treatment with the official product – Pantestin ointment and to the non-treatment scenario where no anti-burn treatment was applied. Treatment of deep burn wounds on the body of rats in the experimental group I resulted in effective cleansing of wounds, formation of granulations, appearance of marginal epithelialization and the development of complete epithelialization ($P < 0.05$).

According to the results of pathomorphological and histological studies, it was found that the infected burn wound was manifested not only by local inflammation, but also by a systemic inflammatory response, which as the wound process progressed, resulted in the damage to organs of the reticuloendothelial system, and accompanied by circulatory and hemorheological disorders. Alterations in the liver, perhaps, were due to the toxic effect of the association of parasitocenotic ubiquitous opportunistic prokaryotic microorganisms. Against the background of acute systemic inflammation, the response of the immune system organs manifested as a number of changes in the affected skin area of the experimental animals. Regional lymph nodes in these animals were characterized by the development of reactive hyperplasia, as part of the inflammatory infiltrate, a rapid replacement of neutrophilic granulocytes by a monocyte and macrophage cell population was observed, which led to rapid wound clearing from purulent necrotic detritus and, as a result, to accelerated wound epithelization.

The chemical properties of the experimental drug ingredients, which are the basis of its medicinal properties mediated by the active substances are as follows:

– ionol, butylhydroxytoluol, dibunol, agidol-1 - 2,6-di-tert-butyl-4-methylphenol, a lipophilic organic substance of phenol class. Its antioxidant properties are due to the ability to neutralize free radicals and interrupt chain reactions by eliminating free radicals. The formula is $C_{15}H_{24}O$. Molecular weight – 220.35 g/mol. Melting point – 70 °C. Boiling point – 265 °C. Density – 1.05 g/cm^2. It has anti-burn and anti-inflammatory properties;

– dimethyl sulfoxide (DMSO) is an organosulfur compound, formula – $(CH_3)_2SO$. Colourless high-boiling-point liquid, acts as an aprotic highly polar solvent, low-toxic. When mixed with water, it heats noticeably, reacts with methyl iodide to form a sulfoxonium ion capable of reacting with sodium hydride. In aqueous solutions (10–50.0%), the preparation has anti-inflammatory and analgesic properties. Moreover, 10.0% concentration can be cryoprotectants, and as a component of the ointment, DMSO increases transdermal penetration of active substances;

– PEG-400 (polyethylene glycol – 400). The molecular formula is $C_{2n}H_{4n+2}O_{n+1}$. A polymerization product of ethylene oxide and ethylene glycol. The chemical class – polymers. Colourless or yellowish liquid, highly hydrophilic, non-toxic, soluble in glycerol and glycol, insoluble in ether and greasy lubricants. It is a material with an average degree of polymerization and viscosity, density of 1.1–1.2 g/cm^2. It has the properties of a preservative and a thickener and creates gel-like structures;

– PEG-1500 (polyethylene glycol – 1500). Polyester molecular formula – $C_{2n}H_{4n+2}O_{n+1}$. The product of polymerization of ethylene oxide and ethylene glycol, has an average degree of polymerization. The chemical class – polymers. Colourless or yellowish liquid. Depending on the molecular weight of the polymer, it is a viscous liquid, gel-like or solid, in the form of scales or a dense mass of white colour. Combustion temperature – 182–287 °C. It is soluble in water, acetone, alcohols, benzene, glycerol, glycol and aromatic carbohydrates. It is an absolutely harmless and non-toxic substance, promotes mixing of incompatible components.

The preparation promotes deep hydration, alleviating inflammation, accelerated formation and restoration of skin cells, wound healing and restoring of mucous membranes. The experimental drug softens and moisturizes rough areas of the skin, relieves redness and reduces inflammation.

Due to the complex physiological effect on metabolic processes, the drug acts as a regulator of the reproductive potential of somatic cells

of epidermal origin, is an adaptogen of cellular metabolism, an antioxidant and inhibitor of free radical compounds and their sequential chain reactions, a stimulant for the expression of intracellular ribosomal reproduction of nitrogen-containing substances and post-translational modifications in the maturation of protein macromolecules, a rapid-acting molecular antidepressant with cryoprotective properties, has adhesive and transdermal potencies, intensively stimulates the regeneration of the skin epithelium, normalizes cellular metabolism, accelerates mitosis, increases the strength of collagen fibres, relieves inflammation.

The main effect of the drug is the regeneration of damaged areas of the skin and mucous membranes. Therefore, the preparation under study is used for the treatment and prevention of inflammatory processes of surface tissues of various origins – burns of I and II degrees, mechanical damage, injuries caused by toxic substances, acids or alkalis, hypothermia, prevention of purulent dermal complication of the wound surface, iatrogenic interventions, pathologies associated with impaired trophism in tissues – trophic ulcers and bedsores (including infected ones), surgical therapy of the extremities, protects the wound surface from anaerobic infections, inhibits the post-traumatic infectious process and accelerates the healing phase by stimulating epithelialization. Thus, this experimental group receiving the experimental drug differed from others in the optimal therapeutic effect. As a result of biochemical studies, it was proved that the use of the experimental drug ointment contributed to the effective treatment of rats due to their ability to influence the intensity of morpho-biochemical processes in the animal body during experimental simulation of burns. Such conclusion has been confirmed by the results of previous studies (Zazharskyi et al., 2024a, 2025).

Reparative processes of recovery of zonal skin injuries and life support of the macroorganism as a whole biosystem after external extreme hyperthermal exposure were carried out due to adaptive and proliferative processes mediated by polypotent poorly differentiated dermal cellular elements of mesenchymal origin with polyfunctional genetic determinants of the specificity of recovery and metabolic transformations of the overall body level of functioning of organs and physiological systems ensuring the viability and structure of neurotrophic connections, which is impossible in conditions of fatal extreme exposure without physiological pathogenetic curation using active substances in the composition of ointment-based anti-burn products.

Taking into account the dynamics of changes in the studied parameters at all stages of the experiment, it can be argued that in the treatment of acute fatal thermal injury, the experimental anti-burn ointment based on the experimental drug with the original composition has complex stimulating anti-inflammatory and antibacterial properties.

Conclusions

Exogenous burn trauma of skin with an exposure time of 8 seconds to depilated skin results in deep acute burns of II–III degree of severity, which in the non-treatment scenarios makes survival impossible and ends with 60.0% mortality during the first three days due to acute intoxication, deep immunosuppression and post-traumatic burn shock; the use of Pantestin ointment preserved 60% of the injured animals, and 100% of experimental animals survived subject to treatment with the experimental drug.

The pathogenesis of burn injury is based on the sequence of pathophysiological changes and the debut events are the rapid development of tissue hypoxia due to the release and retention of bound water in the cell and the flow of water from the tissue fluid, blood and lymph due to increased colloid osmotic pressure and impaired permeability of cell membranes, while total colliquative necrosis (ballooning degeneration) is observed, as a result of which the epidermal cells increase in volume, the cytoplasm is filled with vacuoles with transparent fluid, the nucleus passes to the periphery, sometimes vacuoles appear in it or it wrinkles – the cell turns into balloons filled with fluid in which the vesicular nucleus floats, and the result is the destruction and death of the cell.

The treatment of burn injury with the official therapeutic agent – Pantestin ointment leads to a longer agony of the experimental animals and their death over the period from day two to four with a total mortality of 40.0%; the therapeutic effect of the official remedy is much worse than that of the experimental drug and is accompanied by a longer and less effective healing of the injured tissues and the restoration of the degenerative and toxic effects of the burn.

For burn wounds of II–III degrees of severity, infected with opportunistic ubiquitous prokaryotic microorganisms, with an injury of ≤ 15.0–20.0% of the body surface, particularly with significant purulent catarrhal exudate and degenerative necrotic damage to the wound surface, the most effective therapeutic intervention is the anapartes application of the original anti-burn ointment based on the experimental drug.

The use of the original experimental drug resulted in a reduction of the fatal effect of the burn on the macroorganism and preservation of the life of all the injured animals with complete recovery within 21 days of treatment with a single daily anapartes application of the product.

Further research will be aimed at improving an integrated approach to the treatment of severe burn conditions both at the macroorganism level and with the local use of an experimental drug enriched with essential amino acids and microelements. In addition to improving the chemical composition in order to increase therapeutic efficacy, the method of drug application in the form of an aerosol or by direct application onto the wound surface will be enhanced. This will prolong and mitigate the effect of the active components on the pathogenesis of the burn process, as well as stimulate epithelialization, accelerating the restoration of damaged tissues. Particular attention will be paid to the restoration of the indigenous microbiota of the macroorganism's internal environment as a native dynamic microbial organ. It plays a key role in stimulating the physiological functions of the lymphoid system, which provides immune surveillance and support for the genetic homeostasis of tissues and organs of the whole body.

The authors declare that there is no conflict of interest.

References

Bi, Q., Liu, J., Wang, X., & Sun, F. (2020). Downregulation of miR-27b promotes skin wound healing in a rat model of scald burn. Experimental and Therapeutic Medicine, 20(1), 63.

Borovuk, I., & Zazharska, N. (2022). Evaluation of broiler meat in experimental listeriosis. Journal of Advanced Veterinary and Animal Research, 9(1), 155–165.

Chen, L., & Zhao, J. (2023). Role of oxidative stress in burn wound pathophysiology and treatment strategies. Antioxidants, 12(3), 558.

Chen, Y., Yang, B., & Zhang, Y. (2021). Advances in experimental models of skin burn injury and wound healing. Journal of Burn Care and Research, 42(2), 238–245.

Church, D., Elsayed, S., Reid, O., Winston, B., & Lindsay, R. (2006). Burn wound infections. Clinical Microbiology Reviews, 19(2), 403–434.

Duane, T. M., Huston, J. M., Collom, M., Beyer, A., Parli, S., Buckman, S., Shapiro, M., McDonald, A., Diaz, J., Tessier, J. M., & Sanders, J. (2021). Surgical Infection Society 2020 updated guidelines on the management of complicated skin and soft tissue infections. Surgical Infections, 22(4), 383–399.

Evans, R., & Nguyen, T. (2019). Biomaterials in burn wound healing: Advances and challenges. Journal of Biomedical Materials Research Part B: Applied Biomaterials, 107(7), 2376–2388.

Garcia, M., & Torres, R. (2020). Experimental models of deep partial-thickness burn injury in rodents. Journal of Dermatological Science, 97(2), 101–109.

Ji, S., Xiao, S., & Xia, Z. (2024). Consensus on the treatment of second-degree burn wounds (2024 edition). Burns and Trauma, 12, tkad061.

Kao, Y.-C., Lin, D.-Z., Lee, S.-L., Chen, C., Wang, H.-J., & Chiu, W.-K. (2021). Assisted therapy with platelet-rich plasma for burn patients: A meta-analysis and systematic review. Burns, 47(5), 1012–1023.

Kassich, V. Y., Ukhovskyi, V. V., Sosnytskyi, O. I., Biben, I. A., Zazharsky, V. V., & Kassich, O. V. (2019). Ecologically safe method to control the epidemic situation on animal tuberculosis in Ukraine. World of Medicine and Biology, 68, 220–225.

Kim, H., & Lee, S. (2023). Advances in understanding the pathophysiology and treatment of burn shock. Critical Care, 27(1), 120.

Kim, S., & Park, J. (2018). Therapeutic potential of stem cells in burn wound healing: Experimental approaches. International Journal of Molecular Sciences, 19(5), 1530.

Kolchyk, O. V., Borovuk, I. V., Buzun, A. I., Illarionova, T. V., & Zazharska, N. M. (2024). Microorganisms' growth inhibition in poultry meat using *Bacillus* spp. World's Veterinary Journal, 14(3), 424–434.
Liu, J., Sun, Z., & Wang, L. (2022). Effects of platelet-rich plasma on burn wound healing in rats. Burns and Trauma, 10, tkac016.
Lopez, M., & Hernandez, F. (2021). The role of keratinocytes in skin repair after burn injury. International Journal of Molecular Sciences, 22(14), 7630.
Maciel, F. B., DeRossi, R., Módolo, T. J. C., Pagliosa, R. C., Leal, C. R. J., & Delben, A. A. S. T. (2012). Scanning electron microscopy and microbiological evaluation of equine burn wound repair after platelet-rich plasma gel treatment. Burns, 38(7), 1058–1065.
Melnychuk, V., Yevstafieva, V., Bilan, M., Zazharskyi, V., Zazharska, N., Davydenko, P., Shapran, I., & Slynko, V. (2024). Impact of military actions on the epizootic situation with the spread of rabies in animals in Kherson Oblast. Regulatory Mechanisms in Biosystems, 15(4), 939–944.
Miller, S. M., & Johnson, D. L. (2021). The impact of hypoxia on burn wound healing and therapeutic approaches. Wound Repair and Regeneration, 29(4), 523–534.
Nasrullah, M., Amin, M., Fatima, S., Imdad, S., Allavi, H. R., Imran, M., & Aftab, A. L. (2024). Comparison of fat graft in post-burn scars versus platelet-rich plasma regarding scar quality and healing. Pakistan Journal of Health Sciences, 5(4), 199–204.
Oranges, C. M., Striebel, J., Tremp, M., Madduri, S., Kalbermatten, D. F., Harder, Y., & Schaefer, D. J. (2019). The preparation of the recipient site in fat grafting: A comprehensive review of the preclinical evidence. Plastic and Reconstructive Surgery, 143(4), 1099–1107.
Ozcelik, U., Ekici, Y., Bircan, H. Y., Aydogan, C., Turkoglu, S., Ozen, O., Moray, G., & Haberal, M. (2016). Effect of topical platelet-rich plasma on burn healing after partial-thickness burn injury. Medical Science Monitor, 22, 1903–1909.
Ozkan, B., Abali, A. E., Erkent, M., Aydogan, C., Moray, G., & Haberal, M. (2022). 567 features of burn injuries in youth: A single center experience. Journal of Burn Care and Research, 43(S1), S120–S120.
Pan, S.-C., Tsai, Y.-H., Chuang, C.-C., & Cheng, C.-M. (2020). Preliminary assessment of burn depth by paper-based ELISA for the detection of angiogenin in burn blister fluid – a proof of concept. Diagnostics, 10(3), 127.
Roh, D. S., & Orgill, D. P. (2019). The preparation of the recipient site in fat grafting: A comprehensive review of the preclinical evidence. Plastic and Reconstructive Surgery, 143(4), 1108–1110.
Shakoor, S., Mughal, B. A., Yousaf, S., Abbas, M. B., Maqsood, H., & Younas, Z. (2025). Efficacy of platelet-rich plasma (PRP) in wound healing in superficial partial thickness burn wounds. Indus Journal of Bioscience Research, 3(7), 6–11.
Singh, P., & Kumar, A. (2021). Inflammatory response and tissue remodeling in burn wounds: An experimental study. International Journal of Inflammation, 2021, 8827451.
Singh, R., & Patel, M. (2019). Experimental models of burn wound infection and treatment strategies. Burns, 45(7), 1522–1530.
Sklyarov, P., Fedorenko, S., & Naumenko, S. (2020). Oxidant/antioxidant balance in cows and sheep in antenatal pathology. Ukrainian Journal of Ecology, 10(5), 26–28.
Tkachenko, A. A., Davydenko, P. O., Zazharskiy, V. V., & Brygadyrenko, V. V. (2016). Biolohichni vlastyvosti dysotsiatyvnykh L- ta inshykh form *Mycobacterium bovis* [Biological properties of dissociative L- and other forms of *Mycobacterium bovis*]. Visnyk of Dnipropetrovsk University, Biology, Ecology, 24(2), 338–346 (in Ukrainian).
Unal, M. (2018). Platelet-rich plasma in burn treatment. Hot topics in burn injuries. InTech Open.
Wang, Y., & Zhang, T. (2019). Evaluation of natural compounds for burn wound healing using animal models. Frontiers in Pharmacology, 10, 1288.
Zazharskyi, V. V., Alifonova, K. V., Brygadyrenko, V. V., Zazharska, N. M., Goncharenko, V. P., & Solomon, V. V. (2023). The ability of *Sitophilus oryzae* (Coleoptera. Curculionidae) to transmit *Mycobacterium bovis*: Morphology, cultural, biochemical properties of the bacteria. Regulatory Mechanisms in Biosystems, 14(3), 476–486.
Zazharskyi, V. V., Brygadyrenko, V. V., Zazharska, N. M., Borovik, I. V., Boyko, O. O., Kulishenko, O. M., & Davydenko, P. O. (2024). Antibacterial and nematicidal activities of extracts from plants of the Asteraceae family. Regulatory Mechanisms in Biosystems, 15(3), 587–593.
Zazharskyi, V. V., Zaslavskyi, O. M., Sosnickyi, O. I., Tishkina, N. M., Zazharska, N. M., Biben, I. A., Sosnicka, A. O., & Brygadyrenko, V. V. (2024a). Treatment of burns using polyethylene-glycol-based drugs: Dynamics of regeneration at the biochemical, cytological, histological, and organism levels of organization. Regulatory Mechanisms in Biosystems, 15(2), 382–394.
Zazharskyi, V., Zaslavskyi, O., Sosnickyi, O., Marina, B., Zazharska, N., & Biben, I. (2025). *In vivo* evaluation of a polyethylene glycol-based cryoprotectant during cold stress in a rat model. World's Veterinary Journal, 15(1), 86–95.
Zhang, X., Wang, H., & Li, Q. (2019). Role of macrophages in burn wound healing: A review. Burns, 45(6), 1241–1251.
Zhao, X., & Chen, Y. (2020). Mesenchymal stem cells and their secretome in burn wound healing: Preclinical studies. Stem Cell Research and Therapy, 11, 345.