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Preclinical Studies of the Antimicrobial and Wound-Healing Effects of the High-Intensity Optical Irradiation “Zarnitsa-A” Apparatus

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Abstract: In this paper, a new approach to treating infected wounds is proposed. It is based on treating the wound surface with high-intensity pulsed optical radiation with a continuous spectrum, continuously covering the entire UV range (from 200 to 400 nm) and the visible and near-infrared regions of the spectrum. A pulsed xenon lamp is used as a radiation source. A description of the design and technical characteristics of the device, “Zarnitsa-A”, is presented, implementing the proposed medical technology for wound therapy. The results of our studies of the apparatus in vitro and in vivo are also presented. The article shows that exposure to the apparatus leads to pronounced bactericidal- and wound-healing properties. The apparatus’s action reliably provides higher rates of wound healing compared with only a typical antibacterial or wound healing agent, such as “levomekol” ointment.

Keywords: high-intensity broadband optical irradiation; pulsed xenon lamp; phototherapy device; preclinical studies; bactericidal effect; wound healing



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1. Introduction

The prevention and treatment of wounds and wound infections are urgent tasks in modern practical medicine. Infections in wounds can be caused by various microorganisms, leading to disruptions of the healing process of varying severity. Moreover, each wound infection can spread and cause severe consequences, including life-threatening sepsis. According to statistical data, incidents of purulent-inflammatory diseases and infectious complications occupy one of the highest positions in the list of surgical diseases: the proportion of patients with purulent infections among all surgical patients is 35–40%, and mortality can reach up to 42% [1].

In recent years, the problem of treating wounds and wound infections has been sharply aggravated by the emergence and wide spread of pathogenic microflora with multidrug resistance [2]. In addition, a pronounced modern trend is an increase in the contingent of people with reduced immunity. They tend to suffer various concomitant diseases (allergic diseases, diabetes, etc.) and have restrictions on the use of pharmacological methods of treatment [3].

These factors necessitate the research and development of new, highly effective methods and medical technologies for wound treatment. Non-invasive methods of treating wounds based on their physical impact on damaged tissues are considered promising [4–7]. In this context, therapeutic methods using optical technologies are of significant interest. Among these technologies, in recent years, low-intensity laser therapy [8–10], chromotherapy (treatment with various parts of the visible spectrum range [11], including the treatment of wounds with red [12] and blue [13] light and with infrared radiation [14]), and photodynamic therapy have become popular [15]. The most representative optical technology is

ultraviolet (UV) therapy. This is because UV radiation has maximum photochemical and photobiological effects, primarily because of the significant energy of UV photons, which are commensurate with energy in the chemical bonds in biomolecules.

In photobiology, ultraviolet radiation is divided into three spectral regions, including the UV-C range ($\Delta\lambda = 200 \dots 280$ nm), the UV-B range ($\Delta\lambda = 280 \dots 315$ nm), and the UV-A range ($\Delta\lambda = 315 \dots 400$ nm) [16]. Various biological effects inherent to these ranges are determined by the energy of photons and the depth of their penetration into biological tissues. Photons in the UV-C range have maximum energy, though their penetration ability is practically limited to the upper layer of the epidermis (the stratum corneum, a layer of inanimate (dead) cells). UV-B radiation penetrates deeper into the basal layer and upper dermal cells, i.e., living cells. UV-A radiation has even greater penetrating power and reaches the upper layers of the dermis (a penetration depth of up to ~1 mm); however, its photon energy is noticeably lower than that of the UV-C and UV-B ranges. UV-C and UV-B radiation are strongly absorbed by DNA and protein molecules, causing various destructive modifications and ultimately leading to cell death. This process determines the strong biocidal effect of short-wavelength UV radiation, with UV-C radiation having the maximum biocidal effect [3,6,17]. In this regard, it is widely used to disinfect and sterilize inanimate objects [18,19]. It can also be directly used to destroy pathogens in infected wounds. UV-B radiation has significantly lower biocidal potential than UV-C, but it is characterized by a maximum erythral effect, which can expand capillary vessels, increase the permeability of their walls, and increase local blood flow, leading to a local intensification of metabolic processes and stimulating the wound-healing process [3,4,17,20]. Unlike UV-C and UV-B, UV-A radiation is weakly absorbed by DNA; instead, it excites other endogenous chromophores (porphyrins, etc.), generating various reactive oxygen species in cells, which, on the one hand, activate the cell signaling system, initiating inflammatory and proliferative reactions, and, on the other hand, can cause oxidative damage to DNA bases [3,16,21].

The biological (including therapeutic) effect of exposure to UV radiation depends both on the spectral range of the applied radiation ($\Delta\lambda$) and on the energy dose of exposure (D) (J/cm^2). D is determined by the product of illuminating a biological object (wound) $I_{\Delta\lambda}$ (in W/cm^2) in a given $\Delta\lambda$ for a given exposure time (t (s)); i.e., $D = I_{\Delta\lambda} \cdot t$. In clinical practice, the basis for UV radiation dosimetry is the minimum erythral dose (MED)—the lowest energy dose of skin irradiation (in mJ/cm^2)—which causes minimal-intensity erythema but with clearly defined boundaries. The MED is determined individually, using biodosimeters [4,20]. According to international recommendations, the MED for sensitive skin is $25 \text{ mJ}/\text{cm}^2$ [22]. The sensitivity of microorganisms to UV radiation varies widely depending on the species and their condition: for vegetative bacteria, the ability to form colonies is suppressed by 90% at doses of UV-C radiation (with a wavelength of $\lambda = 254$ nm) from 1.1 to $8 \text{ mJ}/\text{cm}^2$; the doses required to reduce the bacterial load by four decimal orders are therefore in the range of $5 \dots 20 \text{ mJ}/\text{cm}^2$ [19]. At the same time, Gram-positive bacteria, as a rule, are more resistant to UV radiation than Gram-negative ones. The doses for the inactivation of pathogens on living tissue are increased due to the peculiarities of the surface condition of living tissue.

The use of optical technologies in medicine has a long history. The most active research and development period for optical technologies (in particular, ultraviolet) was in the 1930s and 1940s. During these years, practical medicine did not yet know about antibiotics, and many scientists pinned their hopes on ultraviolet light for significant progress in treating tuberculosis, purulent-inflammatory diseases, and infectious diseases. Extensive experimental material on the bactericidal and therapeutic effects of UV radiation was collected, and many preventive and therapeutic methods for the clinical application of ultraviolet radiation were developed [17]. These methods, practically unchanged since then, are still recommended for use at present [4,20,23–25].

However, with the advent of antibiotics, interest in UV-based medical technologies for treating wounds has notably weakened. Another factor constraining the widespread

introduction of UV therapeutic technologies is the risk of possible long-term side effects due to UV irradiation, such as carcinogenesis, which may occur with inadequate methodological applications of such technologies [3,17,21].

The “crisis of antibiotics” became acute at the beginning of the 21st century, forcing medical specialists to reconsider the current paradigm of UV radiation for medical use [3]. In [21], the authors proposed a therapy using short-wave UV-C radiation as an alternative approach to the current treatments for localized infectious diseases, especially those caused by pathogens with multidrug resistance. Prospects for using UV-C therapy to treat wounds are associated with several factors, among which, the following are primarily noted:

- Multidrug-resistant microorganisms are equally sensitive to UV irradiation as their “wild-type” counterparts [21,26,27];
- UV-C radiation destroys microorganisms much faster than antibiotics—hours with UV-C therapy versus several days with antibiotic treatments [3,21];
- UV-C therapy could be much more cost-effective than commonly used antibiotics. This is because, in addition to inactivating microorganisms, adequate exposure to short-wave UV radiation promotes accelerated wound healing and restores skin homeostasis. The effects of UV-C radiation on wound healing include hyperplasia and enhanced re-epithelialization, granulation tissue formation, and the shedding of necrotic tissue [3,21,28].

In [3], in an analysis of the potential of UV-C technologies, low-pressure mercury lamps with various types of excitations, excimer lamps, and short-wavelength LEDs were considered as sources of UV radiation. All these sources are characterized by a narrow (monochromatic) emission spectrum (the typical width of the emission line (band) is ~2 nm for mercury lamps and ~10 nm for excimer lamps and LEDs) and the low irradiance of biological objects (on the order of several milliwatts per square centimeter or less). However, these physical factors can lead to several limitations on the applications of therapeutic technologies using them.

Given the monochromaticity of radiation, the inactivation of microorganisms has a pronounced, selective nature (that is, the death of a pathogen occurs only if its absorption spectrum coincides with the emission spectrum of the lamp).

Low-intensity radiation requires long exposure times and does not enable the effective treatment of massively contaminated wounds. Thus, in [29], in an in vivo study on the antimicrobial effects of UV radiation with a wavelength of 254 nm, achieving a 10-fold decrease in the bacterial load in mouse wounds infected with *Pseudomonas aeruginosa* and *Staphylococcus aureus* required sufficiently large (hypererythemic) single doses of UV-C radiation—2.59 J/cm². Similar doses of UV-C radiation (2.8 J/cm²) were also used in clinical trials to study the effectiveness of UV-C therapy in difficult-to-heal ulcers infected with methicillin-resistant *Staphylococcus aureus* (MRSA) [27]. In another study, antifungal UV-C therapy for *Candida albicans* contaminated wounds in laboratory animals was carried out at even higher doses of UV-C irradiation (up to ~6.5 J/cm²) [30].

This research proposes a new approach to the treatment of wound lesions. It involves treating the wound surface with high-intensity, broad-spectrum pulsed optical radiation, continuously covering the entire UV range (from 200 to 400 nm) and visible and near-infrared spectral regions. As a source of radiation, we propose using pulsed xenon lamps, as they are currently the most technologically advanced high-temperature plasma emitters [31]. Such lamps have many physical and technical features that favorably distinguish them from radiation sources traditionally used in modern phototherapy and suggest their high therapeutic potential and safety.

Among the above features, the emission spectrum of a pulsed xenon lamp should be noted first. In optimal power modes, the lamp emits a powerful continuum in the entire spectral transparency window of the quartz bulb of the lamp, i.e., from 0.2 to 2.7 μm. Various biological objects (microbial cells, subcellular structures, biomolecules, etc.) have different absorption spectra because of their different physical and biochemical organizations. As mentioned, photochemical action occurs only when the absorption spectra of an

object and the spectrum of the acting radiation coincide (the first law of photochemistry is the Grotthus–Draper law). By irradiating microbial cells (the bio-object) with a wide spectrum, such a radiation source can effectively affect all types of microorganisms, regardless of their spectral characteristics, as well as all vital cell structures, such as nucleic acids, proteins, biomembranes, etc. Several important conclusions follow from this: (1) a broad optical spectrum potentially provides a wide range of antimicrobial activity: all types of microflora are suppressed, regardless of the individual spectral characteristics of the microorganisms; (2) broad-spectrum UV irradiation of a microorganism has a multichannel destructive effect on all vital cell structures, which reduces the overall resistance of the pathogen (lethal doses decrease) and the possibility of its adaptation to UV exposure; (3) a continuous (identical to solar) emission spectrum could provide synergy to biological effects in different spectral ranges: the strong biocidal effect of short-wave ultraviolet radiation combines with the immuno- and trophostimulating effects of medium- and long-wave UV, visible, and near-IR radiation, potentially creating optimal conditions for the growth of new tissues and accelerated wound healing.

Another fundamental difference between the proposed technology and known optical therapeutic technologies is associated with the high intensity of impact on biological objects: the characteristic values of the pulsed irradiance of treated surfaces are 10–100 W/cm², with a maximum of 1...10 kW/cm², which is several orders of magnitude higher than the irradiation level of intensity for objects using traditional sources of UV radiation. A high pulse intensity provides conditions for a significant excess in the rate of direct processes (in this case, destructive to biomolecules) over the opposite processes (relaxation, recombination, repair), increasing the resulting quantum yield of photochemical reactions and leading to a decrease in the threshold energy doses necessary to achieve a biological effect. On the one hand, this ensures the rapidity of disinfection processes and medical and preventive procedures, and on the other, it potentially increases the level of safety in the use of UV radiation. In addition, at high pulsed intensities of exposure, along with photochemical mechanisms of cell destruction, it is possible to implement additional nonstationary photodynamic [32,33] and photothermal [34,35] destruction processes in microflora, which are fundamentally unfeasible after exposure to traditional continuous-wave radiation sources.

The results of numerous experimental studies conducted in recent years both abroad [32–40] and in our country [41–47] confirm the expected effects. In particular, it has been shown that broad-spectrum, high-intensity pulsed UV radiation has unique biocidal properties: where traditional disinfection methods using standard bactericidal mercury lamps reduce the level of infection by a factor of thousand, this technology reduces the concentration of microbes by several million times or even more [34,36,42,45]. At the same time, all types of pathogenic microflora are suppressed, including the most resistant forms of spore microorganisms, and viruses, including those resistant to antibiotics. The process remains efficient at extremely high levels of initial microbiological contamination. For several pathogens, a significant (up to 30 times) reduction in the threshold energy of doses required to provide a given level of decontamination or achieve a sterilizing effect has been experimentally established [40,42,45].

In addition to the physical features of flash xenon lamps noted above, experience shows that they also have several fundamentally important technical and operational properties, such as the ability to operate in a wide range of ambient temperatures (from minus 60 to plus 60 degrees Celsius), instant readiness for operation, the ability to operate one lamp in a wide range of average power (from fractions of a watt to kilowatts) without changing the spectral characteristics, environmental friendliness (no toxic substances (mercury, etc.) are used), etc.

Currently, the use of pulsed xenon lamps as a biocidal tool is becoming more widespread. Initially, technologies based on pulsed xenon lamps were considered a means of quickly eliminating the consequences of terrorist acts using bioagents [19,40]. Currently, such technologies are being actively studied for use in the food industry to increase the shelf life

of food products, improve product quality, decontaminate packaging, etc. [36–39]. In the medical field, pulsed xenon lamps are currently used in installations for air and surface disinfection [44,45,47,48].

Thus, there are currently objective scientific and medical–technical prerequisites for the development of a new generation of medical devices for the treatment and prevention of wounds and wound infections based on using pulsed high-intensity optical irradiation technology on biological objects. The objective of this research is to use “Zarnitsa-A”, an apparatus for pulsed optical irradiation developed at the Bauman Moscow State Technical University, in order to implement a new medical technology for the treatment of wounds and infectious diseases.

2. Materials and Methods

2.1. Description of the “Zarnitsa-A” Prototype

In the presented research, a prototype of the “Zarnitsa-A” apparatus was used. Its functional diagram is shown in Figure 1.

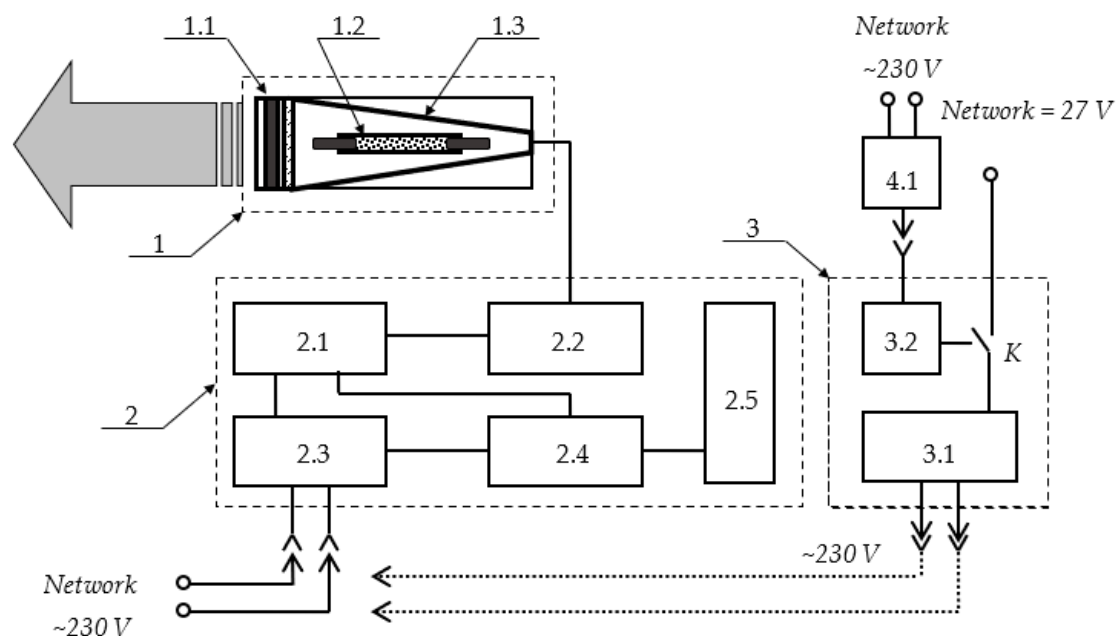


Figure 1. Functional diagram of the “Zarnitsa-A” apparatus: 1—irradiator block; 1.1—replaceable light filter; 1.2—pulsed xenon lamp; 1.3—radiation flux shaper; 2—service block; 2.1—capacitive storage; 2.2—discharge-initiating block; 2.3—capacitive storage charging unit; 2.4—control and monitoring scheme; 2.5—control panel; 3—backup power supply; 3.1—inverter; 3.2—battery; 4.1—battery charging unit; K—power mode toggle switch.

The apparatus consists of a service unit (power and control unit), an irradiator, and a backup power unit. The device’s principle of operation is based on the pulsed irradiation of affected areas of up to 50 cm² (per one setting) with high-intensity optical radiation in a continuous spectrum generated by a pulsed xenon lamp. In the device’s irradiator, we used a tubular xenon lamp with a quartz bulb with an inner diameter of 5 mm and an interelectrode gap length of 60 mm. The lamp operates in a repetitively pulsed mode with a pulse frequency of 5 Hz and an average electrical power of 100 W. The lamp is mounted along the axis of a conical diffuse reflector with an output quartz window with a light diameter of 50 mm. The design of the irradiator enables the installation of additional light filters and/or nozzles outside the exit window, limiting the irradiation area. A set of light filters allows one to cut out the necessary wavelength ranges to work in various therapeutic modes. The service unit (2) includes a capacitive storage unit (2.1) with stored electrical energy of 20 J, a charge initiation unit (2.2), a capacitive storage charging unit (2.3),

a control circuit (2.4), and a control panel (2.5). The service unit is powered by alternating current with a voltage of 230 V directly from the external network or a backup power supply unit. The backup power supply unit (3) consists of an inverter (3.1) and a battery (3.2). The battery is charged using a charging unit (4.1). The backup power unit (3) forms an alternating current of 230 V by converting the voltage of the onboard network of the vehicle or battery cells through the inverter (3.1). The choice of power source (battery/onboard network) is made using the toggle switch (K) located on the front panel of the backup power unit. Service unit dimensions— $192 \times 330 \times 142$ mm; weight—6.5 kg. The dimensions and weight of the irradiator are $\varnothing 72 \times 298$ mm and 0.8 kg, respectively.

On the control panel of the device, the following are installed:

- A key for supplying power to the device: when the key is turned clockwise to the end, operating voltages are applied to the power and control circuits;
- A “Start” button: when it is pressed, the apparatus starts working; the irradiator emits light flashes that follow at a frequency of 5 Hz for 20 s (100 flashes in total) for one irradiation cycle;
- A “Stop” button: when it is pressed, the irradiator stops emitting light flashes, but power continues to be supplied to the circuits of the apparatus. When one presses the “Start” button again, the operation of the device is resumed.

2.2. Spectral Energy Measurements

Photoelectric measurements of the device’s radiation characteristics were carried out using a calibrated set of photodetectors as part of a “Spektr-01K” photoelectronic converter (FEC), which provides radiation detection in narrow spectral ranges of the UV, visible, and near-IR regions [49]. Signals from the FEC were recorded with a Tektronix TDS-2014C digital oscilloscope (bandwidth, 70 MHz). The oscilloscope was set to the signal-averaging mode over 64 pulses.

The time-integrated emission spectra of the apparatus were studied using a Solar S100 wide-range fiberoptic spectrometer with a diffraction grating of 300 lines/mm and an S8378-1024 image sensor (manufactured by Hamamatsu, Hamamatsu City, Shizuoka Pref., Japan). The spectral sensitivity range of the spectrometer was 200...1100 nm; the spectral resolution was 1.5 nm. To register the absolute values of the spectral energy irradiances generated by the device at different distances, we used an Ocean Optics P300-1-SR quartz optical fiber ($\varnothing 300$ μ m, $\Delta\lambda = 200$...1100 nm) together with a CC-3-UV-S cosine corrector ($\Delta\lambda = 200$...2500 nm). The exposure time of the spectrometer was set based on the condition of recording the distribution of energy irradiance averaged over 40 pulses.

In terms of spectral sensitivity, the joint calibration of the optical system of the spectrometer with an optical fiber and a cosine corrector was carried out using a reference radiation source: DH-3P-CAL (manufactured by Ocean Optics).

Figure 2 shows the time dependence of the spectral radiation intensity of the “Zarnitsa-A” apparatus in the short-wave UV region of the spectrum ($\lambda = 257 \pm 14$ nm), which was obtained by processing signals from the Spectr-01K FEC and the results of its integration over time. The thickness of the lines shows the value of the scatter in the values of the measured optical characteristics in 10 different turning cycles on the apparatus, with 64 pulses each. The spread of pulse energies did not exceed 10%. The duration of the UV-C radiation pulse at half-height was 20.0 ± 0.5 μ s. This duration corresponds to the characteristic duration of the energy input to the discharge, which, with an energy of 20 J stored in the capacitors, provides the peak electric power of the lamp at ~1 MW.

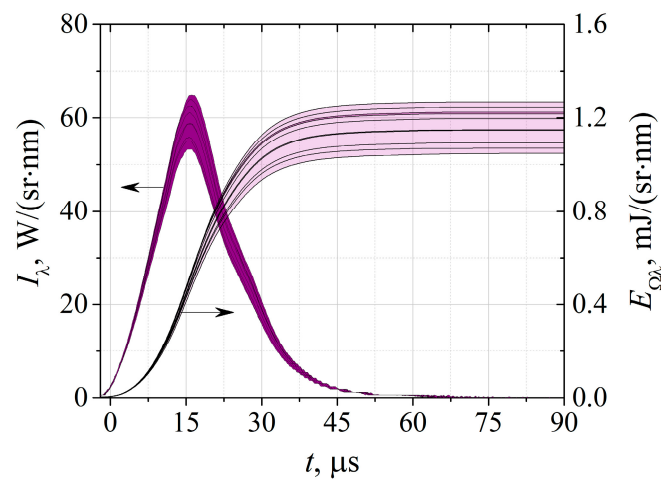


Figure 2. Time dependence of the spectral radiation strength of the “Zarnitsa-A” apparatus and the angular density of radiation energy in the short-wave UV region of the spectrum ($\lambda = 257 \pm 14$ nm).

Similar signal processing from the FEC “Spektr-01K” in other spectral ranges made it possible to reconstruct the photoelectric radiation spectrum of the device and determine the energy–power characteristics of the irradiator integral over the spectrum, which amounted to a radiation power of 31.5 ± 0.1 kW/sr and an angular energy density radiation in the spectral range of 200...1100 nm 0.77 ± 0.02 J/sr. At the same time, up to 40% of the total radiated power and ~25% of the integral radiation energy were emitted in the UV region of the spectrum, $\Delta\lambda = 200...400$ nm. A decrease in the energy efficiency of UV radiation compared with power efficiency is associated with longer light pulse durations in the visible and near-IR spectral regions than in the UV range. The photoelectric measurement data were in good agreement with the results of spectrometric studies performed using the Solar S100 spectrometer.

Figure 3 shows the radiation spectrum of the “Zarnitsa-A” apparatus’s irradiator, as recorded by the Solar S100 spectrometer. The spectrum is provided as a dependence of the spectral energy density of the radiation ($E_{s\lambda}$) on the wavelength (λ) for one irradiation pulse at a distance of $L = 50$ cm from the irradiator cutoff of the apparatus.

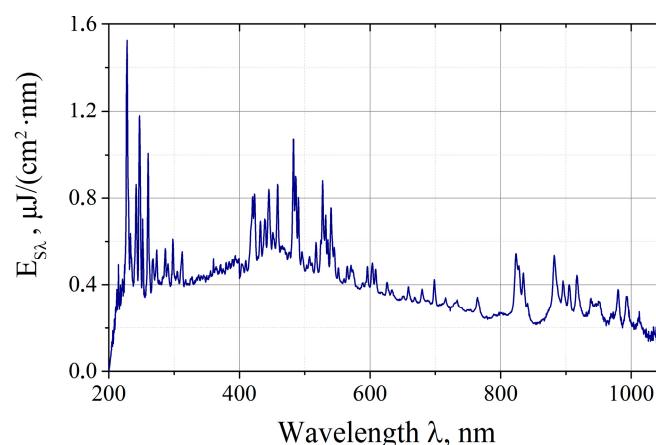


Figure 3. Spectral energy density of the radiation of the “Zarnitsa-A” apparatus per pulse at a distance of 50 cm from the irradiator.

The emission spectrum of the device was predominantly continuous, with powerful lines of atomic and ionized xenon superimposed on it in the short-wave UV region (200...300 nm) and the visible (420...550 nm) and near-IR (800...1000 nm) ranges. The spectrometer registered the emission spectrum in a range of 200...1100 nm; the actual emission spectrum of the xenon lamp (and, accordingly, the irradiator of the device) extends to

the long-wavelength limit of the quartz transmission ($\sim 2.7 \mu\text{m}$). However, as especially conducted measurements show, the fraction of radiation in the $1.1 \dots 2.7 \mu\text{m}$ region does not exceed 10% of the radiation energy in the $0.2 \dots 1.1 \mu\text{m}$ range. By integrating the experimental spectrum over wavelengths, we obtained the radiation energy density of the device in a spectral range of $200 \dots 1100 \text{ nm}$ per pulse at a distance of $L = 50 \text{ cm}$, which was $E_s \approx 0.32 \text{ mJ/cm}^2$. This value determined the angular density of the radiation integrated over the spectrum $E_\Omega = E_s \cdot L^2 \approx 0.81 \text{ J/sr}$ and showed good agreement with photoelectric measurements. A similar procedure makes it possible to determine the spectral efficiencies (or relative shares in the total spectrum) of the actual wavelength ranges. According to the measurements, the share of UV-C radiation was $\sim 10\%$ of the energy emitted in the spectral region ($\Delta\lambda = 200 \dots 1100 \text{ nm}$), and the corresponding shares of the UV-B and UV-A ranges were ~ 4 and $\sim 10\%$, respectively.

At a distance of $L = 5 \text{ cm}$ from the irradiator cutoff of the “Zarnitsa-A” apparatus, given the spectrometric measurements performed using a fiberoptic light guide with a cosine corrector, the diameter of the light spot in terms of the half-intensity level was $D_{05} \approx 4 \text{ cm}$. Furthermore, in one irradiation cycle (20 s, 100 pulses) at the center of the spot, the total radiation energy density in the integrated spectrum ($200 \dots 1100 \text{ nm}$) was $\sim 1 \text{ J/cm}^2$, and the energy dose in the UV-C range was $\sim 100 \text{ mJ/cm}^2$. As the distance increased to $L = 10 \text{ cm}$, the radiation energy density at the center of the spot decreased by ~ 2 times, and the spot diameter increased to $D_{05} \approx 5.5 \text{ cm}$.

2.3. Studies In Vitro

Studies of the bactericidal and wound-healing effectiveness of the Zarnitsa-A apparatus were carried out at the Izmerov Research Institute of Occupational Health. The purpose of the ongoing microbiological studies was to evaluate the in vitro bactericidal action of the “Zarnitsa-A” apparatus in relation to clinically significant microorganisms (MOs).

The following types of MOs (and their strains) were selected as research objects:

- Gram-negative bacteria: *Escherichia coli* (M-17 (O2:H6)) and *Pseudomonas aeruginosa* (ATCC 9027/NCTC 12924);
- Gram-positive bacteria: *Staphylococcus aureus* (ATCC 6538-P/FDA 209-P) and *Streptococcus pyogenes* (ATCC 12344/NCTC 8198).

The MO strains were obtained from the State Collection of Pathogenic Microorganisms and Cell Cultures (“SCPM—Obolensk”) and had a BSL-1 biosafety level. The selected strains were not drug-resistant, except for *Staphylococcus aureus*, which was resistant to the antibiotic novobiocin.

Preparation of the control-strain MOs and the initial suspension was carried out in the following way. We took a pure culture of specific bacteria and sowed it on mown meat–pentone agar (MPA) to obtain a daily culture. From the daily agar culture of the control-strain MOs, a suspension was prepared in physiological saline. We prepared the suspension with a given concentration of cells, using the optical turbidity standard 0.5 according to McFarland. The Densi-La-Meter II device (manufactured by Erba Lachema, Czech Republic) was used to determine the turbidity of the bacterial suspension. To more accurately determine the initial concentration of MOs in the suspension intended for the experiment, serial dilutions of the resulting suspension were performed using the tenfold dilution method.

In the experiments, the following was carried out: (1) positive control (1.0 cm^3 of inoculum was taken from each dilution with an automatic dispenser and transferred to the surface of the agar medium)—this controlled the prepared suspension and further helped to calculate the results; (2) negative control (without inoculation)—this controlled the sterility of nutrient media, utensils, and instruments.

When preparing the experimental Petri dishes, 1.0 cm^3 of seed material was evenly distributed with a spatula over the surface of the agar, $0.5 \dots 1 \text{ cm}$ away from the side walls of the Petri dish, which was necessary to exclude possible parietal effects. Petri dishes

90 mm in diameter were used in the experiments; accordingly, the area of the sown surface was $S \approx 50 \text{ cm}^2$.

The initial density of MOs in the suspensions varied in a range of 10^6 to $4 \cdot 10^8 \text{ CFU/cm}^3$, which provided the initial surface contamination of the agar medium: $N_{S0} = N_0 \cdot \frac{V_0}{S} = 2 \cdot 10^4 \dots 8 \cdot 10^6 \text{ CFU/cm}^2$, where N_{S0} refers to the initial surface contamination of the agar medium; N_0 is the initial density of MOs in the suspensions; $V_0 = 1 \text{ cm}^3$ —volume of the inoculated aliquot; S —the area of the contaminated surface of the agar medium. MPA was used as a nutrient medium on which MOs were inoculated in all experiments. For each irradiation mode, three Petri dishes were prepared to ensure a 3-fold repetition of measurements.

Experimental Petri dishes with the inoculated culture (after drying at room temperature for 30 min) were irradiated using the “Zarnitsa-A” apparatus. The irradiator of the apparatus was fixed in a special stand, which made it possible to adjust the distance from the irradiator to the irradiated object.

Cups with bacterial inoculations (control and workers after irradiation) were incubated in a thermostat at a temperature of $(37 \pm 1)^\circ\text{C}$ for 24 h. After that, the formed colonies were counted.

Most of the experiments were performed at a distance of the irradiator $L = 10 \text{ cm}$ from the contaminated surface to the end plane. The surface (in the plane of the contaminated surface) radiation energy density in the UV-C range ($\Delta\lambda = 200 \dots 280 \text{ nm}$) was taken as a representative dose of radiation. The UV-C radiation energy doses in the experiments ranged from 25 to 150 mJ/cm^2 , and the exposure time ranged from 10 to 60 s.

The effectiveness of disinfection was determined by calculating the logarithm of inactivation, equal to the decimal logarithm of the ratio of the initial number of microorganisms in the sample (N_0) to the number of microorganisms (N_i) that survived after irradiation with a dose of $D_i - \lg\left(\frac{N_0}{N_i}\right)$. The numerical value of the logarithm of inactivation shows how many decimal orders the initial number of bacteria decreased after treatment with a given energy dose.

Another representation of the bactericidal efficiency of the device was the efficiency of disinfection, expressed as a percentage and equal to the ratio of the amount of inactivated (dead) bacteria (N_{ni}) to the number of initially inoculated bacteria (N_0) at a given dose (D_i).

$$\eta_i = \frac{N_{ni}}{N_0} \cdot 100\% = \left(1 - \frac{N_i}{N_0}\right) \cdot 100\%, \quad (1)$$

The number of nines in the numerical value of η_i is equal to the integer number of the inactivation logarithm.

2.4. Preclinical Studies In Vivo

Animal experiments were approved by the ethics committee of the Izmerov Research Institute of Occupational Health.

The studies were carried out using Wistar rats. The age of the animals at the beginning of the experiment was 12 ± 2 weeks with a body weight of at least 215 g, which did not deviate from the average for all groups by more than 20%.

The experiments involved 45 male rats in 3 groups of 15 animals in each. In control group № 1, wound therapy was carried out only with a broad-spectrum antibiotic (cefazolin at an average therapeutic dose, I.M., over a course of 8 days); in control group № 2, wound therapy was carried out with an antibiotic (cefazolin over a course of 8 days) and treatment with levomekol ointment. In the third, main group, the wound was treated similarly to control group № 2, but with additional irradiation with the “Zarnitsa-A” apparatus.

Pathology (Wound) Modeling

Surgical actions to inflict wound defects on the animals were carried out in a state of narcotic sleep. Sevoflurane was used as anesthesia at a concentration of 4%. Anesthesia of the animal was carried out in a special chamber until the visual absence of motor activity

of the animal (on average, after 3...5 min). After placing the animals on the contour of an anesthetic-respiratory apparatus, depilation of the back area with a size of 60×60 mm was performed. Then, a region was marked on the skin of the back region using a marker according to a preprepared template in the form of a square with sides of 20×20 mm, and a simultaneous excision of the skin with subcutaneous fat was performed within the marked contour (Figure 4a):

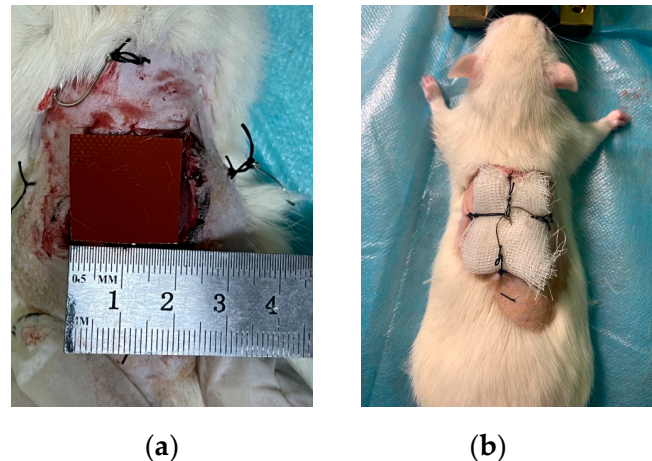


Figure 4. Wound modeling. (a) Applying a wound defect based on the template. (b) Fixing a bandage on the wound surface of the animal.

The skin edges of the wound were fixed to the underlying superficial and deep fascia of the back with simple interrupted sutures using a “Monocryl 3-0” monofilament suture with a cutting atraumatic needle. Interrupted sutures were placed at the corners and in the middle of all sides of intact skin.

Furthermore, all animals were seeded with a wound surface culture of *St. aureus* in an amount equal to 10^3 CFU.

Immediately after seeding the wound surface, an intramuscular injection of a broad-spectrum antibiotic (cefazolin) was performed. The dosage was calculated individually based on the weight of the animal. Calculation was performed with a base dosage of 55 mg/kg. The average dosage was 12 ± 1 mg of the antibiotic.

The next step was applying the “levomekol” preparation (for animals in the 2nd and 3rd groups) and imposing sterile gauze bandages (for animals in all groups). The bandage was affixed using preattached hooks and loops (Figure 4b). To ensure the possibility of atraumatic removal and replacement of dressings and—given the impossibility of their standard fixation via dressing because of the behavioral characteristics of rats—two mutually perpendicular hook-loop pairs were additionally prepared on the backs of the animals, sutured directly to the intact skin.

For the main group, 1 h after seeding the wound surface, the wound was treated with radiation from the “Zarnitsa-A” apparatus. At the same time, the animals’ eyes were covered with an opaque screen to prevent exposure to high-intensity optical radiation.

Irradiating the wound surfaces of rats in the main group was carried out at a distance from the opening of the “Zarnitsa-A” apparatus’s irradiator to the surface of the wound of $L = 5$ cm. At this distance, the diameter of the light spot was 4 cm at half-intensity and exceeded the diagonal (i.e., maximum) wound size (≈ 2.8 cm). The duration of the irradiation procedure was 60 s (three inclusions of 20 s each). Based on the measurements described above, under these conditions, the total dose per therapy session in the UV-C range of the spectrum was ~ 0.3 J/cm²; the corresponding dose in the integrated radiation spectrum ($\Delta\lambda = 200...1100$ nm) was ~ 3 J/cm². Irradiation was carried out daily for 8 days. The total (cumulative) dose in the UV-C range of the spectrum for an eight-day course of therapy was 2.4 J/cm²; the cumulative dose in the integrated spectrum was ~ 24 J/cm².

To assess the effect of the apparatus under study on the organs and vital systems of the laboratory animals, they were observed for 14 days. The dynamics of the animals' body weight, temperature, and wound surface areas were assessed on the 3rd, 7th, 10th, and 14th day. At these time points, blood was taken to perform a clinical blood analysis. To study the bactericidal action, swabs from the wound surface were carried out 4 h after the artificial infection of the wound and after irradiation with the apparatus, as well as on the 3rd, 7th, 10th, and 14th day. On the 14th day of the experiment after euthanasia, all animals were sampled for histological examination.

The wound surface area was determined by applying tracing paper to the wound, on which the edges of the wound were outlined. After that, the outlined area was estimated using graph paper.

Swabs from the wound surface were taken with a sterile swab soaked in Amies transport medium. To determine the total microbial number, the swabs were placed in a sterile test tube with 10 mL of isotonic sodium chloride solution and thoroughly washed via shaking with glass beads for 10 min. The washing liquid was inoculated using the deep method, with 1.0 mL into each Petri dish with MPA (20 mL). The cultures were incubated at 37 °C for 24–48 h. Then, the grown colonies were counted and recorded.

The animals were clinically examined once a day after the study began. During examinations, their general condition was recorded.

The rectal body temperature of each animal was recorded using Oakton 10T electric thermometers (OAKTON Instruments, Vernon Hills, IL, USA) with rectal sensors of the appropriate diameter.

Blood sampling from the lateral tail vein was carried out for hematological studies after 12–14 h of fasting in the animals. Blood samples were collected in 4.0 mL tubes and analyzed within 2 h after collection. For general clinical blood analysis, a URIT-2900 Vet Plus veterinary hematology analyzer (URIT Medical Electronic Co., Shenzhen, China) was used. The hematology analyzer was fully automated to count blood cells and erythrocyte indices.

During histological studies, skin samples were fixed in formalin for 2 days. After passing through alcohols of ascending concentrations and chloroform in a Tissue-Tek VIP5Jr apparatus (Sakura Finetek USA, Inc., Torrance, CA, USA), skin fragments were poured into a histomix using a Tissue-Tek TEC device (Sakura Finetek USA, Inc., Torrance, CA, USA); histological sections 5–8 µm thick were prepared on a Microm HM340E microtome (Thermo Scientific, Waltham, MA, USA). The preparations were stained with hematoxylin and eosin according to Van Gieson. Morphological examinations of the skin wounds were performed using a Leica DM2500 microscope (Leica Microsystems, Wetzlar, Germany) at 200× and 400× magnifications.

2.5. Statistical Processing

The results were statistically processed using Microsoft Excel 2019. The type of data distribution was checked using the Kolmogorov–Smirnov and Shapiro–Wilk criteria. For data with a normal distribution type, the group arithmetic mean (M) and standard deviation (SD) were calculated; for data with a non-normal distribution, the median (Me) and interquartile range (25% and 75% percentiles) were determined.

The statistical comparison criterion was chosen based on the type of distribution; intergroup comparisons of interval variables measurements were performed using ANOVA analysis of variance for data with a normal distribution; for data with a non-normal distribution or a normal distribution in cases where ANOVA was not applicable, we used the nonparametric Kruskal–Wallis test.

For repeated measurements, intragroup comparisons of values of one variable at different measurement periods were performed using ANOVA for data with a normal distribution; for data with a non-normal distribution, we used the Wilcoxon criterion test. Differences were considered statistically significant at $p < 0.05$.

3. Results and Discussion

3.1. Main Results of the In Vitro Studies

As an example, Figure 5 shows the results of microbiological experiments assessing the bactericidal effectiveness of the “Zarnitsa-A” apparatus against *Pseudomonas aeruginosa* and *Staphylococcus aureus* bacteria.

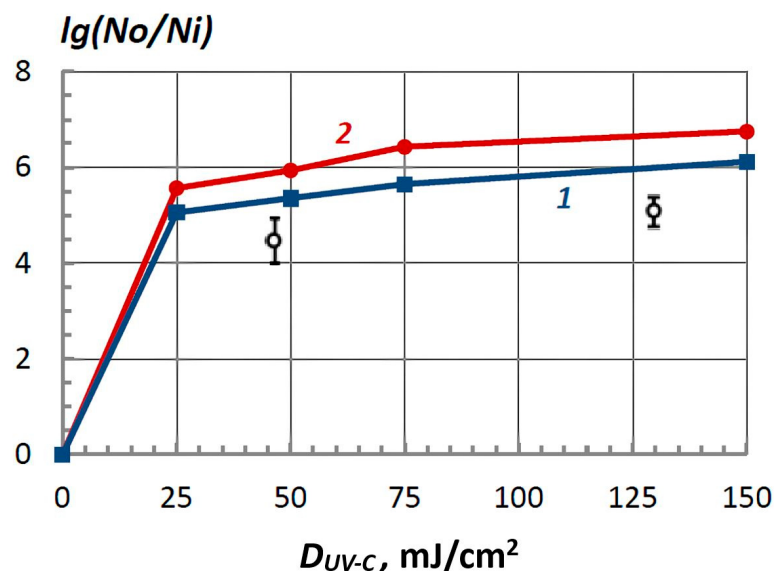


Figure 5. Logarithmic depiction of *Pseudomonas aeruginosa* (1) and *Staphylococcus aureus* (2) bacteria inactivation using the “Zarnitsa-A” apparatus. The initial densities of the bacterial load on the surface of the agar were $N_0(1) = 4 \cdot 10^5$ and $N_0(2) = 2 \cdot 10^6$ (2) CFU/cm². The graph shows the standard deviations (SD) in the area of low and high doses.

The experimental data are represented as a dependence of the logarithm of inactivation on the energy dose of UV-C radiation (the so-called survival curves) and show that the “Zarnitsa-A” apparatus deeply disinfected a surface contaminated with bacteria at doses of ~ 25 mJ/cm², approximately corresponding to one minimum erythemal dose (MED); a decrease in surface contamination of more than five orders of magnitude was achieved (the disinfection efficiency exceeded 99.999%). Similar results were obtained for the other bacteria—*Escherichia coli* and *Streptococcus pyogenes*. No statistically significant differences in the resistance of the selected MOs to high-intensity optical radiation were found over the course of the experiments.

Increasing the dose to 150 mJ/cm² led to the almost complete sterilization of the surface (we observed the absence of colony growth or single colonies).

However, with an increase of more than 25 mJ/cm² in the energy dose, the rate of decrease in the level of infection (inactivation rate) decreased, and the survival curves tended to saturate or reach a plateau. This type of survival curve is determined based on the heterogeneity of the irradiated bacterial population, i.e., the presence of a fraction of microorganisms resistant to a disinfecting factor (in this case, UV radiation). The formation of a “tail” in the survival curve may be due to factors of a nonbiological nature, in particular, the mutual shading of bacteria at their high surface densities, which is characteristic of the conditions of the experiments.

Experimental data representing the bactericidal effectiveness of the “Zarnitsa-A” apparatus at various doses of UV-C radiation are shown in Figure 6.

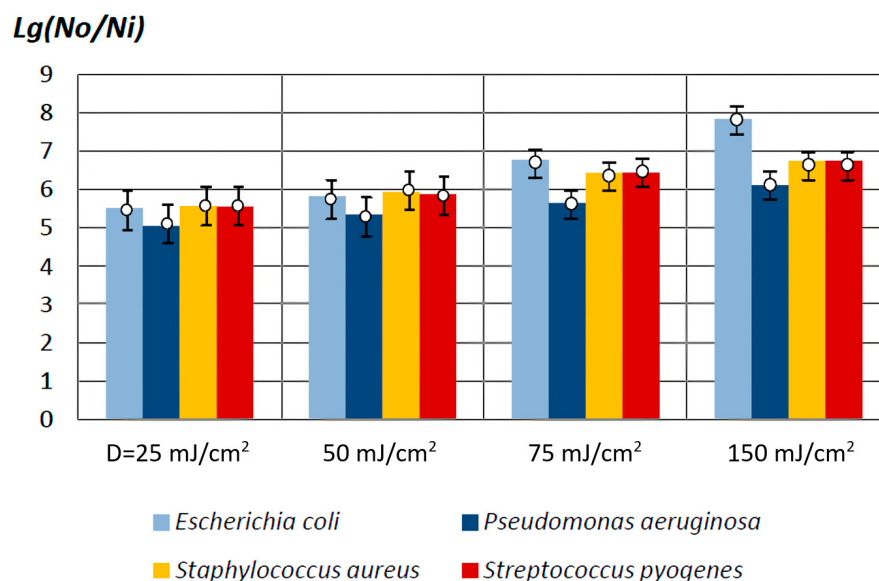


Figure 6. Bactericidal effectiveness of the “Zarnitsa-A” apparatus at various doses of UV-C radiation. The graph shows the standard deviations (SD).

Considering that the microbiological measurements in this methodology had an error within one order, it should be stated that the “Zarnitsa-A” apparatus had approximately the same bactericidal effectiveness regarding all the studied MOs—*Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Streptococcus pyogenes*. In a 10-s irradiation session at a distance of 10 cm from the emitter of the device to the contaminated surface, the level of bacterial contamination for all the indicated microflora decreased by more than five decimal orders; the disinfection efficiency exceeded 99.999%.

At a UV-C radiation dose of $D = 150 \text{ mJ/cm}^2$ (three irradiation cycles of 20 s each), the reduction in agar surface contamination for all considered microorganisms exceeded a million times (inactivation logarithm, $\lg(N_0/N_i) > 6$). Reducing the distance to ~5 cm increased the power density of the UV radiation on the object by about a factor of two, which made it possible to halve the duration of the disinfection process (in particular, to 5 s while maintaining the energy dose of irradiation at $\sim 25 \text{ mJ/cm}^2$).

Thus, the experiments show the high bactericidal effectiveness of the “Zarnitsa-A” apparatus and the possibility of a significant reduction in the duration of preventive and therapeutic procedures.

In this work, microorganisms whose drug resistance had not been studied were mainly used. However, based on the known experimental data on the antimicrobial effects of UV-C radiation [21,26,27], it can be argued that high-intensity optical radiation of a wide spectrum with a significant proportion of short-wave UV radiation (radiation generated by the “Zarnitsa-A” apparatus) will also effectively affect multidrug-resistant microorganisms. The high intensity of irradiation on the surface and the presence of photons with a wide range of energies in the emission spectrum will hopefully effectively inactivate microorganisms in biofilms. However, these questions require experimental confirmation.

3.2. Main Results of Preclinical In Vivo Studies

Daily clinical examinations did not reveal any significant symptoms in the animals in any of the experimental groups. Analyses of data on the rectal temperature (see Appendix A) and body weight (Appendix B) measurements of the laboratory animals also did not reveal significant differences between the experimental groups.

According to the results of hematological analyses (Appendix C) on the 3rd day for all groups, low values for hemoglobin and other indicators of erythropoiesis were noted. Furthermore, almost all the rats had a marked increase in leukocytes, which was probably due to an ongoing inflammatory reaction in their bodies. On the 7th day in the main group,

there was a significant decrease in the number of leukocytes compared with the control groups, an indirect indicator of the bactericidal effect of complex therapy. A significant decrease in erythropoiesis was also noted. In the following days, there was a decreasing trend in the number of leukocytes in all the experimental groups, with the most pronounced in the main group receiving complex therapy. On the last day of the study, there was a significant decrease in the lymphocyte, platelet, and plateletcrit (PCT) values in the main group compared with the control groups.

In general, the selected irradiation regimen did not lead to lethal outcomes or pronounced functional and somatic disorders in the laboratory animals; analyses of their physiological and hematological parameters did not reveal any pathological changes that could be associated with the action of the apparatus under study, which may indicate the potential safety of using this type of apparatus in clinical practice.

The results of microbiological examinations of the animals' wounds are presented in Table 1.

Table 1. Influence of therapy regimens on the average number of colonies per 1 mL (Me (25–75%)).

Experimental Groups	Control Time				
	4 Hours	3 Days	7 Days	10 Days	14 Days
Control № 1 *	44.0 (20.5–300.0)	300.0 (300.0–300.0)	300.0 (250.0–300.0)	300.0 (300.0–300.0)	300.0 (300.0–300.0)
Control № 2	77.0 * (5.5–294.0)	1.0 * (0.0–3.0)	13 * (2.0–58.0)	20 * (9.5–197.5)	7.0 * (0.5–125.5)
Main № 3	3.5 * (2.0–13.0)	0.0 * (0.0–0.0)	0.0 ** (0.0–1.0)	0.0 ** (0.0–2.5)	0.5 ** (0.0–2.5)

*—differences with control group № 1 are statistically significant at $p < 0.05$; #—differences with control group № 2 are statistically significant at $p < 0.05$. The statistical assessment method for the significance of differences in the results of the study was the Kruskal–Wallis test for independent samples.

The results of the microbiological wound examination showed a significant decrease in the average number of microorganism colonies in the main group compared with the control groups throughout the research period. On the first and third days, the microflora was represented by Gram-positive cocci (*Staphylococcus aureus*) in all groups. On the 7th day and later in control group № 2 and the main group, there was no growth in the pathogenic strain (*Staphylococcus aureus*), which prevailed in control group № 1. Microorganism colonies in control group № 2 and the main group were represented by saprophytic microflora. On the last day of the study, there was a slight increase in microflora in control group №; in the main group, there was practically no microflora growth; in control group № 1, the growth of the predominantly pathogenic strain (*Staphylococcus aureus*) was still noticeable. Thus, against the background of these therapy regimens, most animals in the main group experienced pronounced bactericidal effects.

Measuring the size of the wound (Appendix D) showed a significant decrease in the surface area in the main group compared with the control groups; at the same time, starting on the 10th day of the study, a clean wound and no exudate were observed in the main group, while in control group № 2, the presence of exudate in a clean wound was noted; in control group № 1, after removing scabs, there were pronounced wound surfaces with purulent exudate. Figure 7 shows photographs of the state of wound surfaces on animals in different groups on the 14th day of the study.

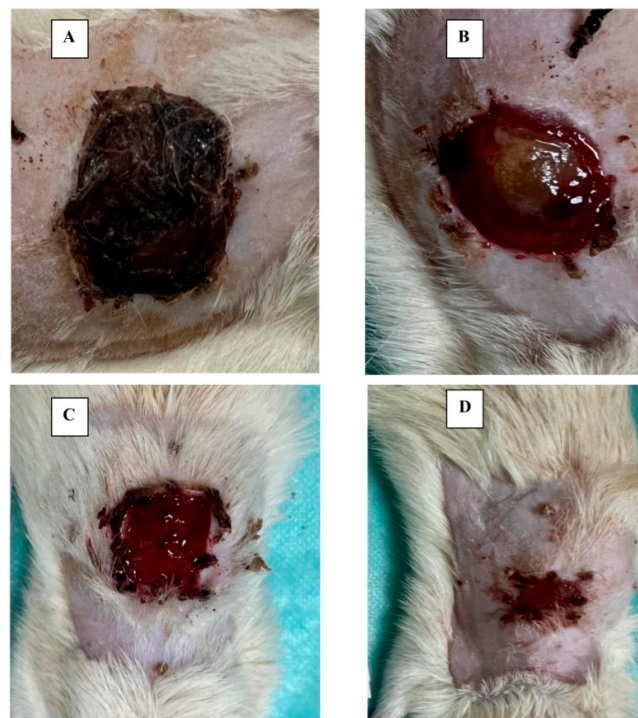


Figure 7. The condition of wound surfaces on the 14th day after introducing the pathology. (A,B)—control group № 1; (A)—wound under the scab; (B)—wound after removal of the scab (there is a pronounced wound surface with purulent exudate); (C)—control group № 2 (levomekol), there is a decrease in the wound surface, the wound contains exudate; (D)—the main group (levomekol + irradiation with the “Zarnitsa-A” apparatus), there is a pronounced decrease in the size of the wound defect and an absence of exudate.

The main results of the histological studies of the experimental groups of animals are as follows.

In control group № 1, on the 14th day of wound healing, granulation tissue was detected (Figure 8), represented by a large number of cells—mainly fibroblasts and fibrocytes, which is typical of phase II in the course of the wound process—proliferation. The granulation tissue contained a large number of plethoric vessels. The vessels were predominantly slit-like; their walls were not formed or only partially formed. Collagen fibers were arranged partially ordered. Inflammatory infiltration by lymphocytes, histiocytes, and neutrophils in the granulation tissue was pronounced in most of the animals.

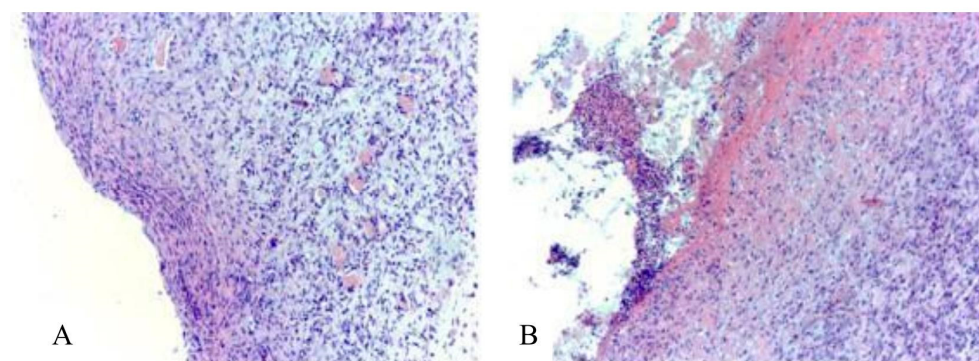


Figure 8. Granulation tissue of a rat wound in control group № 1. Vascular plethora, pronounced inflammatory infiltration by lymphocytes, histiocytes, and neutrophils; cells dominate over the fibers (A,B). Massive overlays of exudate (B). Staining with hematoxylin and eosin. Mag. 200×.

In control group № 2, compared with control group № 1, the granulation tissue in most of the animals contained few vessels (Figure 9). The vessels were predominantly slit-shaped, but some of them were rounded. In the slit-shaped vessels of most of the animals, the walls were partially formed, and collagen fibers were partially ordered. Inflammatory infiltration in the granulation tissue of most of the rats was mild.

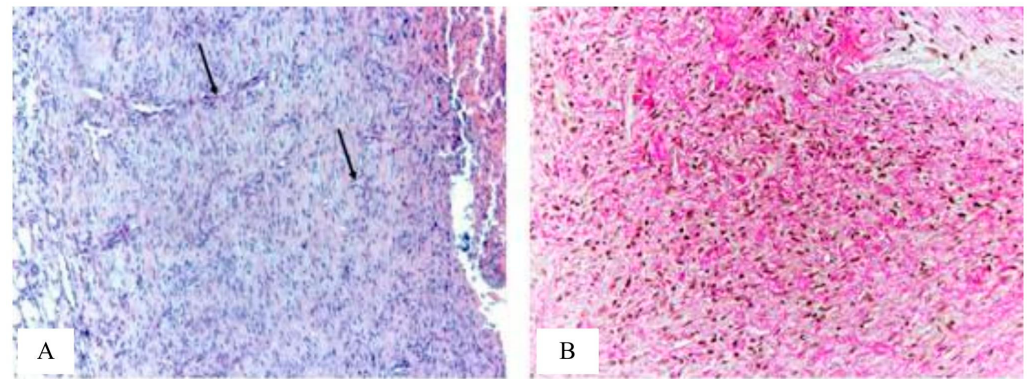


Figure 9. Granulation tissue of a rat wound in control group № 2. (A)—predominantly slit-shaped blood vessels (black arrows); moderate inflammatory infiltration by lymphocytes, histiocytes, and neutrophils. Staining with hematoxylin and eosin. Mag. 200×; (B)—fuchsinophilic collagen fibers are partially ordered; cells predominate over fibers. Coloring according to van Gieson. Mag. 200×.

In general, the histological picture of the wound defects in animals in control groups № 1 and № 2 corresponds to the second phase of the course of the wound process, i.e., proliferation.

In the main group, blood vessels were predominantly round with formed walls in the granulation tissue (Figure 10). Collagen fibers were arranged in an orderly manner. Histologically mature scar tissue was represented by parallel, dense bundles of collagen containing few blood vessels and cells compared with intact tissues. Inflammatory infiltration by lymphocytes, neutrophils, and histiocytes in the granulation tissue of all the animals was weakly expressed. Thus, in contrast to the control groups, given the morphological characteristics, phase III of the wound process in the main group was observed, showing that accelerated wound healing took place.

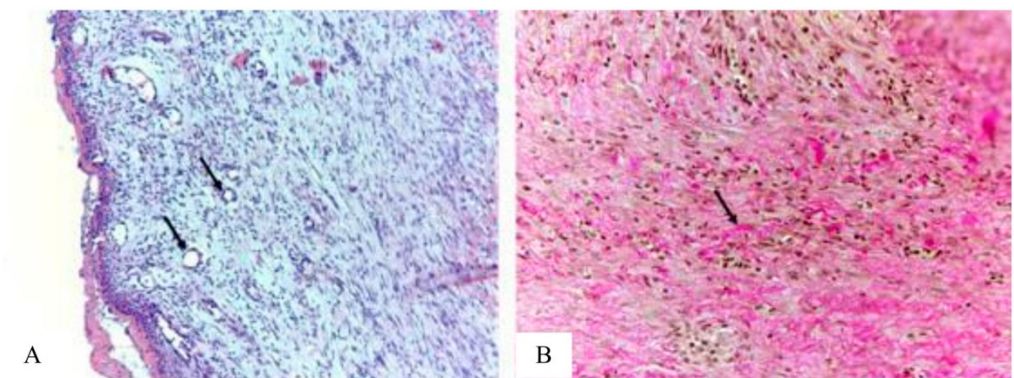


Figure 10. Granulation tissue of a rat wound in the main group (levomekol with irradiation). (A)—blood vessels with predominantly round shapes and formed walls (black arrows); weak inflammatory infiltration by lymphocytes, histiocytes, and neutrophils; fibers predominate. Staining with hematoxylin and eosin. Mag. 200×; (B)—collagen fibers are ordered. Coloring according to van Gieson. Mag. 200×.

4. Conclusions

The problem of preventing and treating wounds and wound infections is acute in modern medicine and largely associated with the emergence of pathogens with multidrug resistance.

In this work, we proposed a new approach to treating complicated wound lesions. It involves treating the wound surface with high-intensity, broad-spectrum pulsed optical radiation and continuously covering the entire UV range (from 200 to 400 nm) and the visible and near-infrared spectral regions. Impulse xenon lamps were used as a radiation source. Such lamps emit a continuous spectrum in a wavelength range of 200 to 2700 nm and make it possible to irradiate objects with extremely high intensity, several orders of magnitude higher than the irradiation intensity of traditional therapeutic radiation sources. Broad-spectrum, high-intensity, pulsed optical radiation is a new and, to date, little-studied physical tool for influencing biological objects, including biomolecules, cells, biological tissues, and the body as a whole. The high-intensity pulsed optical irradiation “Zarnitsa-A” apparatus was studied in this work, and it uses the proposed new medical technology to treat wounds and infectious diseases.

Given the results of the microbiological tests of the “Zarnitsa-A” apparatus, its high bactericidal effectiveness and the possibility of significantly reducing the duration of preventive and therapeutic procedures were shown—after 5–10 s of irradiating heavily polluted microflora surfaces, the disinfection efficiency against clinically significant pathogens was more than 99.999%. In vivo experiments on laboratory animals showed the higher bactericidal efficacy of the “Zarnitsa-A” apparatus compared with the anti-infective and therapeutic ointment levomekol.

In a preclinical study of the wound-healing effect of the “Zarnitsa-A” apparatus, we showed that the selected irradiation regimen—namely, a daily dose of UV-C radiation at 0.3 J/cm²; a fluence integrated over a spectrum of 3 J/cm² with a cumulative dose of short-wave UV radiation for a course of therapy at 2.4 J/cm²; and a cumulative fluence of 24 J/cm²—did not cause death or pronounced functional and somatic disorders in Wistar rats. Analyses of physiological and hematological parameters did not reveal pathological changes that could be associated with the action of the device under study.

At the same time, we found that the “Zarnitsa-A” high-intensity pulsed optical irradiation apparatus has a pronounced wound-healing effect and, in combination with levomekol ointment, reliably provides a higher rate of wound healing compared with official levomekol ointment alone.

The implemented technical characteristics and declared modes of wound irradiation can be recommended as the main ones in treating infected wounds and should be clarified in the course of clinical trials.

Our experimental data indicate the potential prospects of using the “Zarnitsa-A” apparatus for pulsed, high-intensity optical irradiation in medical and veterinary practice to treat and prevent wound lesions.

Author Contributions: Conceptualization, V.V.B., I.V.B. and A.S.K.; methodology, L.Y.V. and E.V.Z.; validation, E.V.Z. and A.V.K.; formal analysis, V.I.K.; investigation, L.Y.V., A.V.K. and S.M.N.; resources, A.S.K. and S.M.N.; data curation, D.O.N., L.Y.V., A.V.K. and S.M.N.; writing—original draft preparation, A.S.K., I.V.B., L.Y.V. and S.M.N.; writing—review and editing, V.V.B., A.S.K. and E.V.Z.; visualization, S.M.N.; supervision, K.A.S., A.S.K. and A.V.K.; project administration, V.V.B. and V.I.K. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: This study was conducted according to the requirements of Directive 2010/63/EU of the European Parliament and of the Council of the European Union of 09/22/2010, on the protection of animals used for scientific purposes and the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (ETS No. 123), Strasbourg, 18 March 1986; Appendix A. Guidelines for the Care of Laboratory Animals. Animal experiments were agreed to and approved by the Ethics Committee of the Izmerov Research Institute of Occupational Health.

Informed Consent Statement: Not applicable.

Data Availability Statement: The authors confirm that the data supporting the findings of this study are available within the article.

Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Table A1. The effect of the compared therapy regimens on the body temperature of rats (°C, Me (25–75%)).

Control Time	Experimental Groups		
	Control № 1	Control № 2	Main № 3
Background	37.6 (37.6–37.8)	37.5 (37.5–37.6)	37.6 (37.5–37.6)
3 days	37.0 (36.9–37.5)	36.9 (36.5–37.0)	37.2 (37.0–37.4)
7 days	37.2 (37.1–37.6)	37.1 (36.9–37.4)	37.3 (37.0–37.5)
10 days	37.2 (36.9–37.8)	36.8 (36.4–37.0)	37.2 (36.9–37.3)
14 days	36.7 (36.4–37.3)	36.8 (36.5–36.9)	37.2 (36.9–37.2)

Appendix B

Table A2. Effect of the compared therapy regimens on the body weight of rats (g, Me (25–75%)).

Control Time	Experimental Groups		
	Control № 1	Control № 2	Main № 3
Background	220.0 (218.5–226.5)	220.0 (218.5–229.0)	220.5 (217.5–235.5)
3 days	217.0 (214.5–220.0)	211.0 (207.5–220.0)	216.0 (210.0–225.5)
7 days	226.0 (219.0–232.5)	221.0 (213.0–224.5)	224.0 (217.0–231.0)
10 days	225.0 (223.5–230.5)	212.0 (209.0–226.5)	215.5 (209.5–225.0)
14 days	221.0 (211.5–231.0)	214.0 (203.0–230.5)	228.5 (217.5–236.0)

Appendix C

Table A3. Effect of the compared therapy regimens on the composition of rat peripheral blood (M ± SD) on the 1st day.

Investigated Indicators	Experimental Groups		
	Control № 1	Control № 2	Main № 3
	1 Day		
Number of white blood cells (WBC), 10 ⁹ /L	14.2 ± 5.0	12.9 ± 4.7	13.4 ± 2.9
Lymphocytes (LYM), 10 ⁹ /L	2.3 ± 0.7	2.1 ± 0.8	2.4 ± 0.7
Monocytes and eosinophils (MID), 10 ⁹ /L	4.8 ± 1.3	4.1 ± 1.6	4.8 ± 1.0
Granulocytes (GRAN), 10 ⁹ /L	7.1 ± 3.9	6.7 ± 3.0	6.2 ± 1.9

Table A3. Cont.

Investigated Indicators	Experimental Groups		
	Control № 1	Control № 2	Main № 3
	1 Day		
Erythrocytes (RBC), $10^{12}/L$	8.9 ± 1.6	8.7 ± 1.5	9.2 ± 1.1
Hemoglobin concentration (HGB), g/L	156.5 ± 27.4	152.0 ± 25.8	159.5 ± 16.2
Hematocrit (HCT), %	35.6 ± 6.3	34.9 ± 5.9	37.0 ± 4.5
Average red blood cell volume (MCV), fl	40.2 ± 0.6	40.0 ± 0.8	40.2 ± 0.6
Average hemoglobin content in erythrocyte (MCH), pg	17.6 ± 0.8	17.4 ± 0.4	17.3 ± 0.6
Average concentration of hemoglobin in erythrocyte (MCHC), g/dL	439.1 ± 23.8	434.9 ± 8.6	$432. \pm 15.7$
Platelet count (PLT), $10^9/L$	311.8 ± 86.0	351 ± 99.7	347.8 ± 81.1
Average platelet volume (MPV), fl	9.5 ± 0.8	9.5 ± 0.9	9.3 ± 0.6
Platelet distribution latitude (PDW), fl	8.1 ± 0.6	8.5 ± 0.8	8.7 ± 1.1
Plateletcrit, (PCT), %	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1

Table A4. Effect of the compared therapy regimens on the composition of rat peripheral blood ($M \pm SD$) on the 3rd day.

Investigated Indicators	Experimental Groups		
	Control № 1	Control № 2	Main № 3
	3 Days		
Number of white blood cells (WBC), $10^9/L$	19.5 ± 7.7	19.1 ± 13.5	19.5 ± 7.7
Lymphocytes (LYM), $10^9/L$	$3.4 \pm 1.4 \#$	3.9 ± 2.6	$3.4 \pm 1.4 \#$
Monocytes and eosinophils (MID), $10^9/L$	5.1 ± 1.6	5.6 ± 2.8	5.1 ± 1.7
Granulocytes (GRAN), $10^9/L$	11.0 ± 5.1	9.6 ± 8.5	11.0 ± 5.1
Erythrocytes (RBC), $10^{12}/L$	6.4 ± 1.9	$6.4 \pm 2.7 \#$	$6.4 \pm 1.9 \#^*$
Hemoglobin concentration (HGB), g/L	122.1 ± 31.3	121.9 ± 48.5	$122.1 \pm 31.3 \#$
Hematocrit (HCT), %	27.6 ± 6.8	28.3 ± 10.9	$27.6 \pm 6.8 \#$
Average red blood cell volume (MCV), fl	$43.9 \pm 3.2 \#$	$44.8 \pm 2.7 \#^*$	$43.9 \pm 3.2 \#$
Average hemoglobin content in erythrocyte (MCH), pg	$19.3 \pm 1.6 \#$	$19.1 \pm 1.0 \#$	$19.3 \pm 1.6 \#$
Average concentration of hemoglobin in erythrocyte (MCHC), g/dL	440.9 ± 19.0	402.3 ± 104.6	440.9 ± 19.0
Platelet count (PLT), $10^9/L$	309.6 ± 86.8	350.7 ± 121.2	309.6 ± 86.8
Average platelet volume (MPV), fl	9.6 ± 0.5	9.4 ± 0.5	9.6 ± 0.5
Platelet distribution latitude (PDW), fl	7.8 ± 1.3	6.7 ± 2.3	7.8 ± 1.3
Plateletcrit, (PCT), %	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1

*—differences with the control group are statistically significant at $p < 0.05$; #—differences with the background are statistically significant at $p < 0.05$.

Table A5. Effect of the compared therapy regimens on the composition of rat peripheral blood ($M \pm SD$) on the 7th day.

Investigated Indicators	Experimental Groups		
	Control № 1	Control № 2	Main № 3
	7 Days		
Number of white blood cells (WBC), $10^9/L$	$19.3 \pm 4.5 \#$	18.7 ± 14.1	$14.7 \pm 3.4 \#^*$
Lymphocytes (LYM), $10^9/L$	3.0 ± 1.0	3.4 ± 2.4	2.5 ± 0.8
Monocytes and eosinophils (MID), $10^9/L$	6.0 ± 1.5	5.3 ± 2.8	$4.7 \pm 1.1 \#^*$
Granulocytes (GRAN), $10^9/L$	$10.4 \pm 3.3 \#$	9.3 ± 9.7	$7.5 \pm 2.0 \#^*$
Erythrocytes (RBC), $10^{12}/L$	8.4 ± 2.6	$7.3 \pm 1.3 \#$	$6.4 \pm 1.7 \#$
Hemoglobin concentration (HGB), g/L	163.8 ± 43.1	143.5 ± 23.1	$127.9 \pm 28.6 \#^*$
Hematocrit (HCT), %	37.6 ± 9.7	33.0 ± 4.3	$29.4 \pm 6.7 \#^*$
Average red blood cell volume (MCV), fl	$45.3 \pm 2.8 \#$	$45.4 \pm 2.2 \#$	40.8 ± 15.3
Average hemoglobin content in erythrocyte (MCH), pg	$19.6 \pm 1.2 \#$	$19.6 \pm 1.0 \#$	19.5 ± 3.2

Table A5. Cont.

Investigated Indicators	Experimental Groups		
	Control № 1	Control № 2	Main № 3
	7 Days		
Average concentration of hemoglobin in erythrocyte (MCHC), g/dL	434.7 ± 15.9	432.6 ± 21.8	434.9 ± 7.4
Platelet count (PLT), 10 ⁹ /L	331.2 ± 70.2	405.4 ± 60.7	406.6 ± 90.4 #*
Average platelet volume (MPV), fl	9.6 ± 0.7	8.9 ± 9.2	9.2 ± 0.3
Platelet distribution latitude (PDW), fl	8.1 ± 1.1	8.9 ± 7.5	7.6 ± 1.3
Plateletcrit, (PCT), %	0.3 ± 0.1	0.2 ± 0.3	0.4 ± 0.1 #

*—differences with the control group are statistically significant at $p < 0.05$; #—differences with the background are statistically significant at $p < 0.05$.

Table A6. Effect of the compared therapy regimens on the composition of peripheral rat blood (M ± SD) on the 10th day.

Investigated Indicators	Experimental Groups		
	Control № 1	Control № 2	Main № 3
	10 Days		
Number of white blood cells (WBC), 10 ⁹ /L	17.2 ± 4.9 #	20.0 ± 7.6 #	15.7 ± 3.5
Lymphocytes (LYM), 10 ⁹ /L	3.9 ± 1.5 #	4.4 ± 1.7 #	3.2 ± 1.7
Monocytes and eosinophils (MID), 10 ⁹ /L	7.1 ± 1.7 #	7.4 ± 2.6 #	6.1 ± 1.1 #
Granulocytes (GRAN), 10 ⁹ /L	6.2 ± 2.7	8.2 ± 4.3	5.8 ± 2.9
Erythrocytes (RBC), 10 ¹² /L	7.7 ± 1.9 #	6.9 ± 2.3 #	7.6 ± 1.9
Hemoglobin concentration (HGB), g/L	141.5 ± 33.0	128.7 ± 43.8	143.6 ± 34.7
Hematocrit (HCT), %	33.4 ± 7.2	30.0 ± 9.3	31.1 ± 11.3
Average red blood cell volume (MCV), fl	44.1 ± 3.1 #	43.8 ± 2.4 #	44.1 ± 1.8 #
Average hemoglobin content in erythrocyte (MCH), pg	18.6 ± 1.2 #	18.7 ± 1.1 #	18.8 ± 0.8 #
Average concentration of hemoglobin in erythrocyte (MCHC), g/dL	400.7 ± 90.0	426.6 ± 18.9	428.9 ± 18.4
Platelet count (PLT), 10 ⁹ /L	326.9 ± 124.0	328.9 ± 108.2	327.1 ± 120.4
Average platelet volume (MPV), fl	9.3 ± 0.3	8.9 ± 9.2	9.2 ± 0.3
Platelet distribution latitude (PDW), fl	7.8 ± 2.3	8.9 ± 7.5	8.3 ± 1.0
Plateletcrit, (PCT), %	0.3 ± 0.1	0.2 ± 0.3	0.3 ± 0.1

#—differences with the background are statistically significant at $p < 0.05$.

Table A7. Effect of the compared therapy regimens on the composition of rat peripheral blood (M ± SD) on the 14th day.

Investigated Indicators	Experimental Groups		
	Control № 1	Control № 2	Main № 3
	14 Days		
Number of white blood cells (WBC), 10 ⁹ /L	13.2 ± 4.4	12.2 ± 3.2	10.8 ± 2.1
Lymphocytes (LYM), 10 ⁹ /L	3.0 ± 0.9	3.3 ± 1.4 #	2.1 ± 0.6 *
Monocytes and eosinophils (MID), 10 ⁹ /L	4.8 ± 1.3	4.7 ± 1.3	4.0 ± 1.0
Granulocytes (GRAN), 10 ⁹ /L	5.4 ± 3.3	4.3 ± 1.0 #	4.7 ± 1.4
Erythrocytes (RBC), 10 ¹² /L	7.7 ± 1.2 #	6.9 ± 2.4 #	7.9 ± 1.2
Hemoglobin concentration (HGB), g/L	140.2 ± 22.7 #	127.5 ± 36.4	141.6 ± 22.1
Hematocrit (HCT), %	33.9 ± 5.6	31.4 ± 5.8	33.7 ± 4.9
Average red blood cell volume (MCV), fl	44.9 ± 8.7	42.4 ± 2.7 #	42.9 ± 1.9
Average hemoglobin content in erythrocyte (MCH), pg	18.3 ± 1.6 #	17.9 ± 1.2	18.0 ± 1.1
Average concentration of hemoglobin in erythrocyte (MCHC), g/dL	415.5 ± 43.2	424.5 ± 16.1	419.4 ± 17.3 #
Platelet count (PLT), 10 ⁹ /L	552.6 ± 154.8 #	532.8 ± 117.1 #	421.0 ± 107.7 #*
Average platelet volume (MPV), fl	9.3 ± 0.8	8.9 ± 9.2	9.1 ± 0.4
Platelet distribution latitude (PDW), fl	8.2 ± 0.9	8.9 ± 7.5	8.2 ± 1.0
Plateletcrit, (PCT), %	0.5 ± 0.2 #	0.2 ± 0.3 #	0.4 ± 0.1 #*

*—differences with the control group are statistically significant at $p < 0.05$; #—differences with the background are statistically significant at $p < 0.05$.

Appendix D

Table A8. Effect of the compared therapy regimens on the wound surface area (mm², Me (25–75%)).

Control Time	Experimental Groups		
	Control № 1	Control № 2	Main № 3
3 days	325.0 (290.0–370.0)	370.0 (327.5–385.0)	340.0 (300.0–385.0)
7 days	420.0 (367.5–452.5)	397.5 * (361.5–479.0)	340.0 * (322.5.0–370.0)
10 days	385.0 (350.0–437.5)	342.5 (311.0–405.0)	320.0 * (262.5–342.5)
14 days	290.0 (227.5–357.5)	292.5 (167.5–317.5)	245.0 (210.0–290.0)

*—differences with the control group are statistically significant at $p < 0.05$. The statistical evaluation method for the significance of differences in the results of the study was the Kruskal–Wallis criterion for independent samples.

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