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The Effect of "Melanin-Gel" on the Wound Healing.

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ABSTRACT

Despite advances in traumatic wound care and management, infections remain a leading cause of mortality, morbidity and economic disruption in millions of wound patients around the world. Animal models have become standard tools for studying a wide array of external traumatic wound infections and testing new antimicrobial strategies. Study of wound-healing action was conducted on model of full-thickness skin wound, purulent necrotic wound and thermal burns. The efficiency of wounds treatment with Melanin that is produced by Antarctic black yeast-like fungi *Nadsoniella nigra* strain X1-M was studied. "Melanin-gel" (0.05% melanin dissolved in 0.5% carbopol) had antimicrobial property against *Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans*, that is one of the mechanisms of wound healing. Application of the "Melanin-gel on wound area enhanced wound cleaning from dead tissue and reduced eschar, stimulated the early growth of granulation tissue, and improved epithelialization of the wound.

Keywords: wound, Melanin-gel, full-thickness skin wound, purulent necrotic wound, thermal burn.

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INTRODUCTION

Every year in the world about 5 million people die from complications as a result of injuries, accounting for 9% of all deaths and 1.7 times the number of deaths from AIDS, tuberculosis and malaria. According to the World Health Organization (WHO), nearly 2% people of the total number of deaths from injuries, die from lesions obtained during the war and 5% - from burns. [1] In addition, the meeting on April 3-4, 2007 WHO decided to develop a strategy to burns prevention and treatment [2]. Search for new, economic methods and drugs for the treatment of wounds of various origins remain relevant and promising area of research. In Ukraine, each year more than 100 thousand cases of burn injuries are registered, with 60-80% of burned having superficial skin burns with II-IIIA point that does not require surgery [3].

An important problem of modern medicine is the growth rate of long no healing wounds. Such wounds often occur in patients with violation of trophic and skin innervations caused by various pathological processes: chronic venous congestion in patients with varicose veins; microcirculation disorder in diabetes and also in cases of deep lesions of the skin and subcutaneous tissue thermal and chemical injuries [4].

Wound hypoxia is always dangerous for wound healing and may be an important factor for the initiation process in chronic wounds, as can be seen in the diabetic foot wounds, bedsores or wounds caused by acute arterial occlusion [5]. The main conditions for wound healing are normal blood circulation in the body, particularly in the area of the wound for sufficient supply of blood and oxygen to the tissues, as well as normalization of metabolism. Injures are often accompanied by significant violation of general and local blood circulation and metabolism.

Wound healing is often accompanied by complications of infectious nature. Often there are also complications of scar-degenerative origin in the area of the wound - hypertrophic and keloid scars; scar restrictions (contractions) of movements scar hernia and diastase on the basis of muscle atrophy, sores and rarely - tumors. Infection has mostly local purulent nature, less often - putrefactive (purulent and putrid inflammation) and much less - anaerobic, spore forming (gas gangrene and phlegmon) and specific (tetanus, diphtheria). Agents of the first two forms - purulent and putrefactive – are staphylococci and streptococci, bacillus, proteus, *Pseudomonas aeruginosa* and anaerobic non spore forming clostridium - bacteroides etc. [6]. Therefore, the surface of wound is optimal healing environment for the development of pathological microflora (which is dominated by species of *Staphylococcus spp.* (42, 7%), a gram-negative microorganisms from which *Pseudomonas aeruginosa* may be found (10,3%) [7]. The surface of wounds of various etiologies may be also infected with microscopic yeast fungi *Candida albicans*.

There are some treatments for open wounds, most common in clinical practice: the use of ointments, gels, bandages [8]. However, the high cost of treatment and lack of effectiveness of existing drugs encourage researchers to seek new methods of treatment. Among the new dermotropic drugs we paid attention to Belarusian drug (Melanin ointment, 0.05%), the active ingredient of which is melanin – the pigment of polyphenol nature, obtained by means of microbiological process.

We have gained extensive experience in melanin, which is produced by Antarctic black yeast-like fungi *Nadsoniella nigra* strain X1-M. It is shown that it has cytoprotective effect on the gastric mucosa of rats, reducing the activity of lipid peroxidation processes and increasing the activity of antioxidant enzymes system. Melanin is able to bind to collagen fibers, stabilizes them and prevents the formation of unwanted cross-links between amino acids, protects the body from ultraviolet and X-ray radiation and has stress protective action. [9]

Some mechanisms of melanin anti-ulcer action were established: enhancement of eNOS expression, anti-inflammatory by reducing secretion of anti-inflammatory and increasing secretion of anti-inflammatory cytokines. These mechanisms are important for dermotropic drug effect, so our research has focused on creating a new dermotropic drug "Melanin-gel", where the main sources are microorganisms that live in extreme conditions and use melanin to protect against harmful UV radiation, and transform energy into a safe amount of heat. Because of its properties melanin absorbs up to 99.9% of UV light and prevents the formation of free radicals at a minimal level, preventing damage of DNA [10]. The purpose of our study was to investigate the impact of new dermotropic drug to speed up healing of lesions of various origins and antibacterial properties of "Melanin-gel".

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MATERIALS AND METHODS

Animals

Research was conducted on white laboratory female rats weighing 200 - 250 g, n = 30, which were divided into three groups, each group was divided into two subgroups. Keeping animals and experiments were conducted according to ethical principles adopted by Ukraine First National Congress on Bioethics, international agreements and national legislation in this area [8]. Before the experiment, the rats were kept in quarantine and were marked by given them notches on ears. When animas were injured they were anesthetized by sodium thiopental (BiochemieGmbH / Austria), at a dosage of 50 mg / kg. Before the experiment epilation was performed in the shoulder-blade area. The animals of experimental group got "Melanin-gel" 0.05% melanin (Melanin) dissolved in 0.5% Carbopol (Carbopol 980) for wounds' healing. In the control series wound healing occurred without drugs.

Microbiological studies

To study antibacterial properties of "Melanin-gel" the method of application of the drug (1 g) to the surface of the nutrient medium was used (NA - Nutrientagar, manufacturer Sigma-Aldrich, Spain). Previously surface environment was covered with suspension of test microorganisms according to the recommendations (on the recommendations, "Determination of the sensitivity of microorganisms to antibiotics" Ukraine Ministry of Health, Order number 167 of 05.04.2007).

Number of colony forming units (CFU) were determined by densitometer «Vitek-2» («BioMerieux» (France). CFU load in microorganism suspensions amounted to 10^5 bc / ml, for bacteria, 10^6 CFU / ml for yeasts. Culture collections of bacteria *Staphylococcus aureus* ATCC 25923*, Pseudomonas aeruginosa* ATCC 27853 and yeasts *Candida albicans* were used as test culture. We investigated the effect of 0.05% melanin gel on these test bacterial cultures of microorganisms.

Skin wound model

Model of full-thickness skin wound. Plate wounds are reproduced on epilated skin in anesthetized rats. To do this, skin is cut using surgical scalpel and forceps, 1 × 1sm^2. Treatment begins immediately after wounds reproduction until healing [11].

Burns caused by calcium salt solution. Chemical skin burn is caused by the introduction of 0,1ml $CaCl_2$. The attention is payed to the standardization of wounds received, the size of which must not exceed 400mm2. In 4-5days necrotomy of the affected area is carried and then treatment of wounds until healing begins [12].

Burns of the 2nd degree. On prepared skin area an appliqué of hot metal plate with the diameter of 20 mm was conducted and during the exposure 4c the temperature was 200^0 C [13]. Every day in experimental groups "Melanin-gel" was applied on the affected areas using a metal spatula, which was annealed before each use. Also every 24 hours the general condition of animals, the condition of the affected surface was evaluated and the size of the wound surface was determined.

Statistical analysis

To assess the state of the wound surface we studied terms of cleaning the wound from purulent necrotic masses, time of granulation occurrence and edge epithelialization, and the terms of full epithelialization of the wound surface. Statistical analysis of data was carried out by the "Statistica 8.0" software package. To determine the reliability of the differences between two samples using Student test (t).Thus deemed reliable difference $P < 0.05$.

RESULTS AND DISCUSSION

During injuries healing, regardless of the origin and presence of exogenous factors, the same cellular elements that provide essentially similar overall dynamics of the healing process are involved [12], which

defines the possibility to use objective criteria for assessing wound healing progress regardless of damage etiology.

Filling wound defect with granulation, epithelization, wound contraction and scarring are the most demonstrative clinical indices of the of wound healing speed, which are expressed in the gradual reduction and ultimately - in closing the wound surface . Thus, the rate of healing is a value that characterizes the change in wound surface area per time unit.

It is known that polyphenolic compounds exhibit a strong biological effect, such as restorative, antioxidant, anti-inflammatory and wound healing. Polyphenolic compounds include melanin - pigment of skin, hair, iris, black substance of the brain, and others. Melanin protects the body from ultraviolet and X-ray radiation, it has stress- and cytoprotective function and antioxidant action. Melanin is able to bind to collagen fibers, stabilizes them and prevents the formation of unwanted cross-links between the amino acids.

Overseas melanin is used as a substance for a number of drugs, including dermotropic. For example, in Belarus there is an ointment which is registered and available in the market and includes melanin (Melanin ointment, 0.05% Byelmedpreparaty).

Our previous studies found that melanin, which is produced by Antarctic black yeast-like fungi Nadsoniella nigra strain X1-M, with oral administration has cyto protective, stress protective, radio protective, antioxidant, anti-inflammatory, immunomodulatory and antitumor properties [14]. The received data is a basis to study dermotropic properties of melanin.

Biocidal effect of 0.05% melanin gel was revealed on test cultures of bacteria *Staphylococcus aureus and Pseudomonas aeruginosa*. In a test culture of *Candida* yeasts melanin gel produced fungistatic effect (areas of growth retardation were noted, where a decreased intensity of yeast growth was observed) (Fig. 1).

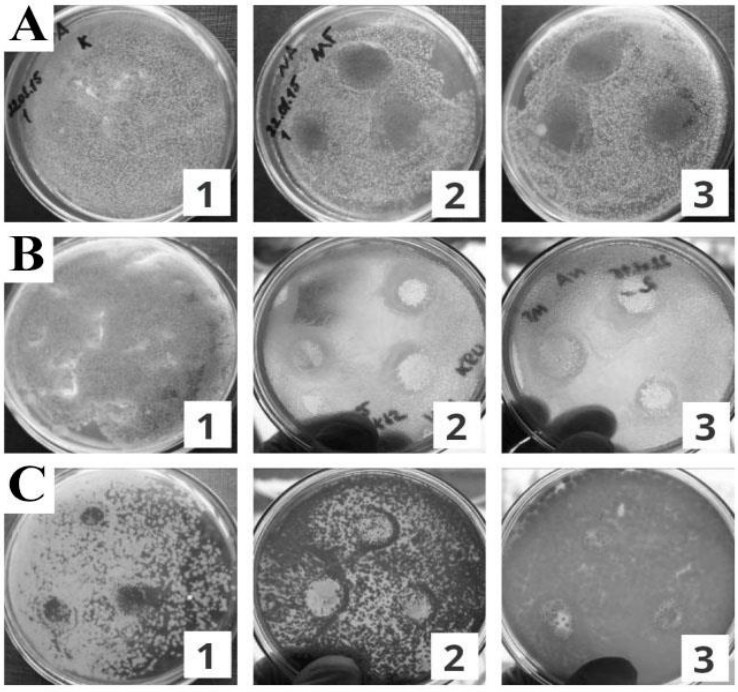

Figure 1: Bactericidal action of Melanin-gel on the bacteria *Staphylococcus aureus* (A), *Pseudomonas aeruginosa* (B), *Candida albicans* yeasts (C). Ingredients: 0.05% melanin, 0,5% carbopol.

A, B: 1- control, 2.3 - growth inhibition zones in the places of application of melanin gel (back side of the Petri dish). The density of the slurry $5*10^5$ CFU / ml.24 hours of cultivation.

C: 1.2 - 24 hours of cultivation, 3 - 72 hours of cultivation, inhibition area of growth overgrew (back side of Petri dishes). The density of the slurry $1*10^6$ CFU / ml.

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The degree of Melanin 0.05 % gel influence in test cultures of microorganisms was evaluated by the presence or absence of growth inhibition zones and their size (diameter). Variants of experiments using gel containing carbopol only (without melanin) and options without making any drugs served as control options.

Evaluation of healing effect of the studied drug "Melanin-gel" was conducted by analysis of active contraction of the wound surface in dynamics on 3, 5, 7, 9, 14, 21 and on 30-th days.

In the model of full-thickness skin wound [11] it was shown that complete wound closure in the control group of animals occurred on the 23,2 ± 1,0 day, in research group- on 21,0 ± 0,5 day [Fig. 2, 3]. In the control group of rats on the 6th day an increase of the injured area was found. Only on the 9th day the wound area began to decrease. In the experimental group of rats decrease of lesion area began on the 6th day. On the 9th and 12th day of the experiment in the above mentioned group healing was stronger compared with the control group.

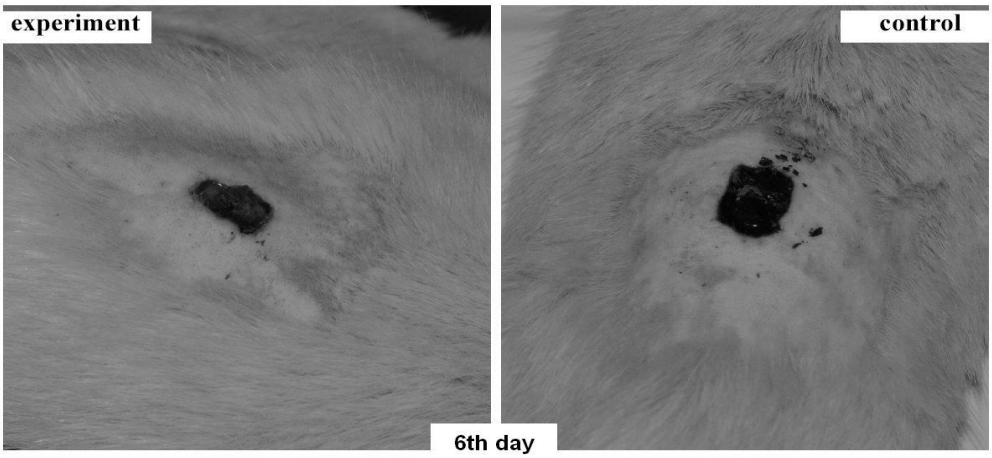

Figure 2: The appearance of the cut wound surface on the 6-th day with the using "Melanin-gel"

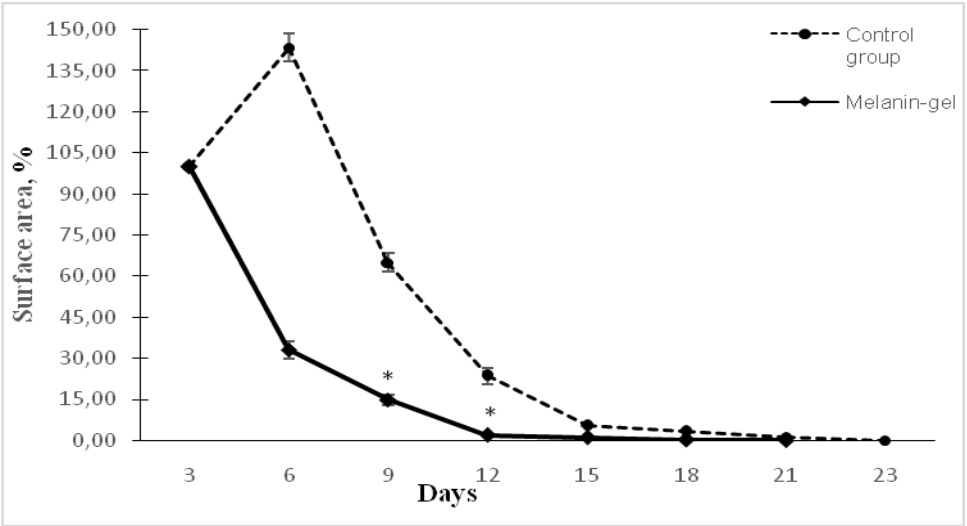

Figure 3: The surface of full-thickness wound area with Melanin-gel action (% of the original size), M±m (n= in each group of animals)

Note: * - p <0.05 compared with the control group of animals

The chemical burns caused by subcutaneous injection of calcium salt, led to extensive tissue necrosis in the explored area of 900mm^2. [12] Necrotomy contributed to the formation of thick scab, under which tissue repair was performed [Fig. 4, 5]. Important in this model is the fact that the surrounding tissues gradually die, increasing the area and depth of the wound until the concentration of chemical injected is

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reduced. In this model, there is a good ability to investigate wound healing drug for cleaning inflammation source from damaged tissue in the wound and toxic products in it.

Complete wound closure in the experimental group was observed on the 36,0 ± 0,7, in the control group – on the 38,1 ± 0,5 days. Reducing the area of purulent necrotic wounds in the experimental group, there was more intense compared to the control group.

Significant reduction of lesion area in the experimental group compared with the control group occurred from the 9th to 30th day. Thus, on the 9th day in the experimental group lesion area was 80% of the original size of the wound, in the control group- 105, 6%.

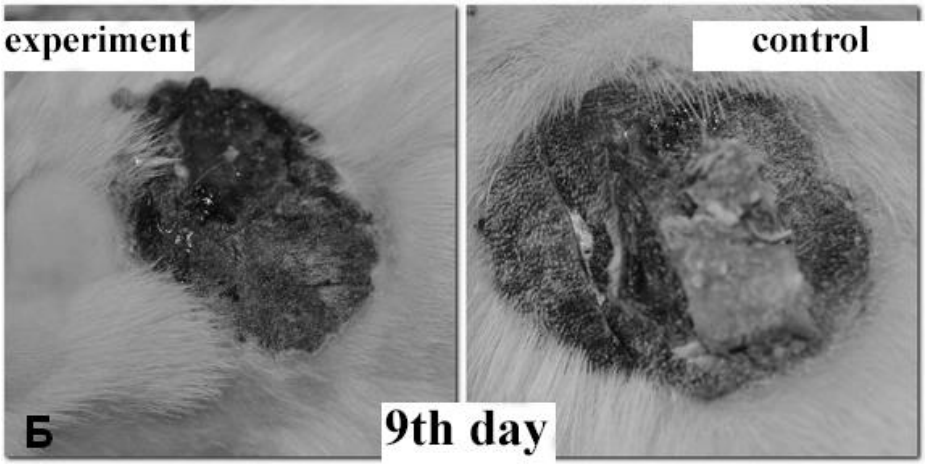

Figure 4: The appearance of purulent necrotic wound surface on the 9th day using "Melanin-Gel"

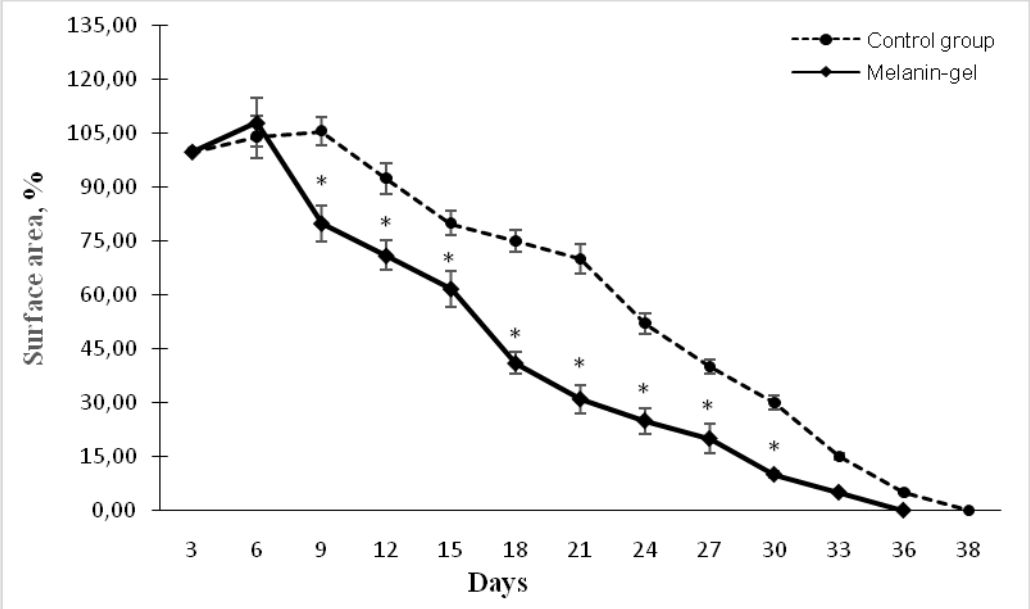

Figure 5: The surface of purulent necrotic wounds area with Melanin- gel action (% of original size) M ± m (n = 10 animals per group)

Note: * - p <0.05 compared with the control group of animals.

Immediately after applying the thermal skin lesions we found that animals which were not treated were slower, had worse appetite. Rats of experimental group were significantly more active. It should be noted that the final closing of wounds in the control group of animals occurred on the 25,0 ± 0,66, and in research – on the 27,2 ± 0,5 days. However, the wound area in the control group on the 6, 9, 12 days increased

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compared with the initial size and was to 156%, 156.25% and 171.88%, respectively (Fig. 6, 7). At the same time, in the group of animals treated with "melanin" the size of the affected area decreased.

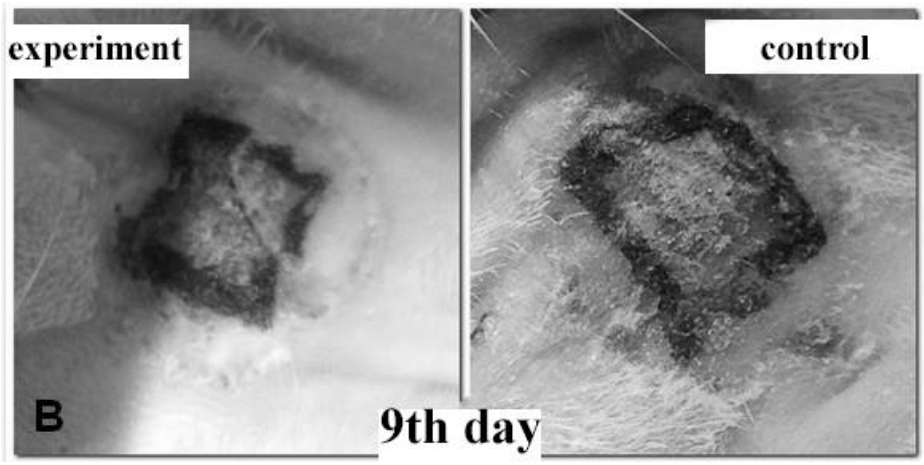

Figure 6: The appearance of burn wound surface on the 9th day using "Melanin-Gel"

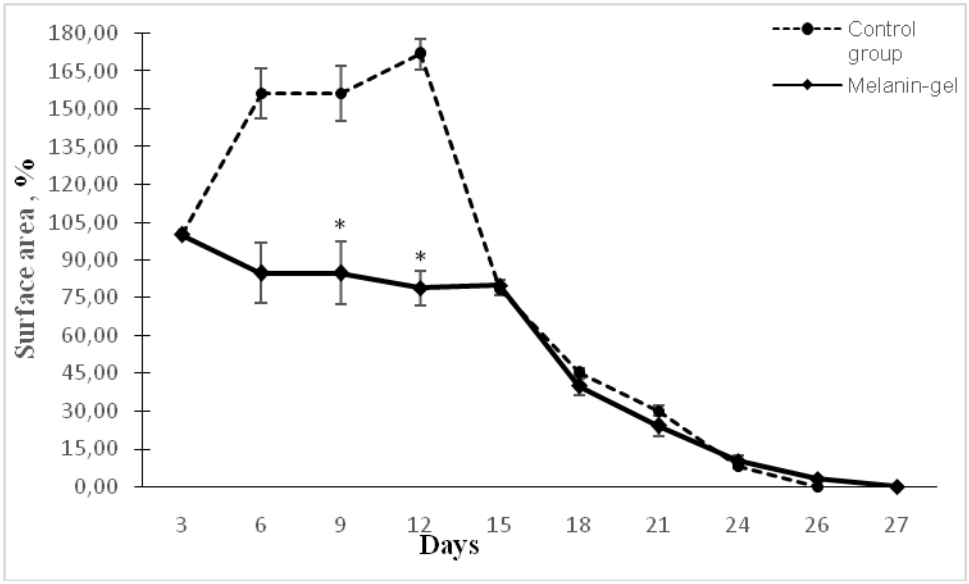

Figure 7: Burn area surface with Melanin- gel action (% of original size) M ± m (n =10 animals per group)

Note: * - p <0.05 compared with the control group of animals.

CONCLUSIONS

It is established that the rapid wounds healing of full-thickness skin wounds and thermal burns using "Melanin-gel" occurred in the initial phase of the regeneration of the skin. The received data indicate that faster purulent necrotic wound healing occurs due to the intense impact on the all phases of the regeneration. "Melanin-gel" has a strong bactericidal effect on *S. aureus,, P. aeruginosa, C.albicans*, that makes the appropriate application of the drug for the treatment of infectious inflammatory processes.

REFERENCES

[1] Injuries and violence: the facts. World Health Organization, Switzerland 2014; 21. (http://apps.who.int/iris/bitstream/10665/44288/1/9789241599375_eng.pdf)

[2] The WHO plan for burns prevention and care. World Health Organization, Switzerland 2008; 21. (http://apps.who.int/iris/bitstream/10665/97852/1/9789241596299_eng.pdf)

[3] Biktimirov VV, Bagriy AV, Himich SD. Morphological aspects of the course purulent-necrotic lesions on the foot of diabetic patients, depending on body weight. Bulletin of morphology 2008; 14(1): 61-65.
[4] Rajsekhar S. Unseen aspects of wound healing: an overview. International Journal of Pharma and Bio Sciences 2011; 2(4): 275-287.
[5] Sakharov SP, Kozlov LB, Ivanov VV. Analysis of the microbial pattern of wound infection at serious thermal injury of children. Fundamental researches 2013; 9(3): 468-471.
[6] Larichev AB. Vacuum therapy in wounds and wound infection: negative pressure wound therapy. Carlsbad, CA, USA: BlueSky Pub. 2005; 248]
[7] Grigoryan SH, Grigoryan AK, Nanyan AS, Grigoryan ES. A comparative study of the effectiveness of different bioactive materials for the treatment of septic wounds. Materials of International Conference, edited by Fedorov VD, Adamyan AA. M, 2001, pp. 101-102.
[8] First national congress on Bioethics. Weekly journal «Pharmacy» 2001; 308 (37) (date 24.09.2001).
[9] Savitsky YM. Effect of melanin on the secretory function of stomach, cytoprotection processes and motility of proximal part of digestive system. Ph.D. thesis in medical science, Lviv, 2002, pp. 133.
[10] Agar N, Young AR. Melanogenesis: a photoprotective response to DNA damage? Mutation research 2005; 571: 121-132.
[11] Henry SL, Concannon MJ, Yee GJ. The effect of magnetic fields on wound healing. Experimental study and review of the literature. Open Access Journal of Plastic Surgery 2008; 8: 393-399.
[12] Bilyayeva O, Neshta VV, Golub A, Sams-Dodd F. Effects of Sea Silon wound healing in the rat. Journal of wound care 2014; 23(8): 140-146.
[13] Ventslus IV, Slutsky LI, Dombrovskaya LE. Experimental test of new drug «Cellocin» for treatment of thermal burns. Rehabilitation system of children with lesions of the musculoskeletal system, Collection of scientific papers edited by Andryyanova VL. L, 1989, pp. 152-155.
[14] Golyshkin DV, Falaleeva TM, Neporada KS, Beregova TV. Effect of melanin on the condition of gastric mucosa and reaction of the hypothalamic-pituitary-adrenal axis under acute stress. Physiological journal 2015; 61(2): 65-72.507.
[15] Schultz GS, Sibbald RG, Falanga V, et al. Wound bed preparation: a systematic approach to wound management. Wound Repair Regen 2003; 11: 23-28.