Gel with Ectoine Improves Wound Healing on a Thermal Burn Model in Rats

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Abstract

Due to the ability to create complexes of water molecules on the surface of the mucous membrane, ectoine is a promising compound for the treatment of burns. Evaluate the reparative activity of Ectoine using the thermal burn model in rats In male rats (n=30), 2 thermal burns were counteracted under counter-anesthesia, one of which was treated by topical application of the test drugs for 7 days, and the other served as a control. On the 8th day, skin samples in the area of the burn were taken for morphological examination. Histological sections were then scored by independent experts. The best histological picture of thermally damaged tissues was demonstrated by animals receiving ectoine. A less significant, but pronounced reparative effect was observed in histological samples of the actovegin group. The least significant reparative effect was demonstrated by contractubex. Ectoine improved the histological picture, improved repair and reduced the inflammatory response of the tissues and can be recommended for further preclinical studies as a treatment for burns.

Introduction

Along with the targeted pharmacological preparations of the new generation, for experimental pharmacology, simpler compounds do not lose their relevance [1-5]. In response to the demands of modern cosmetic and aesthetic medicine, the pharmacological environment forms new approaches to the treatment of morphofunctional skin defects. In case of thermal damage to the skin, one of the most promising strategies is the creation of an artificial barrier between the environment and the damaged area. On this basis, the gel with ectoine is a promising drug for the treatment of burns, since ectoine creates complexes of water molecules on the mucosal surface, forming a “hydration shield” that protects cells of adverse environmental conditions [6-8].

Objective

Evaluate the reparative activity of gel with Ectoine in comparison with Actovegine and Contractubex using the thermal burn model in rats.

Materials and Methods

In male rats (n=30) under anesthesia (chloralhydrate 300 mg/kg) after preliminary depilation of the dorsal surface (70×30 mm) by 15 seconds contact with water in a test tube heated to 99±1° [9-10]. Since the size and severity of the burn can vary significantly depending on the individual reactivity of the organism, as well as from bioethics considerations, it was decided to ignore the possible resorptive effect of the studied gels and not to add a control group to the design of the experiment, the surface of which will not be treated with drugs. Thus, 2 thermal damages of a round shape and a diameter of about 10 mm were applied counter-laterally on the posterior surface at the level of the ribs (Fig. 1).

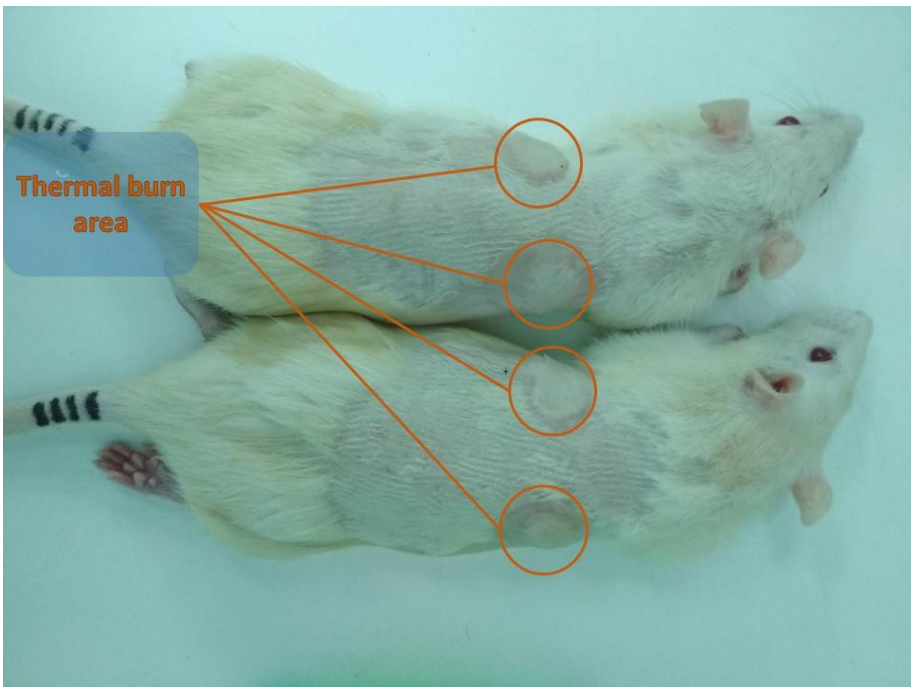


Figure 1. Animals immediately after the application of thermal burns.

Then the animals were divided into 3 equal groups:

I - *Ectoine* - rubbing 500 mg of extoin gel onto the burn area located to the right of the spine 10 minutes after the thermal injury was simulated and for the next 6 days (1 time per day)

II - *Actovegin* - rubbing 500 mg of Actovegin gel onto the burn area to the right of the spine 10 minutes after the thermal injury was simulated and for the next 6 days (1 time per day)

III - *Contractubex* - rubbing 500 mg of cotractubex gel on the burn area to the right of the spine 10 minutes after the thermal injury was simulated and for the next 6 days (1 time per day)

After natural drying of the application area, the animals are placed in individual cages. In the next 6 days, in addition to applying prepartum, photographs of the burn area are made, animals are weighed, as well as an assessment of the clinical condition, physical activity and feed and food consumption. On day 8, animals are removed from the experiment by the method of cranial dislocation under anesthesia.

Evaluation of the reparative activity of the studied drugs was performed by histological examination of thermally damaged tissues. Cutaneous fragments of the thermal burn areas were cut out and fixed in 10% buffered formalin. The preparation of histological preparations was carried out according to standard methods. Sections were stained using hematoxylin and eosin.

Then, the received preparations were assigned code names for an independent evaluation by an expert commission consisting of 5 doctors from the pathoanatomical bureau of the regional clinical hospital in Belgorod (Russia). The assessment was made on a specially developed scale (Tab. 1):

Table 1. Scale for assessing the reparative activity of the studied drugs with the help of a histological picture of the tissue of thermal damage.

Qualitative attribute	Scores and their characteristics			
Violation of cytoarchitecture	0– None	1 - Weakly expressed	2 - Expressed	3 - Pronounced
Intercellular Matrix Architectonics Disruption	0– None	1 - Weakly expressed	2 - Expressed	3 - Pronounced
Violation of epithelialization	0– None	1 - Weakly expressed	2 - Expressed	3 - Pronounced
Presence of scab	0– None	1 - Weakly expressed	2 - Expressed	3 - Pronounced
Leukocyte infiltration	0– None	1 - Weakly expressed	2 - Expressed	3 - Pronounced

Statistical processing of the obtained results was carried out in R medium using a Tukey post-hoc analysis.

Results

After awakening and on further days of the study, the animals were active, the consumption of feed and food was within the normal range. Signs of infection, excoriation and other adverse events from the skin was not observed. Within 7 days of regular differences in the cumulative clinical evaluation of the studied groups was not observed. The morphological study of the obtained samples in various histological preparations revealed:

- ***Ectoine***. A microscopic study of drugs, in the group of observations of ectoine, revealed almost complete recovery of the skin. The fibrous skeleton of the dermis is formed completely. On the surface of the cut of the skin in the area of the burn wound, a scab is visualized, under which is a thin word of the newly formed epidermis (Fig. 2).

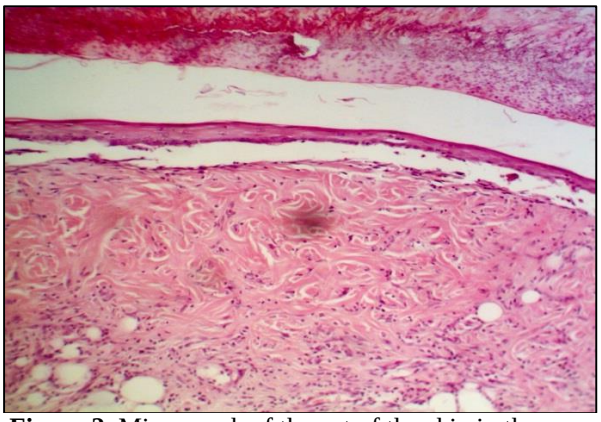


Figure 2. Micrograph of the cut of the skin in the area of the burn in the group of observations with the use of ectoine. Under the scab, the thin newly formed epidermis is well visualized. Stained with hematoxylin/eosin. x200.

The layered structure of the epidermis is preserved (Fig. 3A), three layers are well pronounced. In the basal layer of the cell high prismatic shape. Among the basal epithelial cells, a large number of lymphocytes is detected (Fig. 3B). The stratum corneum is not pronounced, there are no horny scales, which indicates a lack of maturity of the epidermis and full restoration of its functions, including the process of keratinization.

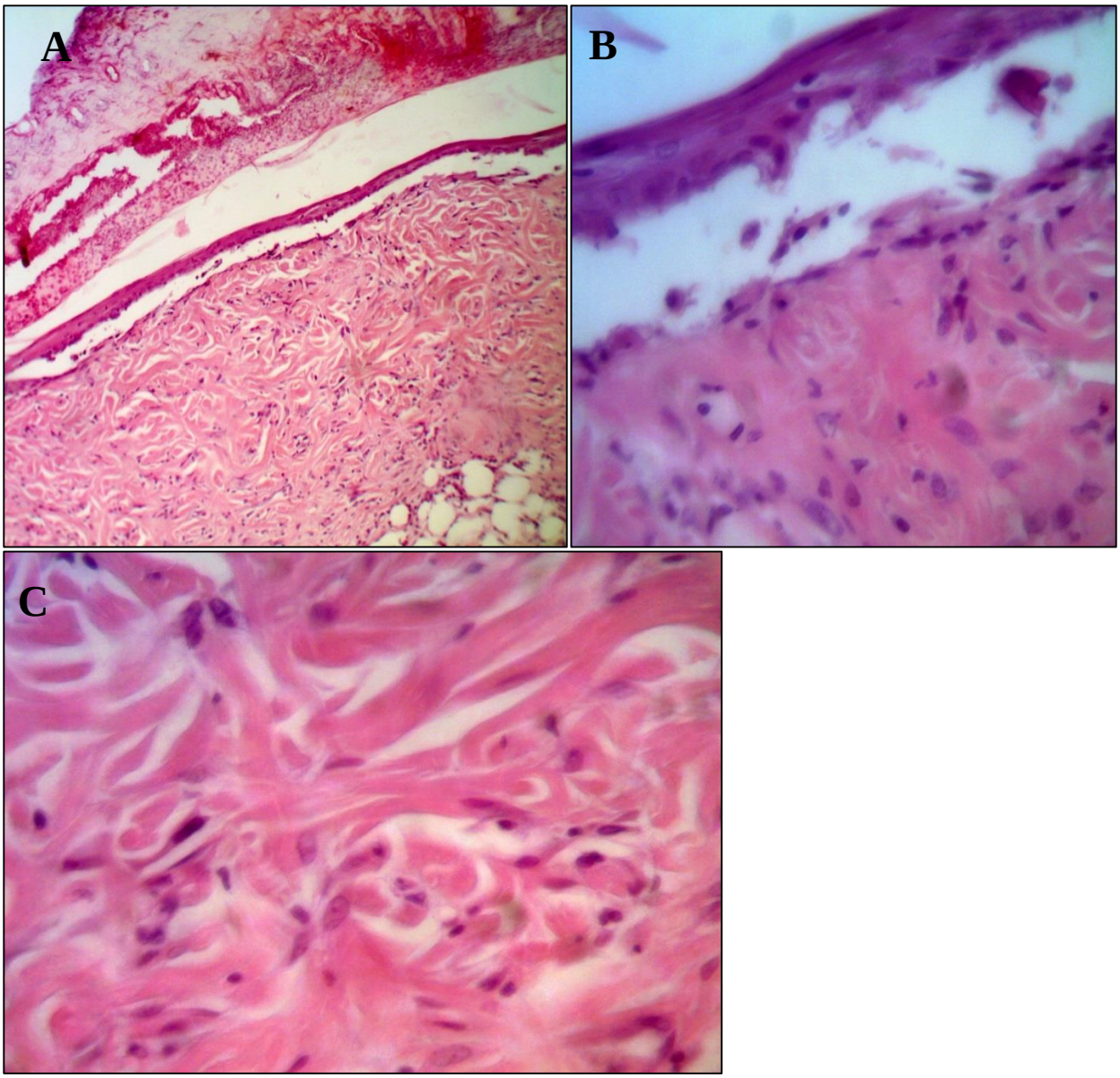


Figure 3. Micrograph of the burn defect zone of the skin in the group using ectoine. The newly formed epidermis is a thin layer located under the scab on the surface of the burn wound. The stratum corneum is not expressed (A, B). x200 (A), x400 (B, C).

In the underlying layers of the dermis in the papillary layer, a fibrous skeleton consists of mature collagen fibers, which have all the morphological and functional criteria necessary for the formation of dense, unformed connective tissue of the dermis.

Regarding the cellular component of the regenerated tissue of the dermis, it should be noted that in the field of view, cells of the fibroblastic differention are predominantly determined, as well as single lymphocytes and mast cells. The fibrous component prevails over the cellular component. Cell density is low (Fig. 3B).

- ***Ectoine-control***. During the histological study of drugs in the group of observations, the ectoine control revealed that there is a scab on the surface of the skin in the area of the burn, which is 3-4 times higher than the experimental group. Epithelization under the scab no. Numerous foci of necrosis are observed over a large area (Fig. 4A, 4B). In the deep dermis, edema, leukocyte infiltration. There are areas of necrosis and soaking with fibrin (Fig. 4B).

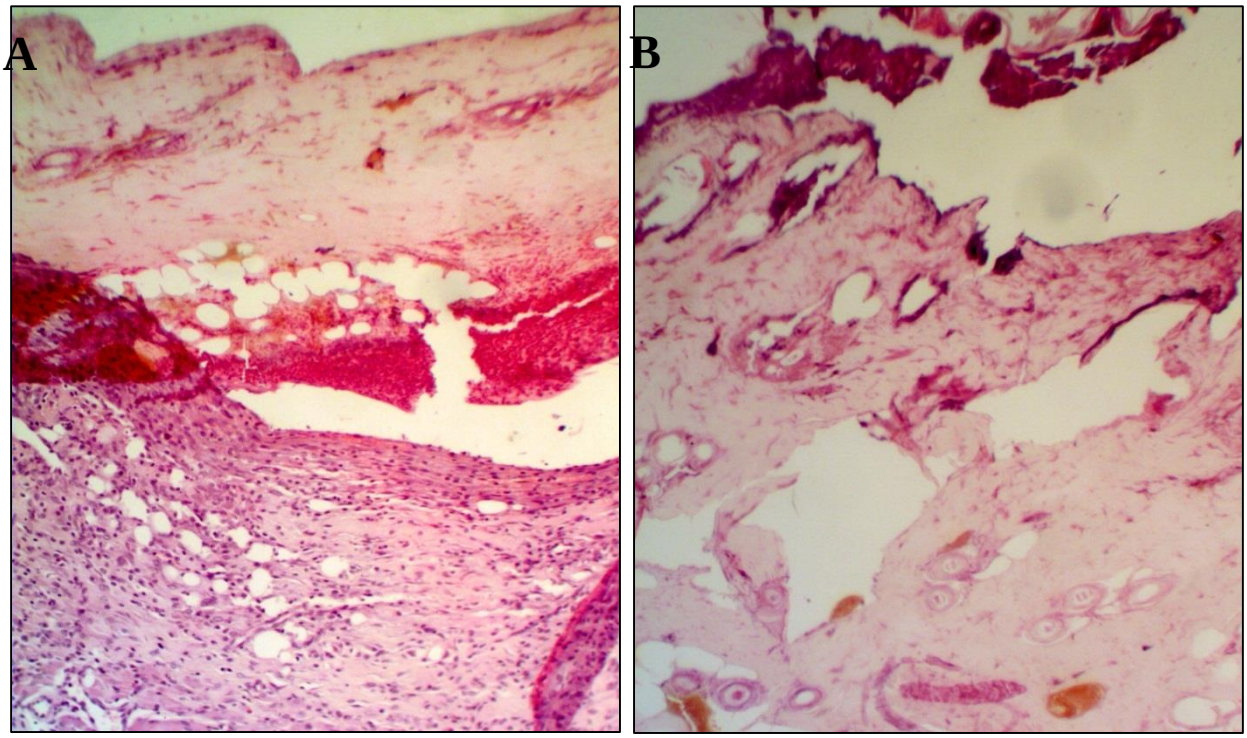
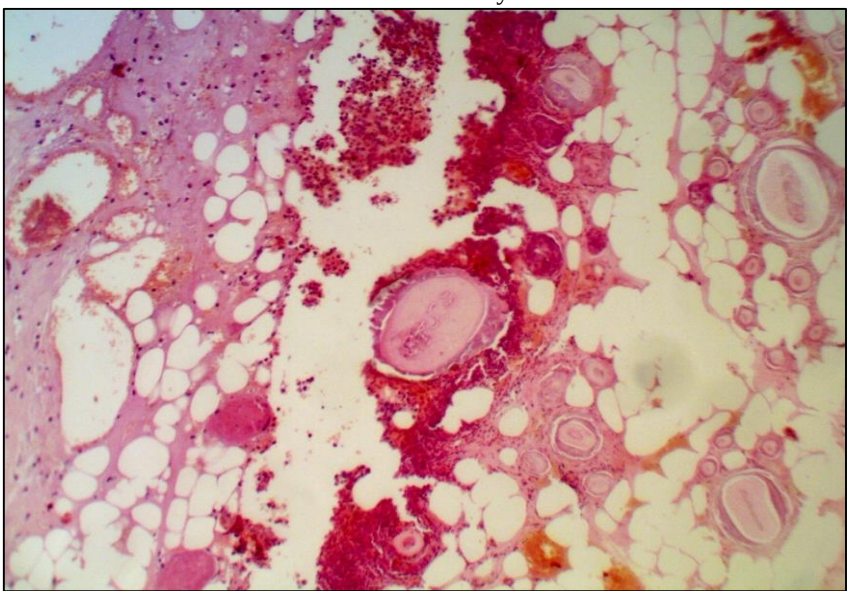


Figure 4. Micrograph of the burn defect zone of the skin in the group using ectoine - control. Epithelization under the scab no. Stained with hematoxylin/eosin. x200.



- *Contractubex*. In preparations in the area of burns on the surface, complete epithelialization of the epidermis is determined (Fig. 5A). The epithelium is thick, layered structure saved. In some epithelial cells in their nuclei, the phenomena of karyopicnosis are expressed. In the zone of epithelialization or connective tissue scar, the hair follicles are not visualized.

In the underlying dermis there is a large number of newly formed blood vessels, with signs of hemorrhage into the surrounding tissue. Collagen fibers are disintegrated (Fig. 5B).

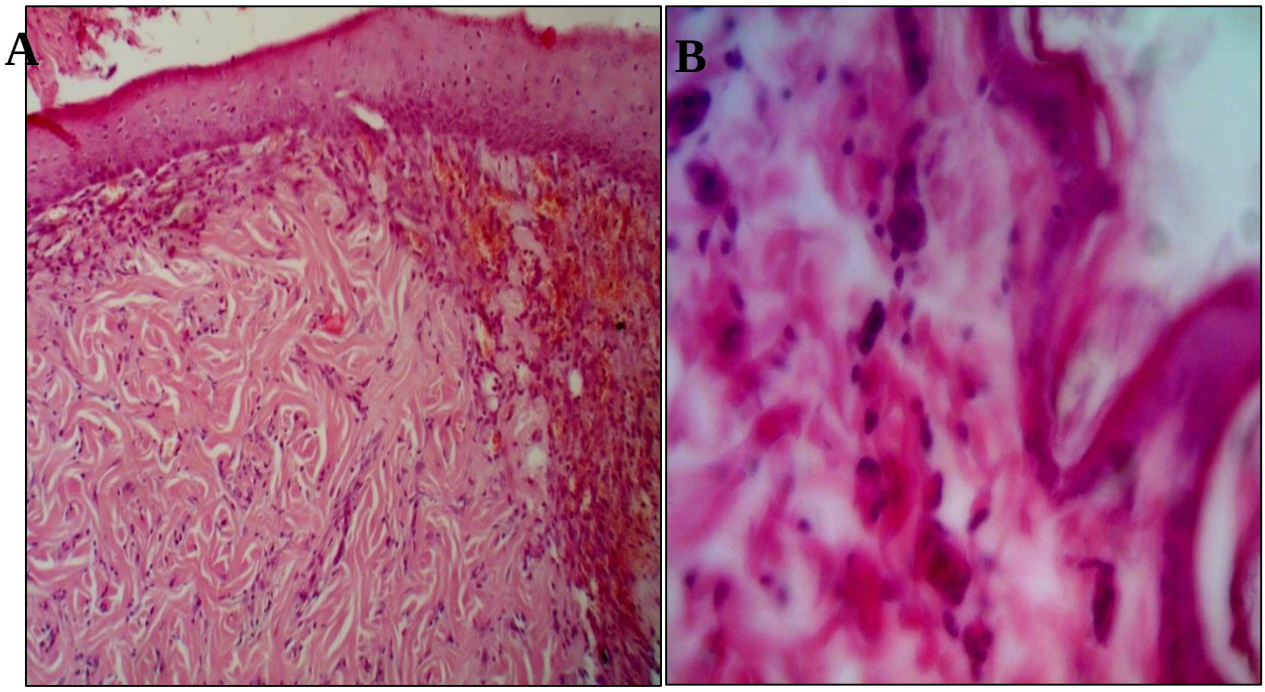


Figure 5. Micrograph of the burn defect area of the skin in the group using contractubex. There is a complete regeneration of the burn defect. In the epidermis, there is a complete restoration of the structural and functional organizationStained with hematoxylin and eosin. H. x 200 (A), x400 (B).

- ***Contractubex-control.*** In the areas of the burn defect layer-by-layer structure of the skin is restored. The eschar in the area of damage is not. There is a complete restoration of the epidermis over the area of the burn wound. The stratified squamous non-keratinized epithelium has a layer-by-layer structure, the layers are clearly differentiated. The newly formed fibers are thin, randomly located. The cellular component is predominant on the fibrous. Lymphocytes and fibroblasts predominate among cells (Fig. 6).

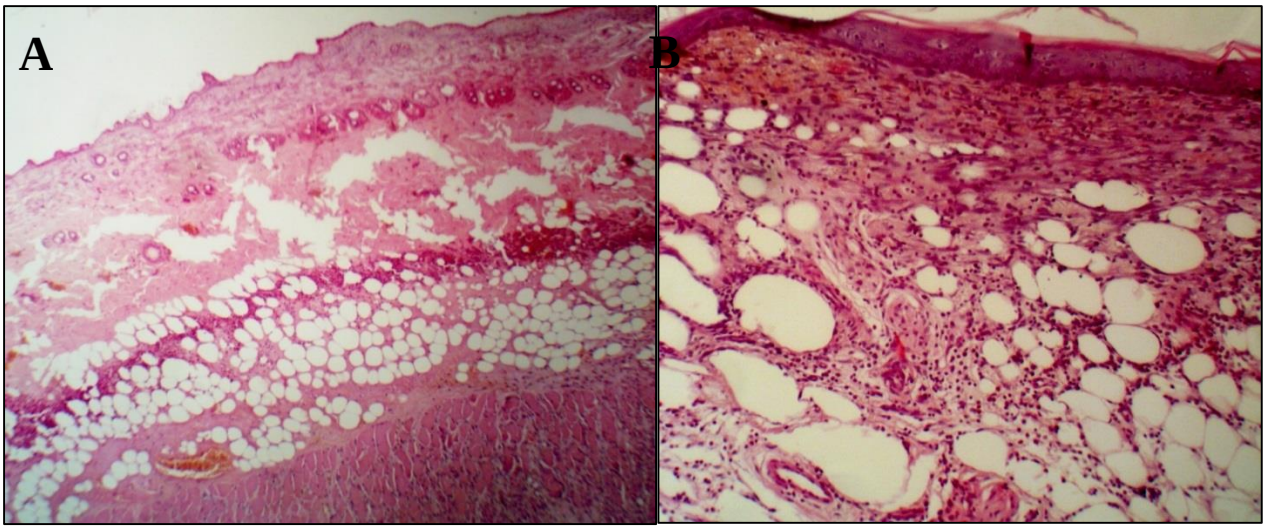


Figure 6. Micrograph of the burn defect area of the skin in the group using contractubex-control. There is a complete restoration of the spatial organization of the skin in the area of the burn defect. Stained with hematoxylin and eosin. x200.

- ***Actovegin***. A thin newly formed layer of the epidermis (Fig. 7A) is visualized at the site of the burn, without a clear division into layers (Fig. 7B). Derivatives of the skin are preserved, which may indicate a shallow depth of the burn. In the underlying dermis, edema is pronounced, the collagen fibers are randomly arranged and in different directions (Fig. 7B).

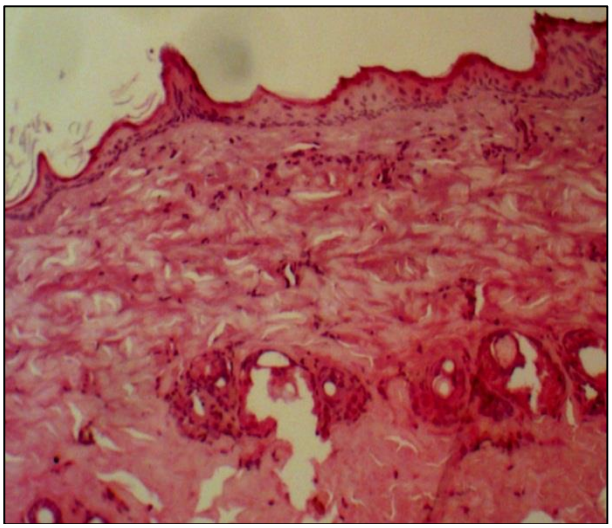


Figure 7. Photomicrograph of the burn defect area of the skin in the actovegin group. Complete regeneration of the burn defect of the wound. Stained with hematoxylin and eosin. x 200.

- ***Actovegin-control.*** The study of microscopic preparations in the control group revealed the presence of complete regeneration of the skin in the place of the burn wound. The stratified squamous non-keratinized epithelium has a well-pronounced layer-by-layer structure. The stratum corneum is quite well expressed and formed by horny scales (Fig. 8).

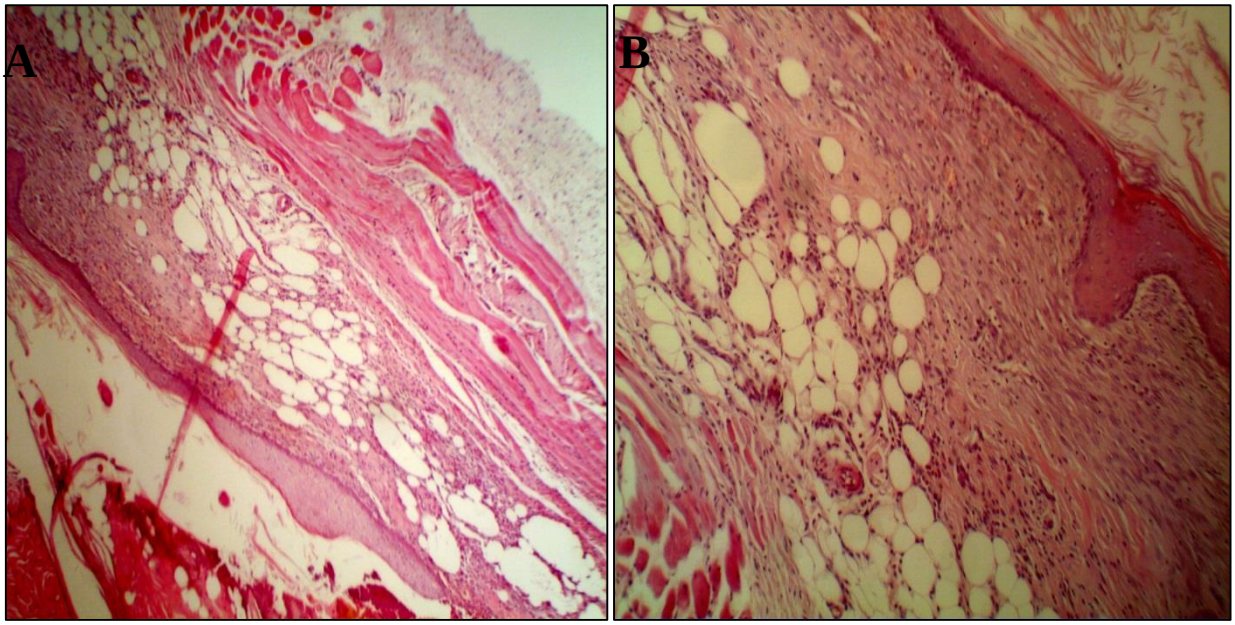


Figure 8. Photomicrograph of the burn defect area of the skin in the actovegin-control group. x200

Comparative quantitative assessment.

When questioning the expert commission in each group, the average score was determined (Tab. 2). It was found that the best histological picture of thermally damaged tissues was demonstrated by animals receiving ectoine. A less significant, but pronounced reparative effect was observed in histological samples of the actovegin group. The least significant reparative effect was demonstrated by contractubex (Tab. 2, Fig. 9)

Table 2. The results of the microscopic specimens of the skin scoring by an expert commission (M±m).

Qualitative attribute	Score					
	Ectoine	E-C	Actovegin	A-C	Contractubex	C-C
Violation of cytoarchitecture	0.91±0.11	1.72±0.19	1.16±0.15	1.64±0.16	1.45±0.19	1.82±0.12
Intercellular Matrix Architectonics Disruption	1.19±0,08	2.11±0.09	1.51±0.11	2.05±0.09	1.62±0.12	2.21±0.10
Violations of epithelialization	1.41±0.10	2.92±0.12	2.29±0.12	2.95±0.14	2.38±0.14	2.87±0.13
Presence of scab	1.77±0.11	2.31±0.11	1.65±0.14	2.26±0.17	2.01±0.15	2.39±0.16
Leukocyte	1.75±0.09	2.15±0.17	1.78±0.10	2.21±0.10	1.87±0.14	2.0±0.12

infiltration						
Average score	1.41±0.10*^	2.24±0.14	1.68±0.12*#	2.22±0.14	1.87±0.16*#^	2.26±0.13

Note: a lower score indicates a more consistent histological pattern; E-C, A-C, C-C - control for ectoine, actovegin and contractubex, respectively; * - $p<0.05$ when compared with the control; # - $p<0.05$ when compared with the group receiving ectoine; ^ - $p<0.05$ when compared with the actovegin group.

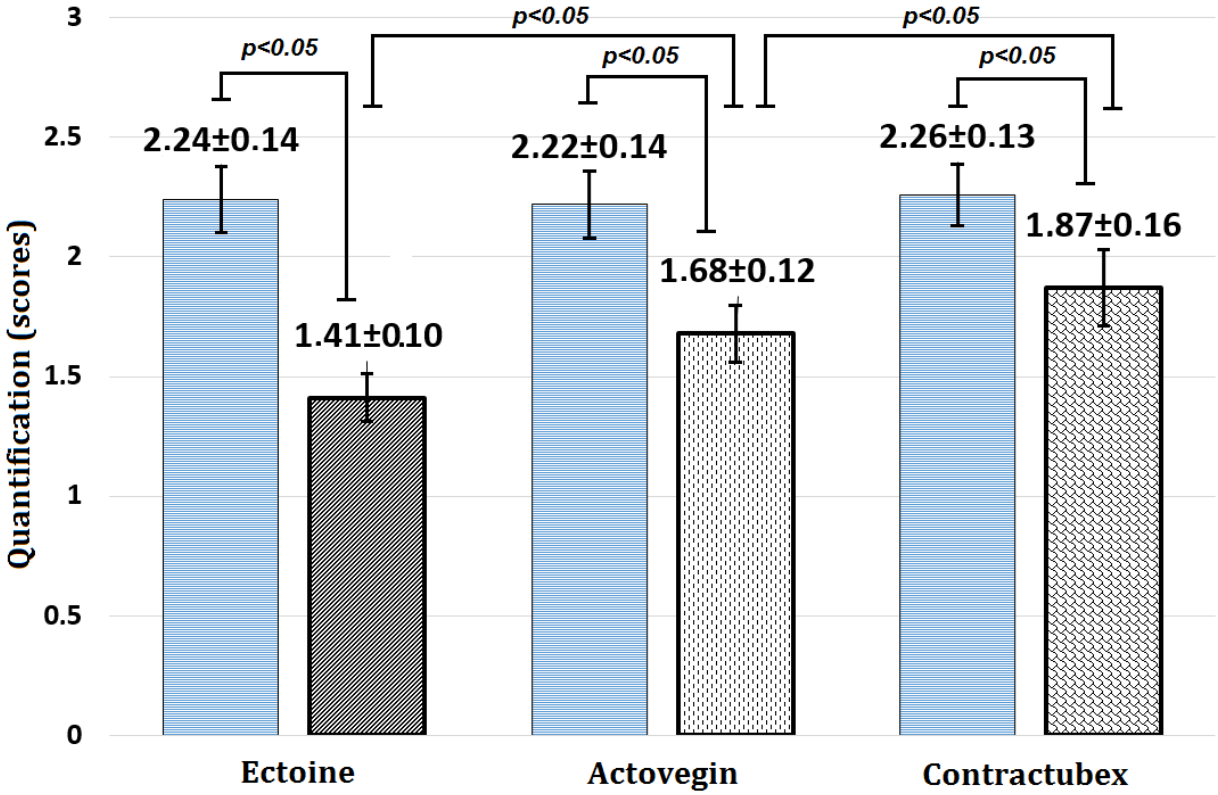


Figure 9. The results of the scoring of skin microscopic preparations by the expert committee.
Note: blue bars (left) - control; gray bars (right) - the studied gel.

Cnclusion

The study showed that the best results were obtained with the external use of ectoine gel. According to the results of histological examination, thermal damage in this group was less pronounced than in the control group. A smaller, but significant reparative effect showed actovegine gel. Finally, contractubex gel showed the lowest efficacy.

Thus, ectoine gel shows positive reparative properties and can be recommended for further study, both on models of other burns, and in modeling skin defects of a different etiology.

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