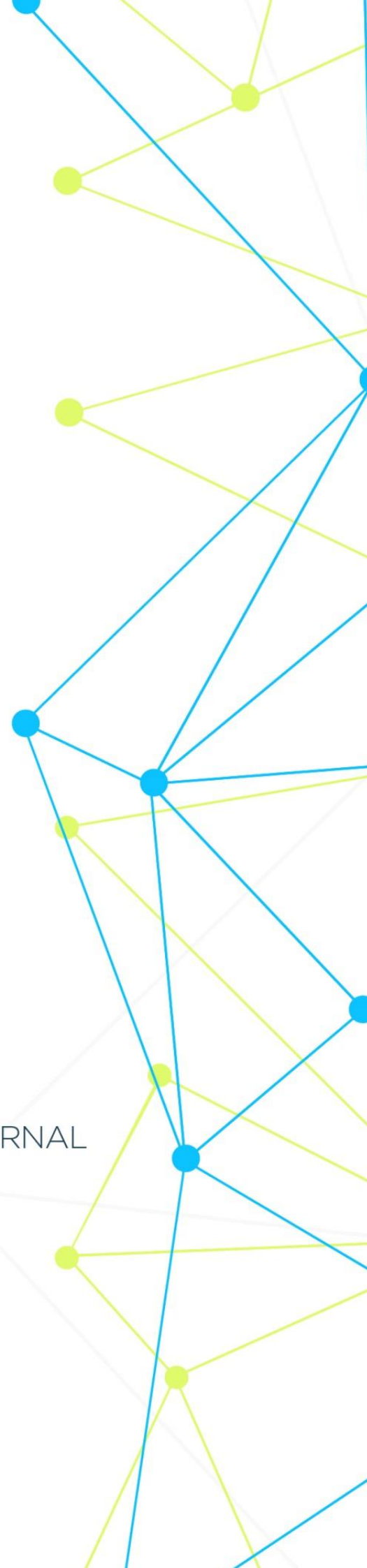


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Comparative characteristics of healing of AFTHOUS stomatitis in experiment

Akbarov A.N., Ziyadullaeva N.S., Irismetova B.D.
Tashkent State Dental Institute Republic of Uzbekistan

Abstract. The work is based on the results of experimental studies on the usage of a new form of Heprocel medication. The experiment has used outbred white rats, which were divided into 2 groups: control and experimental. In the experimental group of animals, HEPROCEL powder was applied to the surface of an artificially induced traumatic ulcer. The developed method for local protection of traumatic lesions of the oral mucosa with Heprocel made it possible to shorten the regeneration time of the erosive process and the period of complete regression of inflammatory changes.

Keywords: Heprocel, treatment of aphthous stomatitis, regeneration of the oral mucosa.

Introduction. Aphtha is a focal deep fibrinous inflammation of the oral mucosa, proceeding according to the Artyus phenomenon, resulting in more or less pronounced destruction of the epithelium, and sometimes the underlying connective tissue part of the mucous membrane [6, 7].

The application of new treatment regimens for local inflammatory appears to be interesting, innovative and promising. It practices manifestations in the oral cavity through the development of differentiated principles of therapy, taking into account the pathogenetic and morphological mechanisms of the erosive process of the oral mucosa, the characteristics of the course and development of recurrence of HRAS [1, 2, 8, 9, 10]. However, the use of most drugs gives a short-term effect with the manifestation of discomfort in the form of a burning sensation and side effects, such as exacerbation of gastric ulcer and duodenal ulcer, leukopenia, anemia, and skin rash [3, 4, 5].

Objective of the study: experimental and morphological substantiation of the effectiveness of a new local method for the treatment of aphthous stomatitis.

Material and methods. In the experiments were used 61 outbred white rats of different sex weighing 220-290 grams. The control group of rats included 32 individuals, experimental 29. The animals were kept in clean, spacious cages. Rooms for animals are equipped with ventilation and air conditioning, constant lighting. The nutrition was balanced with the inclusion of a sufficient amount of proteins, carbohydrates, vitamins. After the ulcer formation, the animals were provided with a diet of non-coarse food to reduce pain.

Daily monitoring of the condition of the animals consisted of examining the oral cavity, washing the area of the formed ulcer in the control group with water (2.0 ml), and in the experimental group, after washing with water, an implant in the form of HEPROCEL powder was applied with the formation of a gel on the surface of the ulcer. The animals were weighed. General condition, mobility, nutrition and physiological functions were assessed.

All operations were carried out in accordance with the rules adopted by the European Convention for the Protection of Vertebrate Animals used for Experiments or Other Scientific Purposes (ETS N 123), Strasbourg, 03/18/1986. Removal from the experiment was carried out by inhalation of an increased dose of halothane vapor. Terms of withdrawal from the experiment: 1 hour, 12 hours, 1 day, 3, 5, 7, 14, 21, 30 days.

The proposed method for the formation of a chronic ulcer is as follows: a chronic ulcer of the oral mucosa is formed by applying filter paper circles moistened with 50% acetic acid to the oral mucosa, characterized in that standard 3x3 mm circles are applied from the side of the lower lip mucosa and fixed with a special holder - a clip of the appropriate size, allowing for fixation in a certain place with a force of 50-100 kPa, and also to prevent dilution of the concentration of the active agent by the separating saliva. The model of such a formation of an ulcer in the oral cavity in a rat makes it possible to cause a lesion with the development of a local aphthous lesion of the mucous membrane, which provides a more comprehensive study to assess the effectiveness of topical application of various agents.

In the experimental group of animals, starting from 3 days after exposure to the surface of the aphthous ulcer, the powder "HEPROCEL" (finely dispersed) was applied with the formation of a gel due to the secretion of the salivary glands. The gel has good adhesion to necrotic tissues, therefore, application is sufficient for 1 minute. Treatment of the ulcer with a wound dressing with the formation of a gel was carried out daily at the same time.

HEPROCEL is a bio-soluble, biodegradable polymer implant with hemostatic properties, manufactured by Turon Silk. Release form - powder.

Physicochemical characteristics of the finished product "HEPROCEL": at t 20°C solubility in water 10 g/l, soluble in water, insoluble in most organic solvents. Melting point 220°C. Stable at pH 5-7. It hydrolyzes quickly in an alkaline environment, more stable in an acidic environment.

The next stage of the study was a morphological assessment of changes in the oral mucosa in experimental animals during the formation of an aphthous ulcer and the dynamic processes of its reparation. Tissue samples were fixed in 10% formalin solution in phosphate buffer. Paraffin sections were stained with hematoxylin and

eosin. Light-optical micrographs were obtained with an Axioscop 40-ZEISS microscope, coupled with a digital camera. All micrographs were processed and data saved on a computer using Microsoft-Windows XP-Professional software.

To assess the adhesion of the cover for use in the oral cavity, we took into account the following circumstances:

- to determine the optimal type and consistency of the coating for better adhesion to the wound surface of the oral mucosa;
- to study the adhesion and durability of the coating depending on the phases of wound healing;
- to study the rate of dissolution of the coating under the conditions of washing with liquid or enzyme solutions.

Results. The results of treatment in the experimental group of rats showed that on the 3rd day after exposure, an aphthous ulcer was detected, formed on the mucous membrane of the lower lip with indistinct contours, undermined edges. On the 5th day, treatment was started, the HEPROCEL powder was applied to the area of aphthous ulcer of the mucous membrane of the lower lip to form a gel on the surface of the ulcer (Fig. 1). On the 7th day of the experiment, the ulcer is covered with a fibrin film, has a fairly clear border, signs of inflammation with subsiding, granulation tissue is visible under the fibrin film (Fig. 2).



Fig. 1. The experimental rats on the 5th day of the experiment. FIELD mucosa of the lower lip aphthous ulcers coated powder "HEPROCEL" to form a gel on the

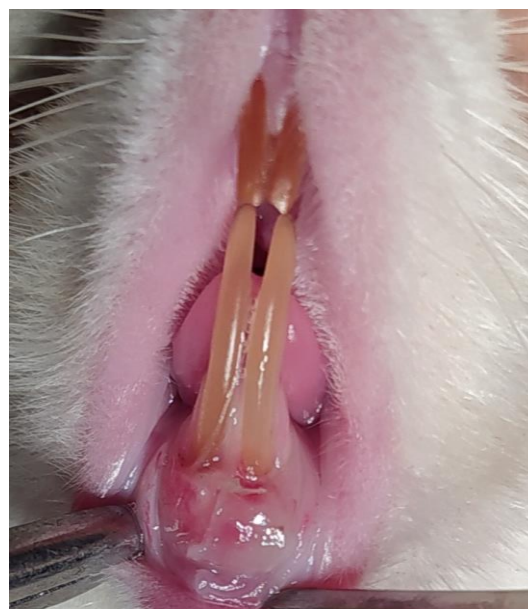


Fig. 2. The experimental rats on the 7th day of the experiment. Ulcer is covered with a fibrin film has enough clear boundary. Signs of inflammation from subsiding.

ulcer surface.

**Granulation tissue viewed under a
fibrin film.**

At the same time, a finely dispersed powder "HEPROCEL" was again applied to the area of the formed aphthous ulcer with the formation of a gel on the surface of the ulcer (Fig. 3). On the 14th day, complete healing of the oral mucosa was determined after the formation of an acetate ulcer and treatment with a gel coating of cellulose derivatives (Fig. 4).

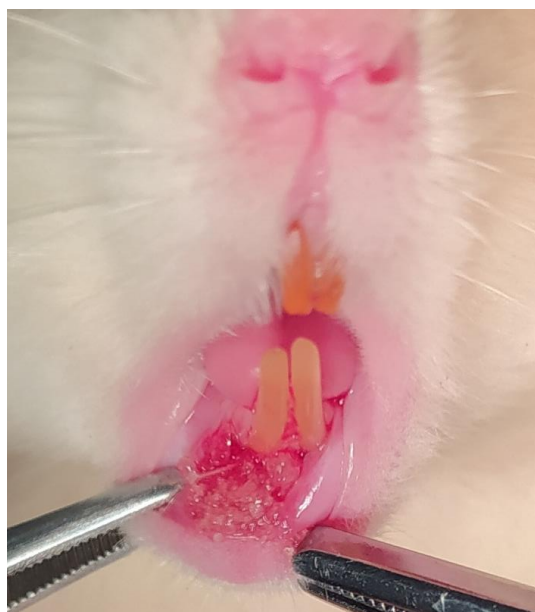


Fig. 3. Experienced rat on the 7th day. On the region formed aphthous ulcer re-applied fine powder "HEPROCEL" to form a gel on the ulcer surface.

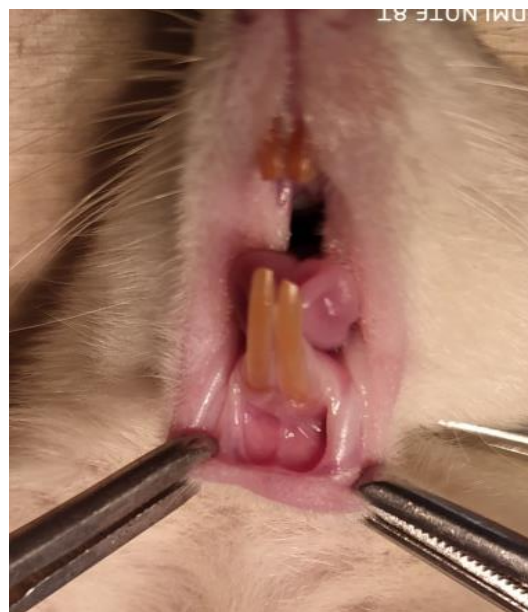


Fig. 4. Experienced rat on the 14th day of treatment. Complete healing of the mucosa of the oral cavity after formation of ulcers acetate and treatment with the gel coating of cellulose derivatives.

Thus, experimental studies on the formation of chronic aphthous ulcers in the oral cavity showed that, in the absence of specific treatment, regression of the inflammatory process begins only by 10-14 days of observation with complete epithelialization by 30 days, in turn, the local application of the powder form of the drug "HEPROCEL" with the subsequent formation of a gel on the surface of the ulcer already 2 days after the start of therapy (on the 7th day of the experiment) leads to the activation of the reparative process with the formation of granulation tissue under the fibrin film and complete healing of the mucous membrane by 14 days.

Analysis of histological changes during the formation and healing of experimental aphthous ulcers in rats showed that 12 hours after the start of the

experiment, it was determined that the stratified squamous epithelium was thinned, the mucous membrane was degraded, the lamina propria was edematous with elements of necrosis, the muscle layer was edematous with degradation, the glands were numerous (fig. 5).

After 36 hours, degradation of the epidermis and stratified squamous epithelium was already noted, the lamina propria was with necrotic masses, the glands were degraded, the striated muscles were edematous and dissociated (Fig. 6).

On the third day, the same pattern persisted (Fig. 7). Further, in the control group of animals, the healing process was characterized by the following picture on the 5th day. The stratified squamous epithelium was eroded in places, the epidermis was altered, the papillae were smoothed, the mucous membrane with elements of degradation and necrosis, the lamina propria is edematous with elements of necrosis, the muscle layer is edematous with degradation, and few glands are degraded. In dynamics, on the 7th day, an actually equivalent picture was determined (Fig. 8).

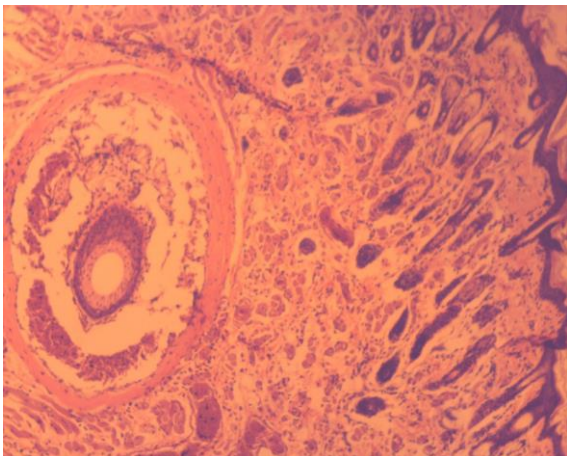


Fig. 5. Micrograph of an aphthous ulcer of the oral mucosa in rats - 12 hours

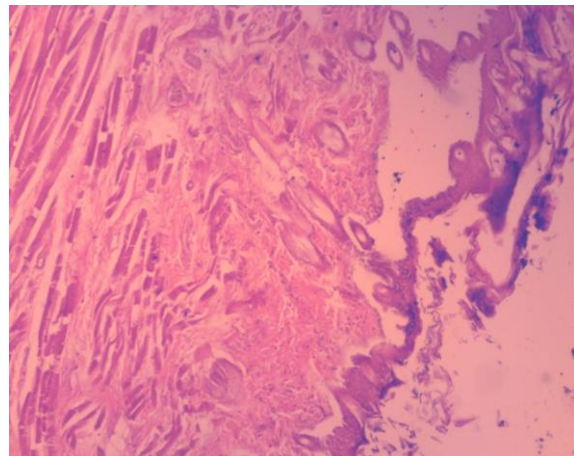


Fig. 6. Micrograph of aphthous ulcer of the oral mucosa in rats, 36 hours

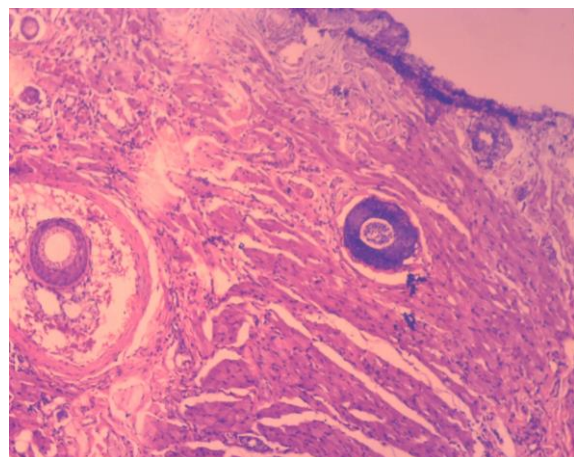
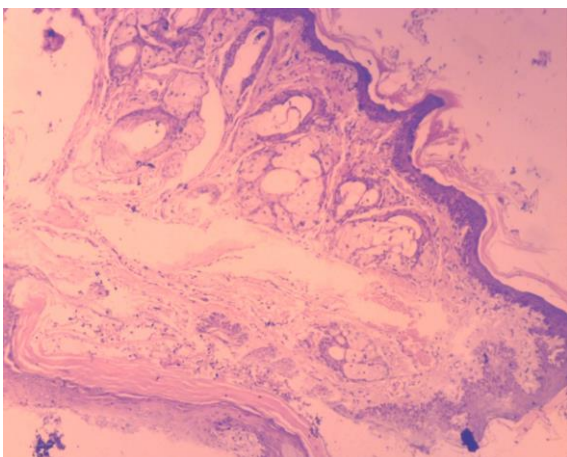


Fig. 7. Micrograph of aphthous ulcer of the oral mucosa in rats on the 3rd day of the experiment.

Fig. 8. Micrograph of the rat lip mucosa in the control group of animals: the beginning of the healing process on the 7th day

On the 14th day, signs of the onset of regression of the inflammatory process were determined, the epidermis was modified, the papillae were high, the stratified squamous epithelium was edematous, the mucous membrane with elements of degradation and necrosis, the lamina propria edematous with elements of necrosis, the muscle layer was edematous with degradation, the glands were numerous degraded (Fig. 9).

On the 21st day, cleansing of necrotic masses was noted, but signs of edema of the epithelium, fibroelastic and muscle tissue, and the glands were partially degraded (Fig. 10).

By the 30th day, complete restoration of the structure of the tissues of the mucous membrane, the absence of signs of degradation, the glands are numerous, the vessels with blood filling.

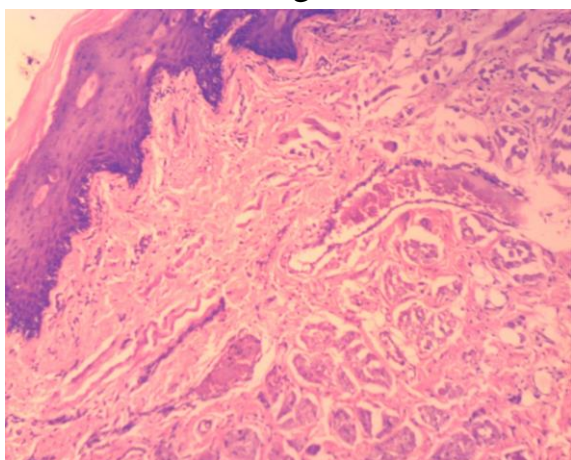


Fig. 9. Micrograph of the rat lip mucosa in the control group: the healing process on the 14th day.

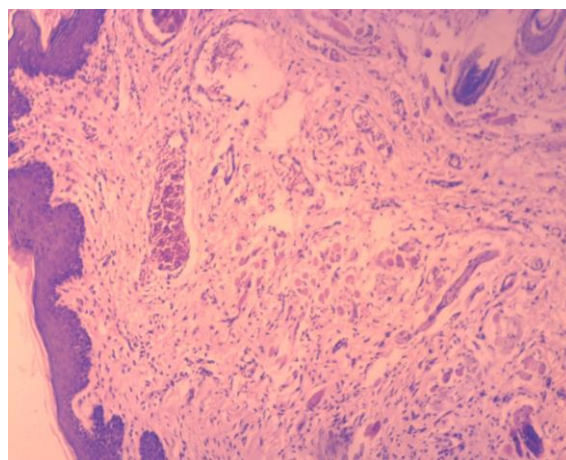


Fig. 10. Micrograph of the mucous membrane of the lip of a rat in the control group of animals: the healing process on day 21

In the main group of animals in the study of the morphology of the proposed interaction with the coating surface of the wound in the space between them is marked formation of thrombotic masses. In the experimental group animals 1 day after exposure of the powder surface aphthous ulcers "HEPROCEL" formed film adheres to the wound surface. The epithelial layer is composed of stratified squamous epithelium, which places thinned, the epidermis is modified, papillae smoothed

mucosa with elements degradation and necrosis, lamina propria of the mucosa with edematous necrosis elements muscular layer edematous with degradation few gland degraded (Fig. 11).

Subsequently on day 7 of the experiment and 2 hours after application of the powder "HEPROCEL" morphological pattern of interaction with the surface of the ulcer GPRS characterized in that the formed film is subjected to fragmentation in the abutment zone defined clusters of blood cells. High epithelium, lamina propria mucosa forms a high papillae, striated muscle slightly swollen in the submucosa mucosal side lips are numerous glands, ducts their associated with the mucosal surface. There was a significant reduction in signs of inflammation, necrotic changes actually determined (Fig. 12).

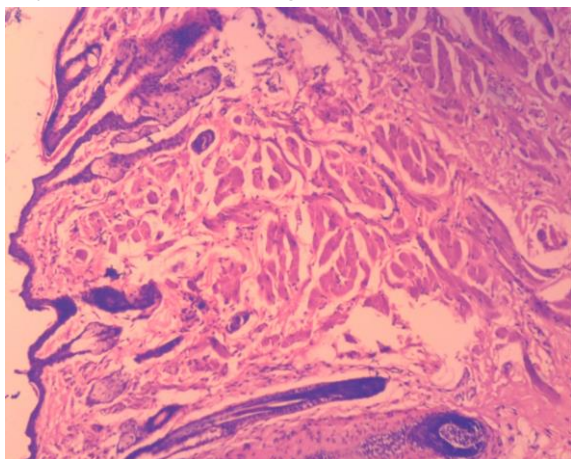


Fig. 11. Micrograph of the mucous lip of the rat in the experimental group of animals: 1 day after exposure to the surface of the aphthous ulcer of the powder "HEPROCEL"

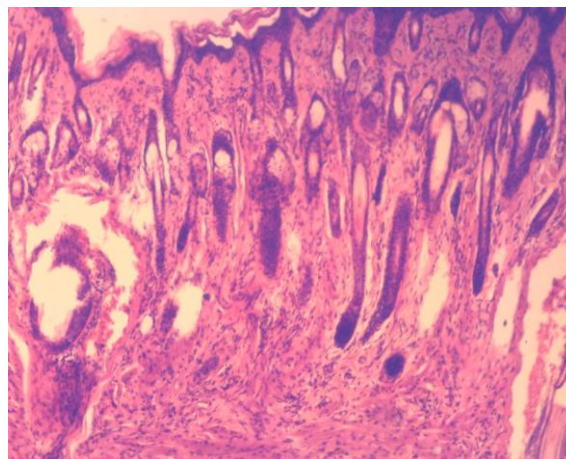


Fig. 12. Micrograph of the mucous lip of the rat in the experimental group of animals: on the 7th day after exposure to the surface of the aphthous ulcer of the powder "HEPROCEL"

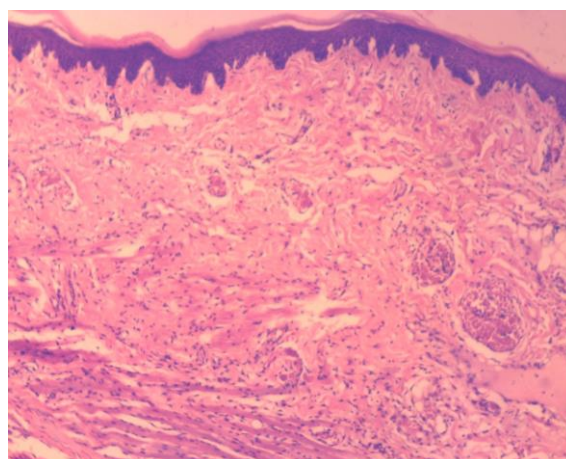
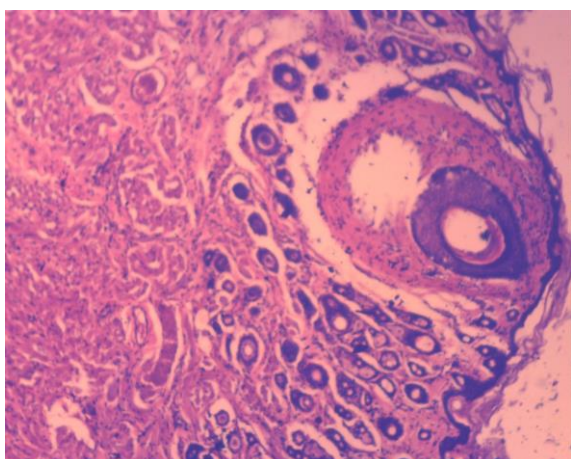


Fig. 13. Micrograph of the mucous lip of the rat in the experimental group of animals: on the 14th day after exposure to the surface of the aphthous ulcer of the powder "HEPROCEL"

Fig. 14. Micrograph of the mucous membrane of the rat lip in the experimental group of animals: on the 21st day after exposure to the surface of the aphthous ulcer of the powder "HEPROCEL"

On the 14th day after exposure to the surface of the aphthous ulcer of the powder "HEPROCEL", only small areas were determined after the fragmentation of the applied coating. The epithelial layer consists of stratified squamous epithelium, the lamina propria forms papillae, the striated muscles are slightly edematous, numerous glands are located in the submucosa of the mucous part of the lip, their excretory ducts are lined with stratified squamous epithelium and are connected to the mucosal surface. There are no phenomena of degradation, necrosis and inflammation, complete repair of the ulcer. (fig. 13).

On the 21st day after exposure to the surface of the aphthous ulcer of the "HEPROCEL" powder, only unchanged tissue was determined. Complete repair of the ulcer (Fig. 14).

Analyzes and discussions. Thus, the model of the formation of an aphthous ulcer of the oral mucosa in rats according to our modified method allows the formation of a chronic ulcer of the mucous membrane in the area of the lower lip and gums. Already 20 minutes after exposure, you can trace visual changes in the mucous membrane in the form of darkening of tissue color and edema. After 3 hours, the affected area has a dark color and clearly differs from the surrounding healthy tissue. After 12 hours, an ulcerative necrotic crater forms on the surface of the mucosa with necrotic tissues and detritus along the periphery of the ulcer. After 1 day, the formed mucosal defect has undermined edges with necrotic plaque and tissue edema. Starting from 3 days after exposure, the ulcer looks like a chronic one, covered with fibrin, the edges are edematous, distinct. There are no signs of granulation. In the control group of animals, the healing process is delayed up to 3 weeks with a gradual rejection of necrotic masses, the formation of a fibrin film and epithelialization starting from the edges of the ulcer. Complete healing of the ulcer can be traced to 30 days after exposure. In the experimental group of animals, the research results showed that the gel on the surface of the ulcer visually lasts for 2-3 hours, then covers the ulcer in the form of a transparent, barely perceptible thin film tightly adhered to the surface of the ulcer, a faster relief of the inflammatory process with epithelization of the surface is noted ulcers within 2 weeks of exposure.

Conclusions. Comparative studies of various forms of the domestic drug "HEPROCEL" with the formation of a gel coating on the surface of a chronic aphthous ulcer of the oral mucosa in rats showed that, depending on the phase of the wound process, the fixation resistance and the strength of the gel structure change, with the best performance achieved when using fine powder in phase of necrotic changes.

The study of the morphological features of the interaction of the proposed coating with the wound surface of the oral mucosa showed that the HEPROCEL powder has a good adhesive ability, adheres tightly to the wound surface, a decrease in the inflammatory process and the beginning of repair is noted already from the second day after application with complete restoration of signs of degradation of cell structures and regression their edema and necrosis by 14 days of the experiment and, accordingly, 7 days of treatment.

The developed method of local protection of aphthous lesions of the oral mucosa in conjunction with a systemic approach to the treatment of stomatitis made it possible to shorten the regeneration time of the erosive process and the period of complete regression of inflammatory changes.

References.

1. Akbarov A.N., Ziyadullaeva N.S., Irismetova B.D. Chronic recurrent aphthous stomatitis: modern approaches to treatment (literature review). RE-HEALTH JOURNAL. 2021; 2(10): P. 196-203.
2. Akbarov A.N., Ziyadullaeva N.S., Irismetova B.D. Chronic recurrent afthous stomatitis. Modern treatment approaches (review article). EUROPEAN SCIENCE 2021. No 4 (60): P: 35-41
3. Marco A Peres and Al. Oral diseases: a global public health challenge. Lancet. 2019 [https://doi.org/10.1016/S0140-6736\(19\)31146-8](https://doi.org/10.1016/S0140-6736(19)31146-8)
4. Queiroz SIML, Silva MVAD, Medeiros AMC, Oliveira PT, Gurgel BCV, Silveira ÉJDD. Recurrent aphthous ulceration: an epidemiological study of etiological factors, treatment and differential diagnosis. An Bras Dermatol. 2018;93(3):341-346. doi:10.1590/abd1806-4841.20186228.
5. Fitzpatrick SG, Cohen DM, Clark AN. Ulcerated Lesions of the Oral Mucosa: Clinical and Histologic Review. Head Neck Pathol. 2019;13(1):91-102.

6. Shah K, Guarderas J, Krishnaswamy G. Aphthous stomatitis. *Ann Allergy Asthma Immunol.* 2016;117:341-343.
7. Hamedi S, Sadeghpour O, Shamsardekani MR, Amin G, Hajighasemali D, Feyzabadi Z. The Most Common Herbs to Cure the Most Common Oral Disease: Stomatitis Recurrent Aphthous Ulcer (RAU). *Iran Red Crescent Med J.* 2016;18:e21694.
8. Edgar NR, Saleh D, Miller RA. Recurrent aphthous stomatitis. *J Clin Aesthet Dermatol.* 2017;10(3):26-36
9. Esmâ Kürklü Gürleyen, Kadriye Peker, Gülsüm Ak. The evaluation of oral health related quality of life in patients with recurrent aphthous stomatitis. *Yeditepe Dental Journal.* 2018; 14(3):91-96.
10. Cardoso JA, et. al. Salivary Alpha-Amylase Enzyme. Psychological Disorders, and Life Quality in Patients with Recurrent Aphthous Stomatitis. *Int J Dent* 2017; 2017:5269856.