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RESEARCH ARTICLE
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Study of the Effectiveness of Drug No. 1 on the Model of Alkaline Eye Burn in Rabbits

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ABSTRACT

Studies have shown that 0.05 mL/kg of drug No. 1 as 1:15 dilution with sterile saline solution has anti-inflammatory, wound-healing, and angioprotective effects if instilled in both eyes of rabbits twice a day for 30 days after an alkaline burn. A stimulation of reparative processes in the cornea was observed with the test dose of drug No. 1. This was manifested by accelerating the recovery of defects in the anterior epithelium and stroma, reducing the frequency of formation of deep defects and the severity of inflammatory reaction and vascularization, and inhibiting the formation of turbidity of its lower intensity and area. A tendency to restore laminarity of the stroma was determined by the action of drug No. 1 throughout the observation period. This contributed to a decrease in the degree of vascularization and prevented ulceration and perforation of the cornea. By the end of experiment, a restoration of strong epithelial–stromal relationships in the experimental group, compared to the control group, was observed due to formation of normal architectonics of fibrous components of intercellular substance. A more pronounced proliferative activity, with an increase in the layering of limbal epithelial cells, was noted in the limbal zone of the cornea in the experimental group rabbits compared to the control group.

Keywords: *alkaline eye burn; anti-inflammatory effect; medicine No. 1; Pletnev drops; wound-healing effect*

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INTRODUCTION

Chemical eye burn is damage to the tissues of the eye because of exposure to chemically active substances (acids, alkalis, some aggressive liquids, etc.).[1–3]

The damaging effect of chemical burns is due to the chemical composition of the burning agent, its concentration, and duration of exposure. Alkalis are particularly aggressive, as they have both hydrophilic and lipophilic properties, cause colliquation necrosis in tissues with the formation of unstable soluble alkaline albuminates, which allow them to quickly overcome cell membranes.[4,5]

Burns can damage the corneal and conjunctival epithelium, nerve endings, lacrimal glands, corneal stroma and endothelium, and the outflow routes of intraocular fluid, sclera, iris, lens, and ciliary body.[3,5]

The frequency of eye burns ranges from 6.1% to 38.4% of all eye injuries. At the same time, chemical burns prevail, which account for about 60–80% of all burns of the visual organ. In manufacturing areas, at least 65–75% of eye burns are industrial, and the remaining are domestic and criminal injuries.[6,3]

Therefore, it is relevant to study the anti-inflammatory, wound-healing, and angioprotective activity of the complex herbal preparation No. 1, for which the horsetail grass is used, on the model of alkaline burn of the cornea of the eye in rabbits.

LITERATURE REVIEW

Horsetail grass is a part of Russian and European Pharmacopoeias and is a raw material for the intra-apical manufacture and production of many medicines.[7]

The drug in the form of a tincture from the above plant has been used in clinical practice for a long time in Russia as well as European countries. Consideration and detailed analysis of the literature data on the chemical composition, biologically active substances, pharmacology, clinical use of the drug from this medicinal plant, and safety was of great interest, since it was a part of the original drug developed by us, and was patented as "Drug No. 1." by the Russian Federation for Inventions.

Tincture of horsetail grass exhibits antimicrobial[8–11] and antioxidant properties.[8,12–17]

The herbal tincture of the plant significantly (by 91.9%, $P < 0.001$) prevents platelet aggregation.[18]

Toxicological examination of horsetail tincture has demonstrated that it is a low-toxic drug as a single intraperitoneal and oral administration to Wistar rats. The 50% lethal dose (LD_{50}) value, calculated in terms of dry residue, was >5000 mg/kg.[19,20]

A single intramuscular injection of horsetail tincture to Wistar rats demonstrated LD_{50} as more than 5000 mg/kg in terms of dry residue.[20]

The study of chronic toxicity of 800, 2000, and 5000 mg/kg of horsetail herb tincture administered orally for 13 weeks to male and female rats of the F344 line showed no damaging effects on the main organs and body systems of experimental animals. Similarly, intramuscular administration of 5000 mg/kg of horsetail herb tincture for 8 weeks to above-mentioned animals showed no damaging effects on vital organs and systems of animals. Pathohistological examination of the internal organs of animals conducted after the end of chronic experiments revealed no pathological changes associated with the toxic effect of the drug.[20,21]

A clinical study conducted on 36 healthy male volunteers showed that horsetail tincture had a diuretic effect, without significantly affecting the urinary excretion of electrolytes, and was recognized as safe for oral administration. The preparation of horsetail grass does not cause toxic damage to the liver and kidneys.[6]

MATERIALS AND METHODS

The study of anti-inflammatory, wound-healing, and angioprotective activity of drug No. 1 was conducted on 30 chinchilla rabbits (60 eyes; males and females, body weight 2.0–2.5 kg). In

order to assess the effect of drug No. 1 on the course of burn eye disease, a model of chemical corneal burn was utilized.[22]

Disks with 7-mm diameter were cut out of sterile cotton fabric. Immediately before the experiment, the discs were placed in a bath with 10% NaOH solution for 5 min. Excess alkali was removed after uniform impregnation. After anesthesia, discs soaked in alkaline solution were placed strictly in the center of the cornea. After 40 sec, the discs were removed, and the eye surface was washed with 20-mL sterile saline solution (SSS) for 30 sec.[22]

After applying a burn of 10% NaOH to all corneas of rabbits in the experimental group, 0.05 mL/kg of drug No. 1 as 1:15 dilution with SSS was instilled, and the control group animals were given an isotonic solution of 0.9% NaCl.[22]

The condition of eyes of the animals was assessed on the 1st, 3rd, 7th, 14th, 22nd, 30th, and 60th day according to the degree of inflammatory reaction, area of epithelial and stroma defect, and intensity of corneal opacity and corneal neovascularization using focal and lateral illumination and biomicroscopy with fluorescein sampling and photoregistration.[22]

The severity of inflammatory reaction was assessed on a point scale (Table 1).[22]

TABLE 1. The Score Scale of the Severity of Inflammatory Reaction of the Eye.

Severity of clinical symptoms	Score in points
Absence of an inflammatory reaction	0
Minor mixed injection of the conjunctiva, scanty discharge, absence of edema of the cornea and surrounding corneal tissue	1
Mixed injection of the conjunctiva, mucopurulent discharge, edema of the cornea and surrounding corneal tissue	2
Pronounced mixed injection of the conjunctiva with chemosis, copious mucopurulent discharge, pronounced corneal edema, hypopion, iridocyclitis	3

TABLE 2. Score Scale of Corneal Lesions by Area and Depth.

Severity of clinical	symptoms	Score in points
Stroma defects by area	Up to ¼ area of the cornea	1
	¼–½ area of the cornea	2
	More than ½ area of the cornea	3
Stroma defects in depth	Absence of stroma defect	0
	Surface stroma defect	1
	Deep defect without descemetocele	2
	Descemetocele	3
	Perforation	4

TABLE 3. Corneal Neovascularization Score Scale after Burn.

Severity of clinical	symptoms	Score in points
Length of vessels from the limb	Absence of vessels	0
	Up to 2 mm	1
	Up to 4 mm	2
	Up to 6 mm	3

Defect in the stroma was determined by area and depth (Table 2).[22]

The degree of corneal neovascularization was assessed by the length of the vessels from the limb to the center of the cornea (Table 3).[22]

The development of burns and the course of reparative processes of animal eyes after alkaline burn was assessed by the following: the number of layers of anterior epithelium and the degree of its differentiation; architectonics of fibrous components of the stroma and its cellular composition; thickness of the cornea; germination of blood vessels and their diameter, and the degree of sparseness of posterior epithelium of the cornea.[22]

RESULTS AND DISCUSSION

The studies were conducted on two groups of chinchilla rabbits (30 animals [60 eyes], males and females, body weight 2.0–2.5 kg). Each group comprised 15 animals (30 eyes): Group 1—control group; and Group 2—drug No. 1 group.[22]

In group 2 animals, 0.05 mL/kg of drug No. 1 as 1:15 dilution with SSS was instilled in both eyes of animals twice a day for 30 days after the application of burn. In group 1, the control group, SSS was instilled in both eyes of control animals twice a day for 30 days.[22]

3rd day of experiment

Desquamation of anterior epithelium of central optical region of the cornea occurred as a result of an ongoing local inflammatory reaction, which was expressed by an increase in the number of lymphocytes and leukocytes, and stroma fibrillation and its swelling (Figures 1A and B).[22]

In paracentral region of the cornea of both groups, sections of epithelial cells migrated from adjacent areas with an average distance of 16.6 microns (μm) between the centers of their nuclei were determined. The surface of the cornea in the paracentral region in animals of the control group was characterized by significant irregularities formed as a result of edema, violation of the stratification of the stroma (Figures 2A and B).[22]

In group 2, the experimental group, there was a slight surface undulation of the stroma and preservation of layering in the underlying layers. In the stroma of paracentral region of the cornea, a significant increase in the number of lymphocytes and polymorphonuclear cells was detected that migrated from the limbal vessels of the cornea (Figures 3A and B). This indicator in the control group was twice higher than their number in the experimental group.[22]

14th day of experiment

The results obtained are presented in Table 4. In the control group, inflammatory reaction in 42.7% of cases corresponded to 3 points and in 57.3% of cases, it scored 2 points. In the control group, a rough scar with pronounced vascularization formed after perforation was observed in 13.3% of cases. By this time, an additional 18.8% of new cases of perforation (defect depth of 4 points) were detected from 42.7% of eyes with descemetocele (Figure 4A).

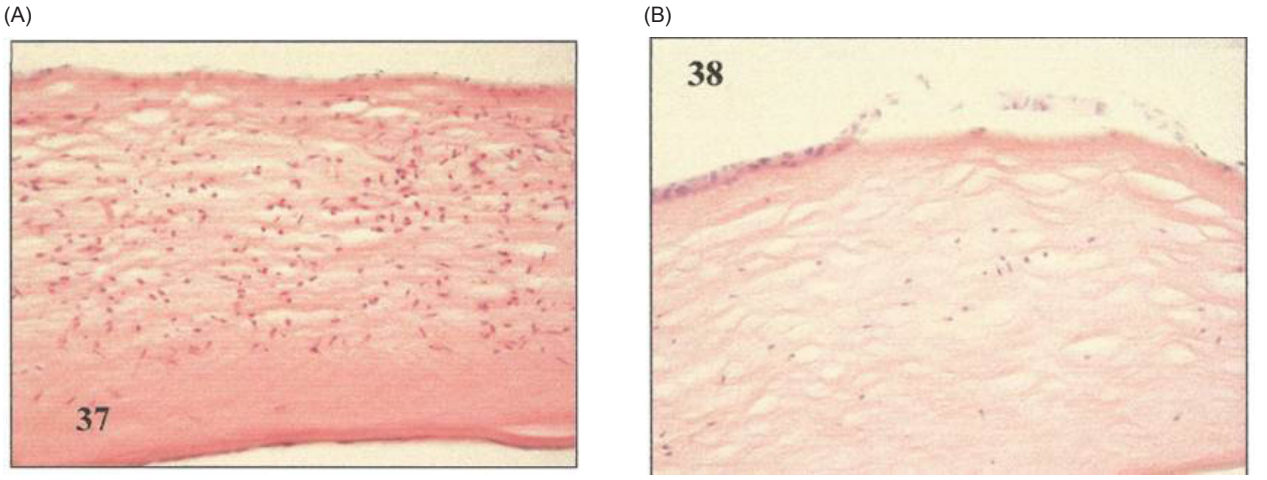

FIGURE 1. (A) Control. 3rd day after the burn. Central optical region of the cornea. A significant increase in the number of eosinophilic leukocytes: 140-fold increase. (B) 3rd day of treatment with drug No. 1. Central optical region of the cornea. Absence of leukocyte infiltration. Restoration of anterior epithelium and its partial desquamation: 140-fold increase.

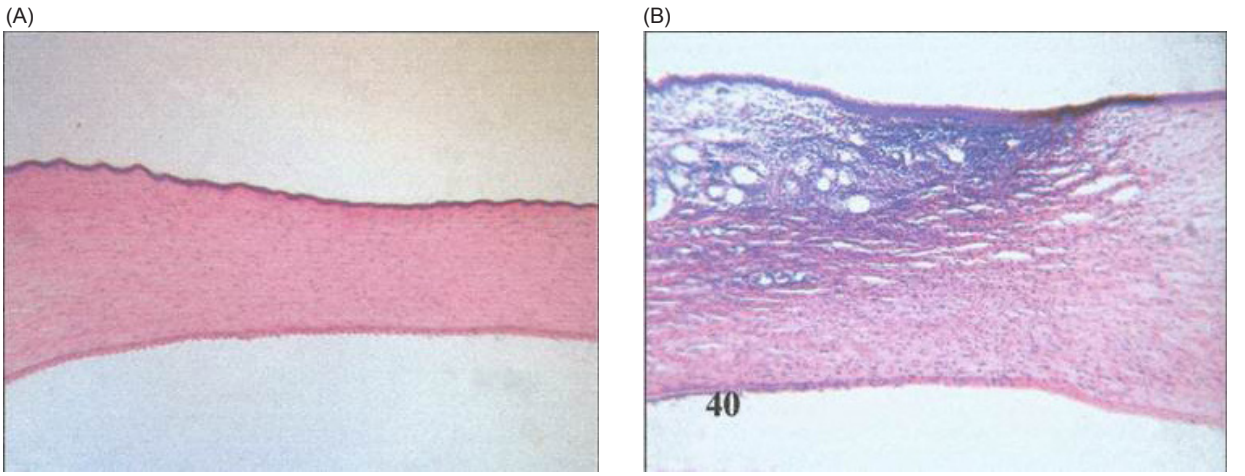

FIGURE 2. (A) 3rd day of treatment with drug No. 1. Paracentral region of the cornea. Slight undulation of corneal surface while maintaining the stratification of the stroma: 140-fold increase. (B) Control. 3rd day after the burn. Limbal region of the cornea. Germination of blood vessels in paracentral region: 140-fold increase.

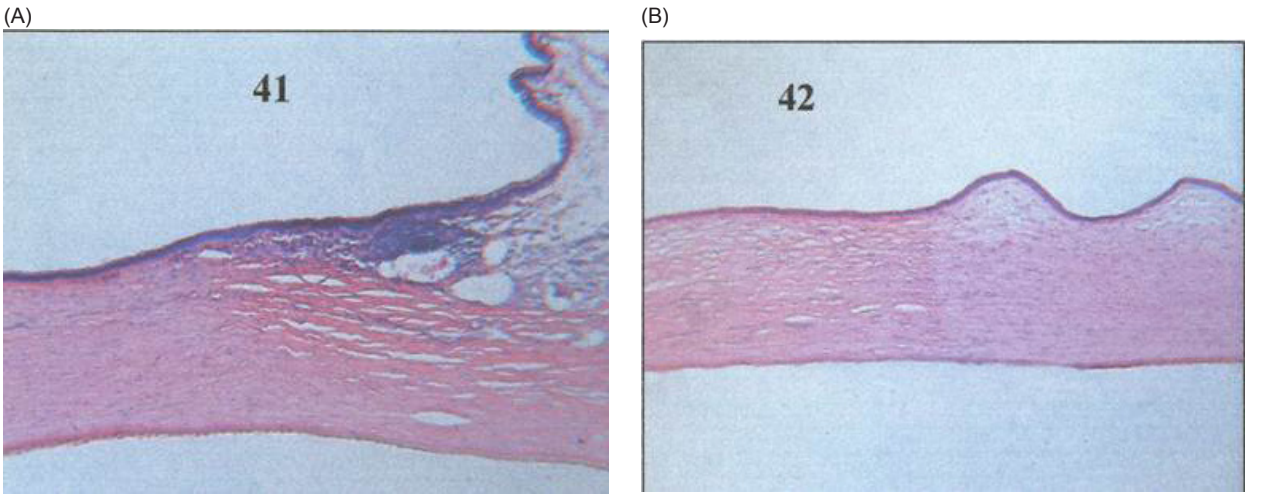

FIGURE 3. 3rd day of treatment with drug No. 1. (A) Limbal region of the cornea. Increase in the number of fibroblasts in the stroma tissue adjacent to the limbal region: 140-fold increase. (B) Paracentral region of the cornea. Wavy corneal surface, significant splitting of stroma plates, and edema: 140-fold increase.

The remaining 54% of eyes retained a deep corneal stroma defect of 2 points. In 27.2% of cases with a stroma defect depth of 3 points, the defect area occupied ½ of the corneal surface. The diameter of epithelial defect averaged 6 mm. Vascularization in most cases corresponded to 3 points.

In the experimental group, on 14th day (Figure 4B), 58.7% of the eyes had an inflammatory reaction of 2 points, and 41.3% of the eyes had a reaction of 1 point. The diameter of epithelial defect averaged 3 mm (1 point). In 69.3% of the experimental eyes, the defect depth corresponded to 1

TABLE 4. Clinical Assessment of the Condition of the Cornea on 14th Day of Experiment.

Evaluation criteria	**Animal groups**	
	1st group: control	**2nd group: drug No. 1**
Inflammatory reaction	3 points: 42.7%	2 points: 58.7%
	2 points: 57.3%	1 point: 41.3%
Area of stromal defect	3 points	1 point
Depth of stromal defect	4 points: 18.8%	3 points: 10.7%
	3 points: 27.2%	2 points: 20.0%
	2 points: 54,0%	1 point: 69.3%
Degree of corneal vascularization	3 points: 86.7%	2 points: 69.3%
	2 points: 13.3%	1 point: 30.7%

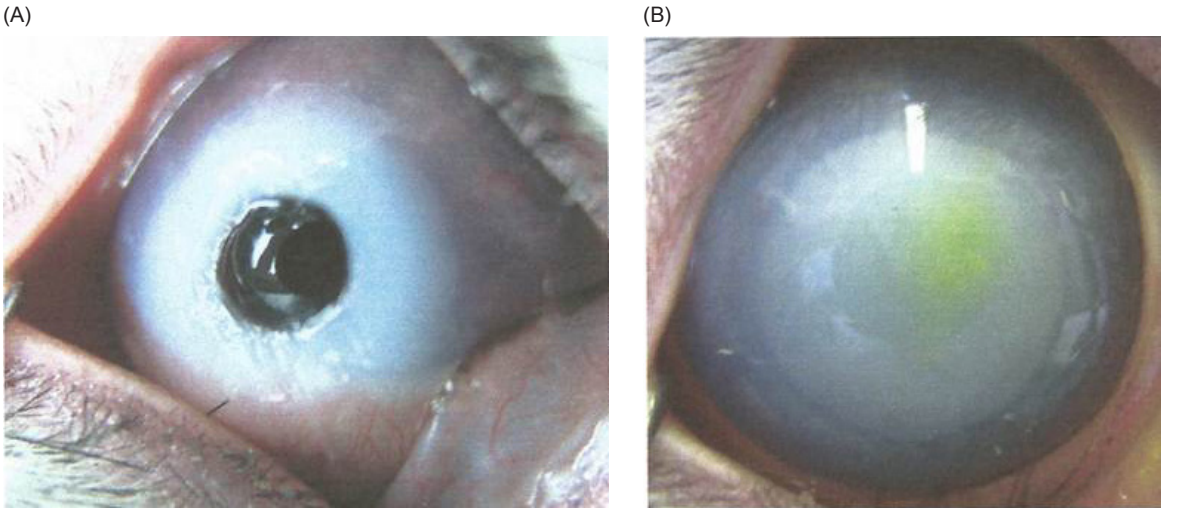

FIGURE 4. 14th day after the application of alkaline burn. (A) Control group. (B) Experimental group with drug No. 1.

point, and the defect area did not exceed ¼ of the corneal surface. A deep stroma defect of 3 points was observed in 10.7% of cases and that of 2 points in 20.0% of cases. Descemetocele was preserved in 10.7% of cases. Vascularization corresponded to 2 points in 69.3% of cases, and that of 1 point in in 30.7% of cases.

Morphological examination of the eyes on 14th day of experiment in the control group showed further destruction of corneal tissues with significant thinning of central optical zone, up to perforation, which was not observed in group 2 of drug No. 1.

In such cases, of all the layers, only the posterior boundary membrane remained, which was edematous and had leukocytes adhered on its surface. Areas with complete perforated layers of the cornea were also observed.

The corneal stroma in the control group acquired friability of fibrous components. This contributed to the germination of vessels from the limbal region to paracentral region, mainly in the anterior third of the cornea.

In the experimental group at this time, in most cases, epithelialization of the cornea into two to three

J Popul Ther Clin Pharmacol Vol 29(1):e26–e39; 1 March 2022.

layers of epithelial cells took place. There was some swelling of the stroma of the central optical region of the cornea with impaired plasticity. In paracentral region of the cornea, edema was reduced noticeably compared to the previous terms of experiment.

In the experimental group, a significant increase in the number of fibroblasts in the central optical region per unit area was marked in comparison with 7 days of observation. In the experimental group of animals, this indicator was significantly higher by the 7th and 14th day of experiment than in the control group, and was more pronounced by the 22nd day, which indicated an increase in proliferative activity.

Thus, on the 14th day of administration of 0.05 mL/kg of drug No. 1 as 1:15 dilution with SSS to rabbits of the experimental group in the form of eye drops, a less pronounced inflammatory reaction was observed compared to the control. In the majority of experimental group animals (about 70%), surface defects of the stroma were determined, the area of which did not exceed 1 point.

22nd day of experiment

The results obtained are presented in Table 5. In the control group, corneal opacity spread beyond the burn injury zone by 2–3 mm. The intensity of the turbidity corresponded to 10 points on the Voino–Yasenetsky scale. Hypertrophied vascularized eyesore was formed in animals with perforation at the site of a deep defect.

Descemetocele with an area of more than ¼ (25%) surface of the cornea (3 points) was preserved in 14.7% of the eyes. A deep stroma defect of 2 points was noted in 41.3% of cases, and a superficial stroma defect was observed in 20% of cases. Vascularization of 3 points was observed in 78.6% of control cases, and in the remaining 21.4% cases, vascularization of 2 points was noted (Figure 5A).

On 22nd day, corneal opacity was within the burn damage in 53.3% of rabbits in the experimental group; in the remaining 46.7% of animals, it spread beyond its limits by no more than 1–2 mm (Figure 5B). The intensity of turbidity corresponded to 7 points on the Voino–Yasenetsky scale. An epithelial corneal defect with an average diameter of 2.5 mm was observed in 58.7% of cases; 33.3% of cases had a superficial corneal stroma defect of 1 point; and a deep corneal defect without descemetocele (2 points) persisted only in 8% of cases. By this time of observation, in most cases (78.7%), the intensity of vascularization corresponded to 2 points; it reached 3 points in 21.3% of cases.

During morphological examination of the eyes on 22nd day of experiment, most animals of the control group had a gradual restoration of thickness of

TABLE 5. Clinical Assessment of the Condition of the Cornea on 22nd Day of Experiment.

Evaluation criteria	**Animal groups**	
	1st group: control	**2nd group: drug No. 1**
Inflammatory reaction	3 points: 16.4%	1 point: 78.3%
	2 points: 83.6%	0 points: 21.7%
Area of stromal defect	2 points	1 point
Depth of stromal defect	3 points: 14.7%	-
	2 points: 41.3%	2 points: 8.0%
	1 point: 20.0%	1 point: 33.3%
	0 points: 24.0%	0 points: 58.7%
Degree of corneal vascularization	3 points: 78.6%	3 points: 21.3%
	2 points: 21.4%	2 points: 78.7%

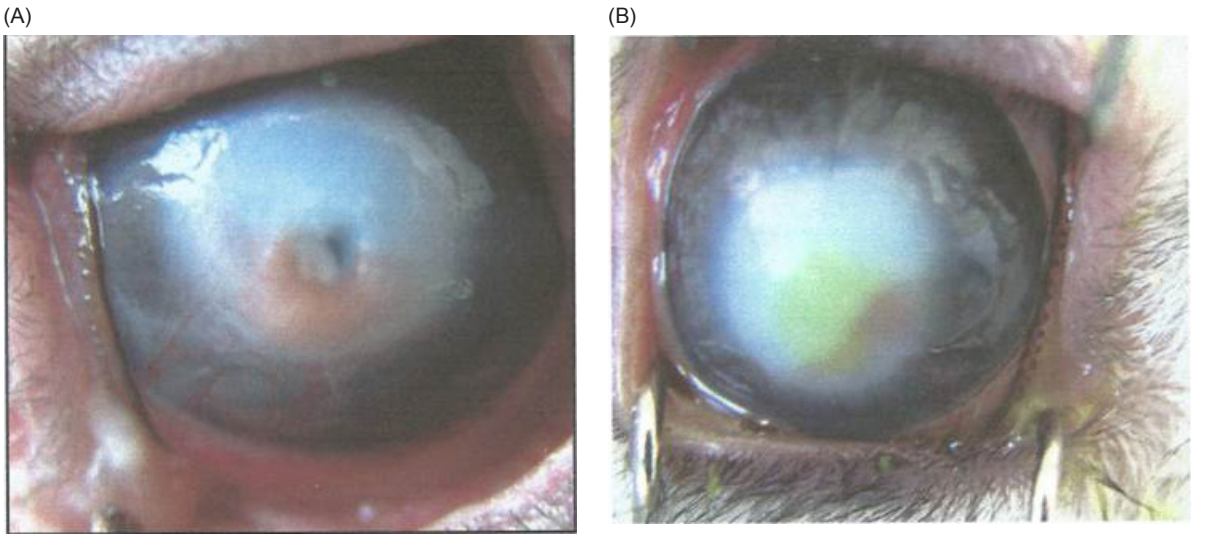

FIGURE 5. 22nd day after application of alkaline burn. (A) Control group. (B) Experimental group with drug No. 1.

the cornea, migration of fibroblasts, and subsequent synthesis of intercellular substance of the stroma. In the anterior third of the stroma, the germination of a dense vascular network and mass leukocyte migration were observed.

In the experimental group, there was a tendency to increase the thickness of the cornea of the eye.

In the stroma of experimental group of animals, there was also a significant increase in vascular infiltrate cells in the anterior region of the cornea and the absence of cellular elements in the deepest layers with the preservation of stromal plates. The anterior epithelium formed 3–4 layers of undifferentiated epithelial cells without a tendency to desquamation.

Thus, on 22nd day of using 0.05 mL/kg of drug No. 1 as 1:15 dilution with SSS, in the form of eye drops in the experimental group, 58.7% of cases had closure of a corneal stromal defect; however, in the control group, deep corneal defects of 2–3 points remained in 56% of cases. In the experimental group, the intensity of neovascularization was four times lower than in the control group.

30th day of experiment

The results obtained are presented in Table 6. Corneal opacity was observed corresponding to 10 points on the Voino–Yasenetsky scale in 49.3% of the control group rabbits; it exceeded the burn damage zone by 1–2 mm on average (Figure 6A). Persistent erosion was observed in 20% of cases, and a superficial stroma defect of 1 point was observed in 49.3% of cases.

A complete closure of epithelial defect by 30th day was discovered in 89.3% of rabbits treated with drug No. 1 (Figure 6B). At the same time, opacity of the cornea did not exceed the damage zone, and the intensity of opacity corresponded to 7 points on the Voino–Yasenetsky scale. The remaining 10.7% of animals retained a superficial epithelial defect with a diameter not exceeding 2.3 mm on average. The degree of corneal vascularization corresponded to 0 point.

During morphological examination on 30th day of experiment, ulceration and initial formation of fibrous tissue were observed in the central optical region of the cornea of control group. In most cases, the anterior epithelium consisted of two rows of epithelial cells without signs of differentiation.

TABLE 6. Clinical Assessment of the Condition of the Cornea on 30th Day of Experiment.

Evaluation criteria	Animal groups	
	1st group: control	2nd group: drug No. 1
Inflammatory reaction	1 point	0 points
Area of stromal defect	49.3%	89.3%
Depth of stromal defect	20.0%	10.7%
Degree of corneal vascularization	1 point: 30.7%	0 points: 100.0%
	0 points: 69.3%	

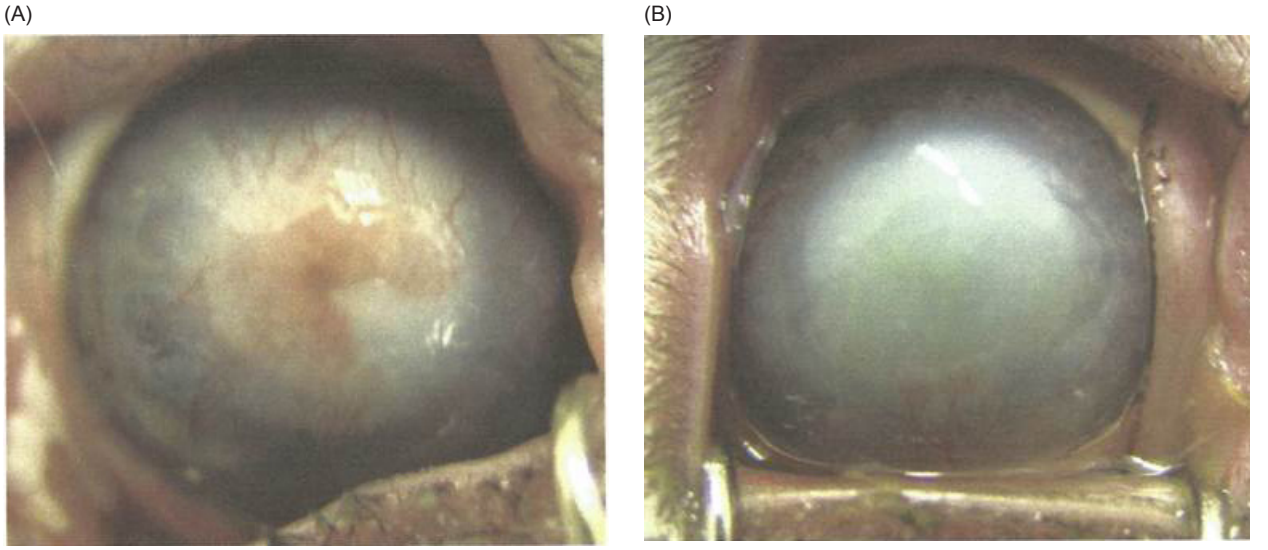

FIGURE 6. 30th day of application of alkaline burn. (A) Control group. (B) Experiment group with drug No. 1.

Gross vascularization with cellular infiltration persisted in all areas of the cornea.

In a number of control group rabbits, endothelium of the central optical region began to recover and had an average distance of 25 μm between cells.

In the experimental group rabbits, anterior epithelium was restored to four to five layers but had no pronounced differentiation into a basal, spiny layer and a layer of flat cells.

The corneal stroma continued to retain some swelling in the central optical region; however, at this moment there was a restoration of stromal plates, suppression of vascularization, and a significant decrease in vascular infiltrate cells, which indicated decrease in inflammatory response.

Thus, on 30th day of using 0.05 mL/kg of drug No. 1 as 1:15 dilution with SSS, in the form of eye drops in the experimental group, epithelial defect closure was observed in 89.3% of cases; however, in the control group, stroma defects remained in 49.3% cases.

The experiment also studied dynamics of changes in the thickness of the cornea in its central and paracentral regions under the influence of drug No. 1.

It is known that normally the thickness of the cornea in rabbits in the central optical zone is not less than 300 μm.

After 1 day of the start of experiment, the thickness of the cornea in the central optical region

of rabbits in both groups corresponded to the norm (Table 7).

On 3rd day of experiment, an approximately equal decrease in the thickness of the cornea was observed in both groups.

On 7th day of experiment, a significant difference in the thickness of the cornea was revealed in the control and experimental groups, with pronounced thinning of the cornea in the control group because of destruction of its tissues after a burn ($P < 0.05$). However, the tendency to ulceration was compensated by edema in some cases.

After 14 days of burn injury, significant ulceration of the cornea, on average 8.3 ± 1.96 μm, was observed in the control group, and there were also cases with complete absence of all layers of the cornea in the area of damage (Tables 7 and 8). In the experimental group, the thickness of the cornea corresponded to the norm.

A gradual increase in the thickness of the cornea in the control group with the preservation of inflammatory edema of stromal tissue and loosening of its plates was observed by 22nd day of experiment. In the experimental group, a significant increase in corneal thickness was observed because of an increase in the number of fibroblasts and their pronounced proliferative activity (Table 7).

From 30th to 60th day of experiment, inversely proportional dynamics of changes in corneal thickness was observed in both groups. In the control group, there was a significant increase in thickness by the 60th day compared to the 22nd day of experiment. In the experimental group, a decrease in the thickness of the cornea to normal values was observed compared to the 22nd day.

The study of dynamics of changes in the number of fibroblasts in central and paracentral regions of the cornea under the influence of drug No. 1 demonstrated that the pattern of changes in the number of fibroblastic cells in the control and experimental groups had similar dynamics. This can be explained by the general pattern of reparative processes, but the intensity of this process in the experimental group exceeded by more than two times than in the control group ($P < 0.05$) (Table 9).

A significant increase in the number of fibroblasts was recorded because of their mitotic activity attaining a maximum peak by the 22nd day of experiment in the central optical region of the cornea in experimental group.

TABLE 7. Corneal Thickness in the Central Optical Region (M ± m).

Animal groups	1st day	3rd day	7th day	14th day	22nd day	30th day	60th day
Control group	311.8 ± 5.10	266.6 ± 1.41	133.3 ± 9.47	8.3 ± 1.96	250.0 ± 0.06	275.0 ± 2.10	500.0 ± 20.47
Experimental group	308.3 ± 5.93	275.2 ± 8.65	350.0 ± 2.53*	341.6 ± 3.21*	616.6 ± 19.24*	425.0 ± 3.60*	350.0 ± 2.53*

**Statistically significant differences with control at P < 0.05. M ± m, arithmetic mean ± error of the arithmetic mean.*

TABLE 8. Corneal Thickness in Paracentral Region (M ± m).

Animal groups	1st day	3rd day	7th day	14th day	22nd day	30th day	60th day
Control group	666.6 ± 25.95	558.3 ± 17.11	391.6 ± 3.50	8.3 ± 27.80	291.6 ± 4.67	325.0 ± 1.94	200.0 ± 12.15
Experimental group	412.5 ± 1.36*	625.0 ± 18.71*	600.0 ± 16.67*	375.0 ± 1.70*	300.0 ± 7.82	225.0 ± 13.95	233.3 ± 13.27

**Statistically significant differences with control at P < 0.05. M ± m, arithmetic mean ± error of the arithmetic mean.*

TABLE 9. Number of Fibroblasts per Unit Area in the Central Optical Region of the Cornea (M ± m).

Animal groups	1st day	3rd day	7th day	14th day	22nd day	30th day	60th day
Control group	0 ± 1.26	0 ± 1.26	8 ± 0.61	28 ± 1.03	32 ± 1.35	28 ± 1.03	12 ± 0.28
Experimental group	0 ± 2.43	0 ± 2.43	16 ± 1.12*	36 ± 0.51*	64 ± 2.80*	56 ± 2.15*	36 ± 0.51*

**Statistically significant differences with control at P < 0.05. M ± m, arithmetic mean ± error of the arithmetic mean.*

TABLE 10. Number of Fibroblasts per Unit Area in the Paracentral Region of the Cornea (M ± m).

Animal groups	1st day	3rd day	7th day	14th day	22nd day	30th day	60th day
Control group	20 ± 0.98	32 ± 0	24 ± 0.65	36 ± 0.33	44 ± 0.98	44 ± 0,98	24 ± 0.65
Experimental group	48 ± 0.14*	24 ± 1.82*	48 ± 0.14*	36 ± 0.84	84 ± 3.08*	52 ± 0.47*	32 ± 0.17*

**Statistically significant differences with control at P < 0.05. M ± m, arithmetic mean ± error of the arithmetic mean.*

By the 60th day, the number of fibroblasts per unit area corresponded to the norm. The parallel increase in the number of fibroblasts and the thickness of the cornea at the same time of experiment, apparently, is explained by the effect of drug No. 1 on corneal tissue.

In the control group, a gradual increase in the number of fibroblasts was also observed in the central optical region of the cornea by the 14th day. This period accounted for the maximum thinning of the cornea in the control group. The maximum increase in the number of fibroblasts per unit area occurred on the 22nd day of experiment; however, this parameter was twice less than that of the experimental group ($P < 0.05$). By the end of experiment, the number of fibroblasts in the central optical region of the cornea in the control group was ⅓ of their number in the experimental group ($P < 0.05$).

In the experimental group, a significant increase in the number of fibroblasts was noted because of increased mitotic activity ($P < 0.05$) in paracentral region on the 1st day of experiment against the backdrop of using drug No. 1 (Table 10).

By the 3rd day of experiment in the experimental group, a noticeable decrease in the number of fibroblasts was noted due to the migration and redistribution of fibroblasts to central corneal zone. The next wave of increase in their number was observed by the 7th day ($P < 0.05$). By the 14th day of experiment, the average number of fibroblasts per unit area corresponded to their number in the control group. On the 22nd day of experiment, the maximum proliferative activity of fibroblasts occurred with the restoration of normal numbers by the 60th day.

In the control group, a slight change in the number of fibroblasts was observed in paracentral region up to 14 days, with a slight increase in their number by the 22nd and 30th day and a gradual normalization by the 60th day of experiment.

While assessing the effect of drug No. 1 on inflammatory reaction in the cornea of rabbits after a chemical burn, the number and caliber of blood vessels of corneal stroma, the density of fibrous components as well as the number of leukocytes, lymphocytes, and macrophages were taken into account.

Normally, these cells do not penetrate into the cornea but appear in its tissues with any damage. There are various ways of migration of vascular infiltrate: directly from conjunctival vessels into edematous stroma of the cornea, and from the moisture of the anterior chamber of the eye. Neutrophils, together with macrophages, affect the rate of wound healing and participate in the resorption of cellular detritus. Leukocytes, selectively adhering to the cells of the leading edge, on the one hand perform a protective function, and on the other prevent the migration of epithelium along the stroma.

TABLE 11. Number of Vascular Infiltrate Cells in the Central Optical Region of the Cornea (M ± m).

Animal groups	1st day	3rd day	7th day	14th day	22nd day	30th day	60th day
Control group	0 ± 2.81	26 ± 0.69	16 ± 1.50	150 ± 9.44	30 ± 0.36	16 ± 1.50	3 ± 2.57
Experimental group	0 ± 0.44	13 ± 0.62*	15 ± 0.78	6 ± 0.05*	2 ± 0.28*	2 ± 0.28*	0 ± 0.44

**Statistically significant differences with control at P < 0.05. M ± m, arithmetic mean ± error of the arithmetic mean.*

TABLE 12. Number of Vascular Infiltrate Cells in the Paracentral Optical Region of the Cornea (M ± m).

Animal groups	1st day	3rd day	7th day	14th day	22nd day	30th day	60th day
Control group	7 ± 0.64	33 ± 1.48	12 ± 0.23	40 ± 2.05	8 ± 0.56	2 ± 1.05	2 ± 1.05
Experimental group	0 ± 0.08*	8 ± 0.33*	10 ± 0.49	4 ± 0.01*	1 ± 0.24*	2 ± 0.16	0 ± 0.33

**Statistically significant differences with control at P < 0.05. M ± m, arithmetic mean ± error of the arithmetic mean.*

While studying the dynamics of changes in the number of vascular infiltrate cells in the cornea of rabbits after a severe alkaline burn, it was possible to note a tendency of active inhibition of leukocyte migration in the experimental group, which was not observed in the control group (Table 11).

In the central optical region, vascular infiltrate cells were detected on day 3 of experiment, and in the control group, their number was twice as large as in the experimental group. By day 14, massive leukocyte infiltration was observed in the control group, accompanied by pronounced lytic processes in the stroma, leading to thinning of the cornea up to its complete perforation. At the same time, in the experimental group, there was a slight increase in the number of inflammatory cells on day 7, with a gradual decrease in their numbers by day 22 of experiment with the use of drug No. 1.

In paracentral region of the eye, cells appeared from dilated conjunctival vessels on the 1st day of experiment in the control group (Table 12), but this happened only on the 3rd day in the experimental group. In the control group, two peaks of pronounced leukocyte infiltration were detected—on day 3 and day 14 of observation.

A significant difference in the number of vascular infiltrate cells in the control and experimental groups ($P < 0.05$) indicated the absence of a pronounced active inflammatory process in experimental animals, which is explained by the positive effect of drug No. 1.

During experiment, the corneal stroma in the control group was characterized by the friability of fibrous components. This contributed to the active germination of vessels from limbal to paracentral region, mainly in the anterior third of the cornea.

Pronounced gross vascularization was observed in all parts of the cornea in control group rabbits. In the animals of experimental group, in comparison to the control group, a tendency to restore laminarity of the stroma was marked throughout the entire observation period. This contributed to a decrease in the degree of vascularization and prevented ulceration and perforation of the cornea.

CONCLUSION

Studies have shown that 0.05 mL/kg of drug No. 1, in 1:15 dilution with SSS, has anti-inflammatory, wound-healing, and angioprotective effects if instilled in both eyes of rabbits twice a day for 30 days after an alkaline burn. A stimulation of reparative processes in the cornea was observed under the influence of test dose of drug No. 1. This was manifested by the following: accelerating the recovery of defects in the anterior epithelium and stroma,

reducing the frequency of formation of deep defects, reducing the severity of inflammatory reaction and vascularization, and inhibiting the formation of turbidity of its lower intensity and area. There was a tendency to restore the stroma layers under the action of drug No. 1 throughout the observation period. This contributed to a decrease in the degree of vascularization and prevented ulceration and perforation of the cornea. By the end of experiment, a restoration of strong epithelial–stromal relationships was observed in the experimental group, compared with the control group, because of the formation of normal architectonics of fibrous components of intercellular substance. A more pronounced proliferative activity, with an increase in the layering of limbal epithelial cells, was observed in the limbal zone of the cornea in rabbits of experimental group compared with the control group.

COMPETING INTERESTS

The author claims that he has no competing interests to declare.

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