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RESEARCH ARTICLE
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Study of the Effectiveness of Drug No. 5 in a Model of Dry Eye Syndrome in Rabbits

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ABSTRACT

It was shown that in the model of dry eye syndrome (DES) in rabbits, if drug No. 5 was instilled in both eyes of animals at a dose of 0.05 mL/kg in 1:15 dilution with sterile saline solution twice a day for 30 days, then it had a strong anti-inflammatory, wound-healing, and angioprotective effects. This positively affected the course of reparative process in the conjunctiva and cornea complicated by nonclosing of the eyelids. It was also found that drug No. 5 in the tested dose promoted the stimulation of reparative processes in the conjunctiva and cornea, clinically manifesting itself in accelerating the recovery of defects in the anterior epithelium and corneal stroma, and in reducing both frequency of formation of deep corneal defects and severity of inflammatory response and vascularization. There was a slowdown in the formation of corneal opacities, a decrease in the amount and appearance of a more liquid mucous discharge of the conjunctiva compared to the control. It was also demonstrated in the stated model of DES that drug No. 5 in the test dose had a pronounced pharmacological effect, contributing to a faster recovery of damage to the superficial epithelium and stroma of the cornea, anterior and posterior chambers of the eye, the vascular membrane and retina as well as goblet cells of the conjunctiva.

Keywords: *dry eye syndrome; medicine No. 5; Pletnev drops No. 5; pronounced anti-inflammatory effect; pronounced wound-healing effect.*

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INTRODUCTION

Dry eye syndrome (DES) is one of the most common chronic eye diseases. According to literature, the prevalence of this pathology among the adult population varies from 18% to 67%.[1,2,3,4]

In people aged more than 50 years, the incidence of DES at an ophthalmologist's appointment can reach up to 67%.[5] DES is observed in most patients who wear contact lenses. Often, owing to the development of DES, patients are advised not to use contact lenses.[6] Currently, DES has been also established in children and adolescents. Cases of the development of DES in these age categories are mainly due to the prolonged use of various gadgets, in particular smartphones, iPhones, and computers.[7]

For this reason, it is relevant to study the anti-inflammatory, wound-healing, and angioprotective effects of complex herbal preparation No. 5, for which leaves of hanging birch are used, in the model of DES in rabbits.

LITERATURE REVIEW

Tincture of hanging birch leaves has an immunomodulatory,[8,9] antimicrobial,[9,10] antiviral,[9,11,12] antioxidant,[14] and hepatoprotective effects,[9] and exhibits anti-inflammatory properties.[13,14]

Toxicological examination has established that the tincture of hanging birch leaves is low-toxic with a single oral administration to small (mice) and large laboratory animals (dogs). The 50% lethal dose (LD_{50}) value of the drug for mice is 2110 mg/kg in terms of dry matter, 2250 mg/kg for rats, and 2100 mg/kg for dogs.[15]

Study of toxicity of birch tincture in rats with 17-week oral administration with a dose of up to 100 mg/kg (by dry residue) demonstrated no damaging effect to the main organs and body systems of experimental animal. This was confirmed by the pathohistological study of the internal organs of animals conducted after the experiment.[16]

In the same manner, study of toxicity of birch tincture with a dose of up to 250 mg/kg with 10-week oral administration to dogs established no damaging effect on the body of experimental animal.[16]

Clinical studies of birch leaf tincture with a dose of 1080 mg per day for 4 weeks in 1066 patients with urinary tract infection demonstrated a pronounced effect, relieving symptoms of inflammation and restoring impaired functions.[17]

A randomized, double-blind, placebo-controlled pilot study confirmed that a dose of 1080 mg per day of birch leaf tincture for 20 days was highly effective in the treatment of 15 patients with urinary tract infection.[17]

MATERIALS AND METHODS

The study was conducted on 45 rabbits (90 eyes) of the chinchilla breed (males and females, body weight 2.0–2.5 kg).[18]

A model of DES was reproduced by stitching the edges of upper and lower eyelids to the skin of frontal and buccal regions, respectively, for persistent eyelid nonclosure.[18]

After 5 days, when there were pronounced signs of dryness of the anterior part of the eye, conjunctival chemosis, pronounced hyperemia, keratopathy, and filamentous keratitis (in some cases, the formation of serous degeneration of the cornea and conjunctiva, complete eyelid closure) treatment was initiated in three groups: experimental group with drug No. 5; comparison drug group with 20% ocular actovegin gel (AGel) (Nicomed, Austria); and control group with sterile saline solution (SSS).[18]

The condition of the eyes of animals was assessed on the 2nd, 5th, 10th, 15th, 20th, 25th, and 30th day of the treatment according to the degree of inflammatory and degenerative reaction, area of the defect of epithelium and stroma, intensity of corneal opacity, fluorescein staining of the cornea, condition of the eyelids and their dilution, and preservation of fixation sutures.[18]

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The severity of changes in the conjunctiva and cornea was assessed by the score system (Table 1).[18]

The width of the eye slit was measured (Table 2).[18]

The areas of corneal stroma defect and stroma defect were estimated by depth (Table 3).[18]

RESULTS AND DISCUSSION

The studies were conducted on the following three groups of chinchilla rabbits with 15 animals (30 eyes) in each group: Group 1—control, SSS; Group 2—comparison drug 20% AGel; and Group 3—drug No. 5. A dose of 0.05 mL/kg of drug No. 5 in a dilution of 1:15 SSS was instilled in both eyes of animals twice a day for 30 days. In group 2, 1 drop (400 mcg of the active substance) of AGel from the tube was put in both eyes of the animals twice a day for 30 days. In group 3, SSS was instilled in both eyes of control animals twice a day for 30 days.[18]

The clinical condition of all experimental animals in 3 groups on the 5th day of the experiment before the administration of drug No. 5 is presented in Table 4.

TABLE 1. Score Scale of the Conjunctiva and Its Separable.

Severity of clinical symptoms of the conjunctiva and its separable in the DES model	Score in points
Absence of inflammatory reaction	0
Scanty, gentle mucous discharge, weak conjunctival injection	1
Scanty, separated by separate dense purulent or dry lumps or crusts, mixed injection of the conjunctiva	2
Pronounced mixed injection of the conjunctiva with chemosis, copious mucopurulent discharge, or dense crust, may occupy the entire surface of one eyelid	3
Discharge is very strong, up to the complete closure of the ocular slit of the mucosa of different densities, chemosis, or initial necrosis	4

DES: dry eye syndrome.

TABLE 2. The Point Scale of the State of the Eyelids.

Severity of clinical symptoms according to the position of the eyelids in the DES model	Score in points
The eyelids are free and close easily, no seams and fixation	0
The seams are loosened or cut through the fabric, the eyelids partially closed	1
The seams are loosened, the eyelids do not close up to half	2
The seams are partially weakened, the mobility of the eyelids is limited, they do not close	3
The seams are preserved, the eyelids do not close and are motionless, even if there are no seams (deformation)	4

DES: dry eye syndrome.

TABLE 3. Score Scale of Corneal Condition and Its Fluorescein Staining.

Severity of clinical symptoms in the cornea of the DES model	Score in points
Lack of reaction.	0
Absence of edema of the corneal epithelium; the cornea is transparent, stained with fluorescein superficially, not fixed anywhere, easily movable	1
Fixed color of the cornea with fluorescein with foci up to ¼ of the area of the cornea, lumps of colored mucous discharge cover the surface of the cornea, corneal erosion is easily formed; the cornea is transparent, but there is a slight fleur	2
Corneal fluorescein staining up to ½ of the corneal area, corneal xerosis phenomena, corneal tissue structure fluoresces, its permeability changes, descemetocele without perforation, hypopion	3
Corneal fluorescein staining of more than ½ of the corneal area, corneal xerosis with foci up to 6–8 mm, corneal ulcer, corneal structure opalescent, matte, corneal perforation	4

DES: dry eye syndrome.

TABLE 4. Condition of the Objects of Eye Examination on the 5th day of Experiment before Starting Administration of Drug No. 5.

Evaluation criteria	Score in points	Animal groups (%)		
		1: Control	2: AGel	3: Drug No. 5
Condition of seams	4	93.8	92.1	92.3
	3	6.2	7.9	7.7
Condition of divorced eyelids	4	94.6	91.3	94.4
	3	5.4	8.7	5.6
Condition of eye discharge	3	15.2	–	47.9
	2	46.9	61.8	29.4
	1	19.6	23.7	14.5
	0	18.3	14.5	8.2
Conjunctival condition	3	34.2	43.7	24.9
	2	44.8	31.3	39.2
	1	19.2	17.8	29.7
	0	1.8	7.2	6.2
Staining corneal surface with fluorescein	3	–	35.3	35.0
	2	20.0	28.1	36.7
	1	20.4	36.6	27.3
	0	59.6	–	–
Condition of corneal stroma	3	41.5	43.8	39.8
	2	27.8	37.8	39.4
	1	23.4	5.7	18.9
	0	7.3	12.7	1.9

AGel: 20% ocular actovegin gel.

The revealed changes in the position of the eyelids, absence of their closure, state of the conjunctiva in the form of stagnant phenomena, an abundant amount of discharge of different consistencies, initial keratopathy and the formation of serous degeneration, appearance of a corneal abscess, and formation of an ulcerative focus indicated the possibility of using this experimental model of SSS to study the effect of drug No. 5 and AGel on the pathological process (Figure 1).[14]

The 5th day of treatment

The results of the study on the 5th day of administration of drug No. 5 are presented in Table 5.

In the control group with SSS, stagnant phenomena that did not stop on the 5th day began to manifest—the conjunctiva was loose, edematous, and hyperemic (3–4 points), approaching chemosis (pronounced swelling of the conjunctiva of the eye).

On the anterior surface of the eye, mostly on the conjunctiva of the eyeball, there was a wet discharge—up to 3–7 mm in length (in almost all cases, scored 3 points), and in some cases the discharge was greasy in consistency.

The cornea remained in an intact state of surface structures, and the fluorescein test scored 0 point, but in half of the animals the condition of the cornea worsened, the edema of the stroma near the

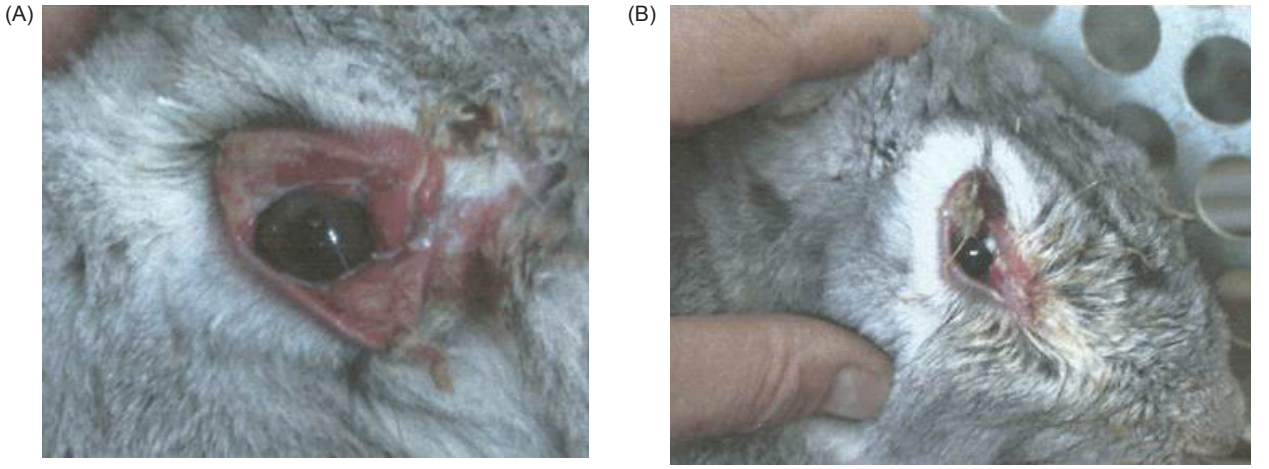

FIGURE 1. Rabbit eyes on the 5th day of DES development with auto-traumatization before the administration of drug No. 5.

limb was more pronounced outside and the epithelial staining on the entire central surface, as with the rejection of its epithelium and the fluorescein test scored 3 points.

Total edema of the stroma with the formation of a focus with a deep lesion of the stroma, neovascularization (3 points) and the fluorescein test (2 points) were determined, a corneal abscess occurred (4 points) up to one-third of its area and the fluorescein test (3 points).

In group 2 with AGel, the general condition of the conjunctiva was much better compared to the control, it had a pinkish color and reduced swelling of the tissue: separated wet, dry, or greasy.

Bullous keratopathy and its staining were noted on the cornea, the stroma was matte and opalescent, and the areas of serous formations decreased by two times, but the fluorescein staining scored 2 points.

In group 3 animals with drug No. 5, conjunctival loosening persisted: nodular seals and hyperemia scored up to 3 points.

There was not much to separate, it was wet and greasy; a dry form was also present, which was loose and without conglomeration.

The condition of the cornea was calm, the stroma was clean, weak stains from fluorescein were detected on the surface, but at places surface was well-stained with fluorescein. With corneal ulcer, the condition was stable, the corneal stroma was at the same level—there was a defect in the wound in the form of depressions, the ulcer flattens (swelling subsided), epithelized. The stroma was translucent, there was no hypopion in the anterior chamber (accumulation of pus).

On the 5th day of the treatment in group 3 with SSS, the conjunctiva suffered the most, the discharge was more abundant and dry. Drug No. 5 and AGel had approximately the same positive effect on the conjunctiva and the amount of discharge. The quality of the discharge was better with drug No. 5 and the condition of the conjunctiva during instillation of hypertension.

Thus, drug No. 5 in the test dose had a significant positive effect on the conjunctiva and cornea, reducing the amount of discharge in DES. The effectiveness of the reference preparation of AGel was 12.5% lower compared to the effectiveness of group 1 rabbits receiving drug No. 5.

The 10th day of treatment

The results of the study on the 10th day of treatment are presented in Table 6.

TABLE 5. Condition of the Objects of Eye Examination on the 5th Day of Administration of Drug No. 5.

Evaluation criteria	Score in points	Animal groups (%)		
		1: Control	2: AGel	3: Drug No. 5
Condition of seams	4	86.4	81.3	75.3
	3	10.2	16.5	21.4
	2	3.4	2.2	3.3
Condition of divorced eyelids	4	77.0	69.2	71.2
	3	19.5	24.4	20.8
	2	3.5	6.4	8.0
Condition of eye discharge	3	11.7	13.2	4.6
	2	64.2	67.3	68.3
	1	24.1	19.5	21.2
	0	–	–	5.9
Conjunctival condition	4	4.8	–	–
	3	19.8	4.6	12.7
	2	44.4	49.3	59.4
	1	31.0	34.7	24.7
	0	–	11.4	3.2
Staining corneal surface with fluorescein	3	19.0	24.7	–
	2	48.8	42.9	–
	1	23.7	20.6	81.8
	0	8.5	11.8	18.1
Condition of corneal stroma	3	21.2	4.8	–
	2	42.4	42.4	2.6
	1	27.6	35.2	32.2
	0	8.8	17.6	65.2

AGel: 20% ocular actovegin gel.

In the control group of animals with SSS instilled in the conjunctiva, stagnant phenomena increased along with dry hyperemia and chemosis, with extensive detachability and wetness.

Unstable condition of the cornea was observed. The eyes were determined without pathological changes and stroma edema, which in quite a number of rabbits expanded to ⅔ the volume of the cornea, there was fixed mucus in the lesions of the limb and a positive fluorescein test but only in the keratosis zone. In some cases, the cornea was marked by total edema of the stroma and foci with deep stroma damage and keratosis, clouding of the corneal stroma, neovascularization (scored 4 points) and the fluorescein test (3 points). The condition of corneal abscesses worsened.

In group 2 of animals with AGel, condition of the conjunctiva stabilized, almost all rabbits had its lesions, not exceeding 2 points. There was a dry discharge; if it was wet, then it was smeared on the surface of the cornea.

The condition of the cornea was satisfactory; when stained with fluorescein, the discharge

TABLE 6. Condition of the Objects of Eye Examination on the 10th Day of Administration of Drug No. 5.

Evaluation criteria	Score in points	Animal groups (%)		
		1: Control	2: AGel	3: Drug No. 5
Condition of seams	4	84.3	61.3	68.5
	3	15.7	38.7	31.5
Condition of divorced eyelids	4	45.5	31.2	29.7
	3	54.5	68.8	70.3
Condition of eye discharge	4	2.2	6.7	–
	3	35.2	47.8	4.4
	2	47.7	28.8	36.6
	1	14.9	16.7	39.5
	0	–	–	19.5
Conjunctival condition	4	4.3	–	–
	3	42.2	20.5	–
	2	47.9	64.4	24.4
	1	5.6	15.1	68.2
	0	–	–	7.4
Staining corneal surface with fluorescein	4	–	–	–
	3	14.4	–	–
	2	78.8	79.5	7.5
	1	6.8	20.5	24.3
	0	–	–	68.2
Condition of corneal stroma	3	35.6	16.2	–
	2	42.8	31.4	7.8
	1	21.6	52.4	38.8
	0	–	–	53.4

AGel: 20% ocular actovegin gel.

spread out, focal changes decreased to 1–2 mm, the bed itself was mined out, and stroma folds were observed. In some cases, the stroma was opaque and opalescent, serous degeneration decreased in the area by three times compared to the initial values.

The use of drug No. 5 for 10 days stabilized the conjunctiva along the limb, hyperemia persisted on the eyelid, chemosis was determined in several cases. Hyperemia was expressed in the conjunctiva, papillae were preserved (3 points). The general condition of the conjunctiva was calm from 0 to 3 points, especially on the eyeball; separated dry and wet: closes to liquid state.

Thus, by the 10th day of the treatment, there was a tendency to reduce eye damage in animals in group 2 with AGel. In rabbits of group 1 with drug No. 5, the phenomena of inflammation, irritation, and the amount of discharge decreased significantly, and the transparency of the stroma was preserved without staining the anterior epithelium of the cornea. In group 2 with AGel, 11.2% of eyes had ulcers and symptoms of endophthalmitis.

The 20th day of treatment

The results of the 20th day of study are presented in Table 7.

The conjunctival condition scored 2–3 points in group 2 with AGel and group 3 with SSS in 67.0% and 69.2% of the eyes, respectively. Group 1 with drug No. 5 scored 1 point, which was noted in 60.7% of cases, no eye irritation was detected in the remaining animals of this group.

In group 1 with drug No. 5, the discharge was wet and very small (1 point), but was enlarged in three eyes.

Three-quarters of the cornea had an epithelium with minor inflammation, the rest had a slight

TABLE 7. Condition of the Objects of Eye Examination on the 20th Day of Administration of Drug No. 5.

Evaluation criteria	Score in points	Animal groups (%)		
		1: Control	2: AGel	3: Drug No. 5
Condition of seams	4	67.8	18.8	20.8
	3	32.2	49.5	37.6
	2	–	31.7	41.6
Condition of divorced eyelids	4	37.6	17.6	18.8
	3	48.2	37.6	37.6
	2	14.2	44.8	43.6
Condition of eye discharge	4	–	16.8	–
	3	31.4	16.8	–
	2	56.0	50.4	58.6
	1	12.6	16.0	22.0
	0	–	–	19.4
Conjunctival condition	4	20.5	24.5	–
	3	28.8	18.8	–
	2	40.4	48.2	29.3
	1	10.3	8.5	60.7
	0	–	–	10.0
Staining corneal surface with fluorescein	4	4.6	–	–
	3	41.2	13.2	–
	2	46.6	65.8	19.8
	1	7.6	21.0	48.6
	0	–	–	31.6
Condition of corneal stroma	4	18.8	–	–
	3	38.6	18.8	–
	2	37.6	18.8	–
	1	5.0	48.2	36.6
	0	–	14.2	63.4

AGel: 20% ocular actovegin gel.

staining with fluorescein. The stroma retained transparency.

The cornea was more strongly protected and restored by drug No. 5, the score of which remained as 1 point (48.6%), while lesion of the anterior epithelium scored 3 points in group 3 with SSS and group 2 with AGel in 41.2% and 13.2% of the eyes, respectively. The state of the anterior epithelium was also calm with drug No. 5. The stroma suffered in group 3 with SSS (4 points—18.8%), which was accompanied with opalescence, impaired transparency, descemetitis, abscessing keratitis, corneal perforation, and endophthalmitis. Drug No. 5 reduced the effect of dry eye by up to 45% and maintained the cornea of the remaining eyes without pathological manifestations.

In the eyes of group 1 with drug No. 5, there was no corneal perforation in any case despite the presence of serous degeneration in the initial stages of the experiment—abscessing keratitis, and endophthalmitis in two cases.

Thus, by the 20th day of the treatment with drug No. 5, the elimination of corneal stromal defect was observed whereas in the control groups and hypertension, deep corneal defects of 2, 3, and 4 points remained by this time. In addition, in group 1 with drug No. 5, there were no signs of neovascularization, conjunctival irritation phenomena, and decrease in the amount of mucosal discharge.

The 30th day of treatment

The obtained results characterizing the condition of the eyes are presented in Table 8.

In the groups with SSS, AGel, and drug No. 5, the discharge was absent in 8.0%, 54.0%, and 80.5% of the eyes, respectively (Figure 2).[18]

A completely transparent corneal stroma was observed in group 1 with drug No. 5, while in the groups with SSS and AGel, it ranged from 1 to 3 points.[18]

In group 1 with drug No. 5, stable conditions of all the indicators used were visible: almost complete absence of mucosal discharge and a decrease in corneal opalescence.[14]

FIGURE 2. Condition of anterior segment and accessory apparatus of the eye in rabbits of group 1 with drug No. 5 on the 30th day of treatment.

Group 3 with SSS: Macroscopically, an eyeball of normal size and shape was present. In seven cases out of 30 (23.3%), an inflammatory focus with a pronounced lymphoplasmocytic infiltrate with the inclusion of a large number of eosinophils, which also infiltrated the epithelial layer, was determined on the periphery of the cornea. In the projection of the epicenter of the focus of inflammation, the phenomena of nonpurulent expression were observed. A large number of newly formed vessels, foci of perivascular infiltration in the form of couplings around the vessels were present (Figure 3A).

In all cases, in the epithelial cover above the focus of inflammation, cell layering was disrupted, the basal layer was disoriented, and the entire epithelial layer was infiltrated by eosinophils. There was hypertrophy of the epithelium along the periphery of the focus, an increase in its thickness relative to the stroma.

In the central part, some thinning of the cornea was observed, with separate inflammatory cells between the stromal plates. In general, the structure of all layers of the cornea was preserved (Figure 3B) and the posterior epithelium was not involved in the pathological process.

TABLE 8. Condition of the Objects of Eye Examination on the 30th Day of Administration of Drug No. 5.

Evaluation criteria	Score in points	Animal groups (%)		
		1: Control	2: AGel	3: Drug No. 5
Condition of seams	4	18.8	–	6.6
	3	18.8	18.8	21.8
	2	45.6	18.8	48.8
	1	16.8	62.4	22.8
Condition of divorced eyelids	4	16.8	–	6.4
	3	46.2	15.2	21.8
	2	26.0	32.8	48.8
	1	11.0	52.0	41.8
Condition of eye discharge	4	4.6	–	–
	3	13.8	2.9	–
	2	25.0	18.8	–
	1	48.6	24.3	19.5
	0	8.0	54.0	80.5
Conjunctival condition	4	–	–	–
	3	9.8	–	–
	2	37.2	26.4	–
	1	38.2	62.6	15.6
	0	14.8	11.0	84.4
Staining corneal surface with fluorescein	3	16.8	4.6	–
	2	16.2	18.8	–
	1	54.4	56.3	–
	0	12.6	20.3	100
Condition of corneal stroma	4	–	–	–
	3	17.6	9.1	–
	2	43.4	37.6	–
	1	39.0	53.3	–
	0	–	–	100

AGel: 20% ocular actovegin gel.

Normally, the thickness of the cornea was 530.27 microns (μm), but the thickness of the presented and modified cornea was 1508.66 μm. The unchanged part of the stroma was 210.61 μm. The size of the hearth: depth 1259–1500 μm, width along the plane: 3.5 mm.

In 17 eyes (56.7%), the phenomena of ulcerative necrotizing keratitis were observed, with foci of abscess formation and mixed infiltration (Figure 4A). The corneal epithelium was infiltrated far beyond the focus by polymorphonuclears (cells with polymorphic nuclei, i.e., each cell of this type had a morphologically different nucleus from the nuclei of cells of the same type, mainly neutrophils, basophils, and eosinophils). Polymorphonuclears as cellular effectors of inflammation absorbed and cleaved the remains of dead cells.

(A) (B)

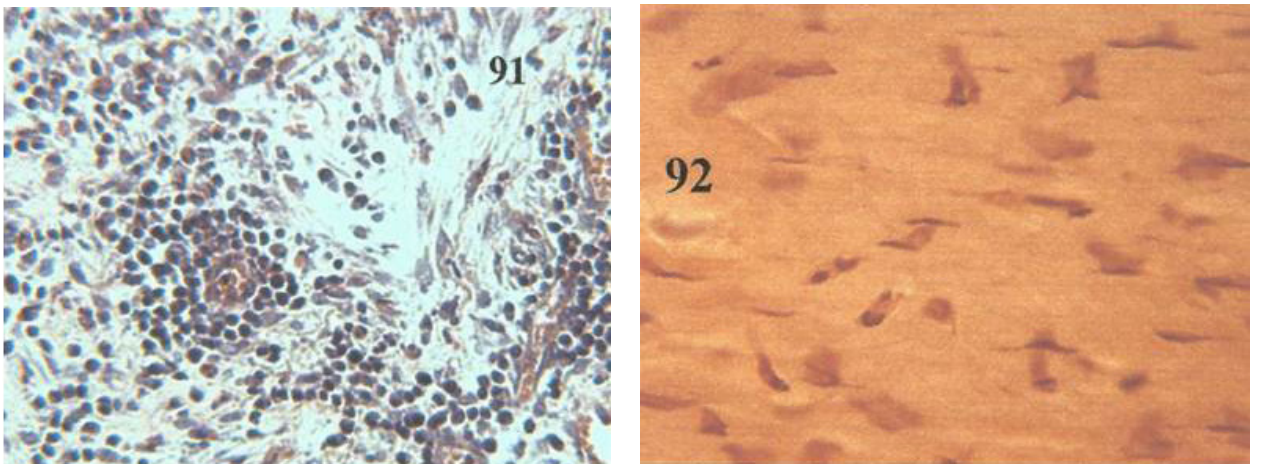

FIGURE 3. Control with sterile saline solution: (A) In projecting the epicenter of the focus of inflammation—the phenomenon of nonpurulent ulceration, a large number of newly formed vessels, foci of perivascular infiltration in the form of couplings around the vessels; 250-fold increase. (B) Corneal thinning in the central part, inflammatory cells between stroma plates; 250-fold increase.

(A) (B)

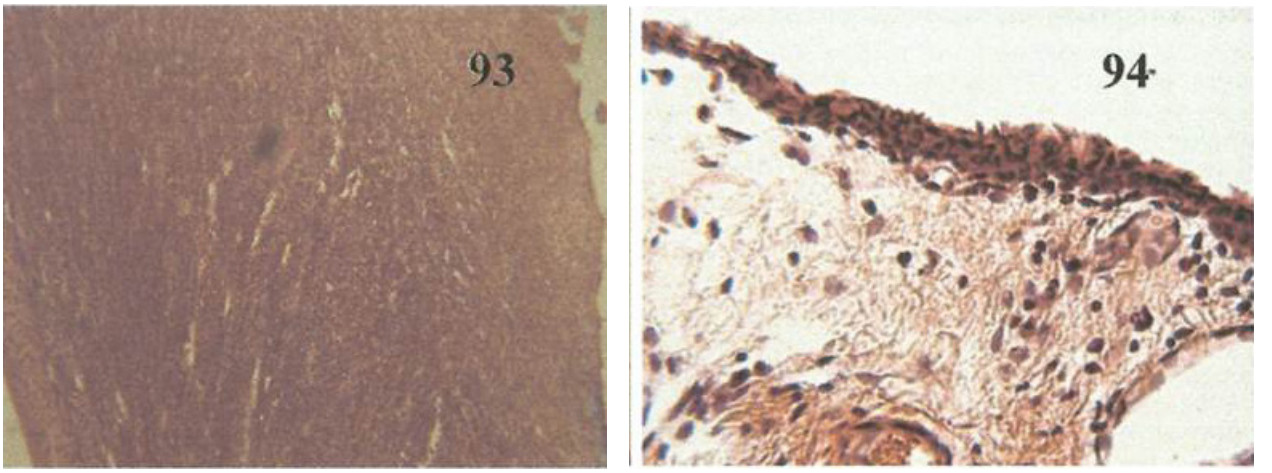

FIGURE 4. Control with sterile saline solution: (A) Ulcerative keratitis with necrosis, a focus of abscess formation and mixed infiltration. The corneal epithelium outside the focus is infiltrated by polymorphonuclears; 140-fold increase. (B) Single goblet cells in the thickened conjunctiva. Lymphocytic infiltration around pericorneal vessels; 140-fold increase.

In three eyes (10%), corneal perforation was marked, followed by death of the eyeball. In other cases, the posterior stromal layers were preserved, the corneal epithelium did not change, and there was no threat of perforation.

Lymphoplasmocytic infiltration of the posterior wall was detected under the conjunctiva of 10 eyes (33.3%). Single goblet cells in the conjunctiva of the remaining eyes were established (a significant decrease in the number of goblet cells, 0–2 in the

field of vision), the conjunctiva of the eyeball had thickened due to edema. Small lymphocytic infiltration around the pericorneal vessels was observed (Figure 4B).

The drainage zone was preserved. There was a weak hyperemia in celial processes. Choroid and retinal pigment epithelium remained unchanged. The layer of sticks and cones was well preserved.

Thus, during the morphological examination of the eyes of the control group of rabbits using SSS for 30 days in the DES model, the phenomena of pronounced ulcerative–necrotic keratitis were observed due to the violation of barrier function of the epithelium in 56.7% cases, the addition of secondary infection occurring against the background of constant exogenous irritation and lack of irrigation of the cornea. In 10% cases, the eye died after perforation. The thickness of the cornea reached 1508 μm, which exceeded its normal thickness by three times. In all cases, the row of cells in the epithelial cover above the focus of inflammation was disrupted, the basal layer was disoriented, and the entire epithelial layer was infiltrated by eosinophils. Weak hyperemia in ciliary processes was observed.

These results indicated the absence of protective and therapeutic properties of SSS to eliminate the induced changes in the DES model.

Group with 20% AGel: Macroscopically, an eyeball of normal size and shape. In 14 of the 30 eyes (46.7%), the cornea was thickened due to pronounced peripheral abscessing ulcerative keratitis affecting up to ½ the thickness of the cornea. As part of the infiltrate were granulocytes (including eosinophils), plasma, mast cells, macrophages, and lymphocytes (Figures 5A and B).

The thickness of the cornea was 434.62 μm. The infiltration became less pronounced toward the center of the cornea (Figure 6A). On the affected side, vessels of the limbal region were sharply expanded and filled with blood. Heterogeneous, newly formed vessels going to the focus of inflammation were observed in 19 of the 30 cases (63.3%). Owing to the porosity of the vascular wall, hemorrhage foci were determined in 11 of the 30 cases (36.7%).

The posterior corneal epithelium was preserved but flattened and with a reduced cell density (Figure 6B).

Along the periphery of inflammatory focus, the phenomena of stroma *fibrotization* were

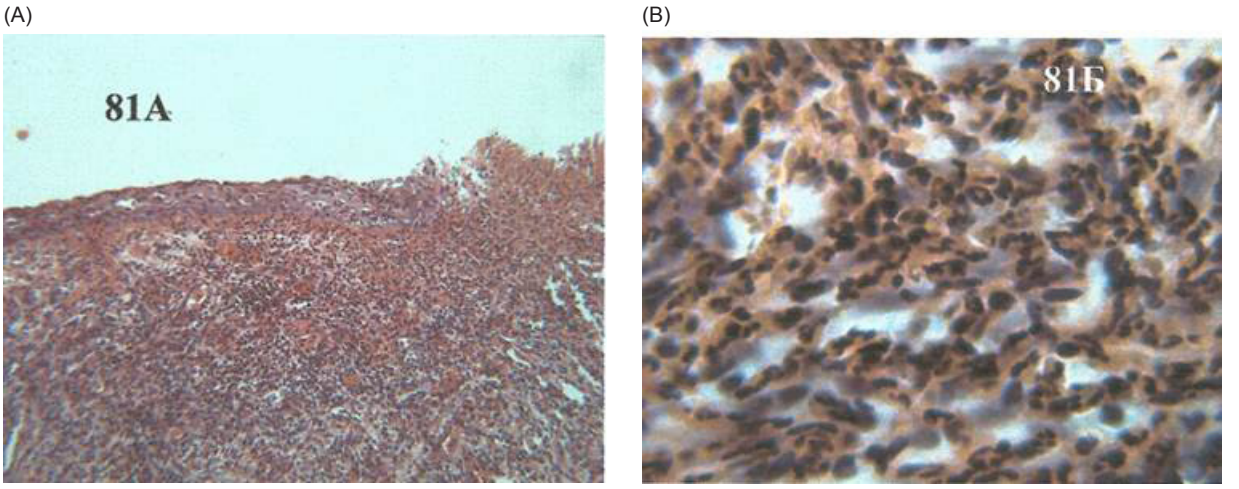

FIGURE 5. Experience with AGel: (A) Peripheral abscessing ulcerative keratitis; 140-fold increase. (B) In infiltrate, granulocytes noticed are eosinophils, plasma and mast cells, macrophages, and lymphocytes; 250-fold increase.

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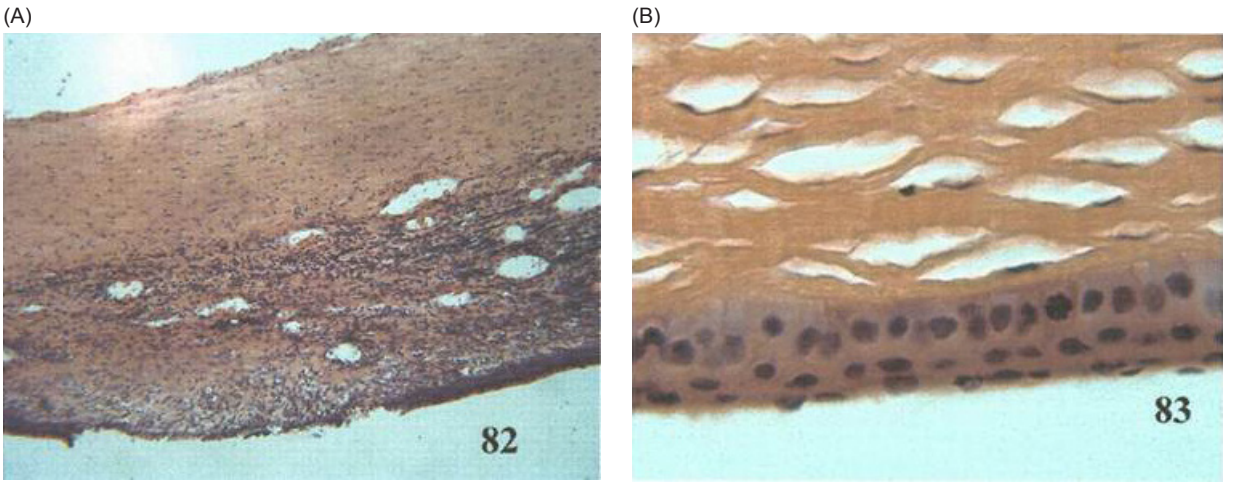

FIGURE 6. Experience with AGel: (A) Infiltration to the center of the cornea is reduced, vessels of the limbal region are dilated and filled with blood; 140-fold increase. (B) The posterior corneal epithelium is preserved and flattened with a reduced cell density; 250-fold increase.

determined—because of developed abscessing process. Within this zone, there was a weakening of epithelial–stromal contact with a tendency to develop subepithelial edema (Figure 7).

The conjunctiva was edematous (Figure 8A) due to vascular dilation and moderate cellular infiltration. Goblet-shaped cells were not found in the epithelium (Figure 8B).

Attention was drawn to the dilation of vessels in the iris of 7 of the 30 cases (23.3%)—perspiration of fibrinous exudate of the iris (Figure 9A). Individual inflammatory cells were detected in the anterior chamber (Figure 9B).

The ciliary processes (or processes of the ciliary body—numerous protrusions on the anterior inner surface of the ciliary body responsible for forming watery moisture of the eye) were also edematous, with signs of local detachment of the pigmented epithelium. In the posterior chamber, the phenomenon of protein exudation was marked. There were moderate changes in the drainage zone.

In the choroid (vascular membrane of the eye), 15 of the 30 eyes (50%) (Figure 10A) had dilated vessels of medium and large caliber (it was possible to increase blood flow as a protective reaction to an increase in light (thermal) energy on the retina because of eyelid nonclosure). The retina was close to normal, a slight hypertrophy of pigmented epithelial cells (Figure 10B) and edema in the photoreceptor layer were found in its posterior parts. The optic nerve disc remained unchanged.

FIGURE 7. Experience with AGel. Phenomena of stroma *fibrillization* with weakening of epithelial–stromal contact with a tendency to develop subepithelial edema; 140-fold increase.

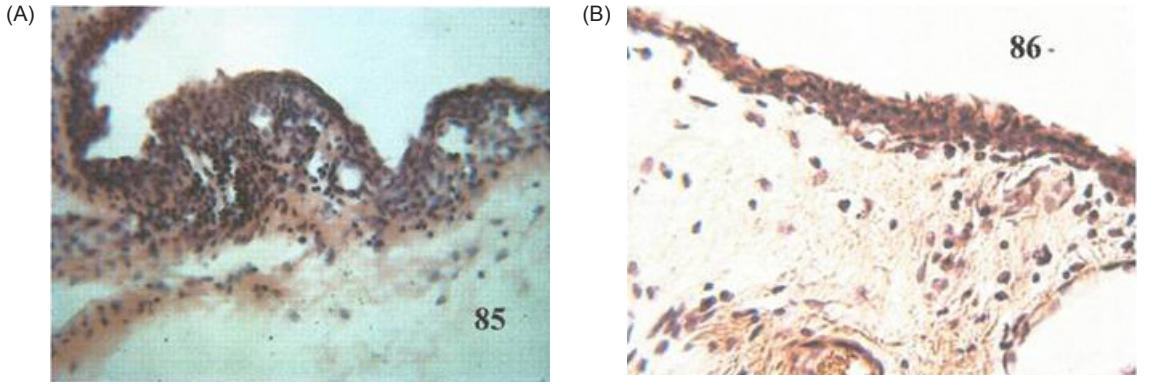

FIGURE 8. Experience with AGel: (A) The conjunctiva is edematous; vascular dilation, cellular infiltration; goblet cells are not detected; 140-fold increase. (B) Single goblet cells in the epithelium; 140-fold increase**.**

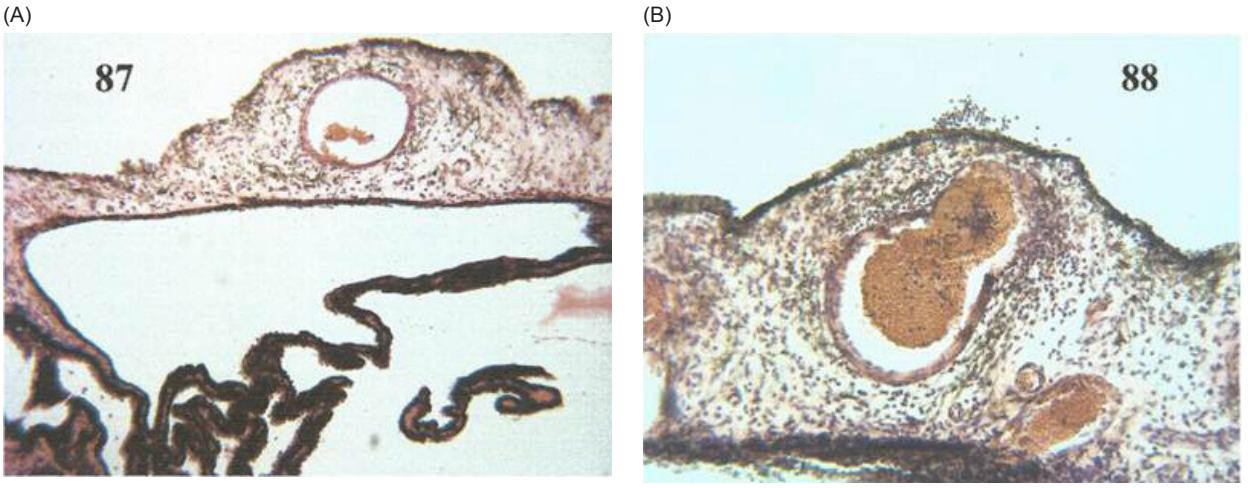

FIGURE 9. Experience with hypertension: (A) Dilation of vessels of the iris, sweating of fibrinous exudate of the iris; 140-fold increase. (B) inflammatory cells in the anterior chamber; 140-fold increase.

Thus, during morphological examination, usage of AGel for 30 days in the DES model somewhat weakened the manifestations of pathological processes. In 46.7% of rabbit eyes, there was a thickening of the cornea because of pronounced peripheral abscessing ulcerative keratitis affecting up to half the thickness of the cornea while maintaining the posterior corneal epithelium but with its flattening and reduced cell density, dilation of vessels of the limbal region, and filling with blood. There was swelling of the conjunctiva with rare goblet cells and sweating of fibrinous exudate of the iris.

Group with preparation No. 5: Macroscopically, an eyeball of normal size and shape. Newly formed vessels were found on the periphery of the cornea in 6.7% eyes. The vessels remained dilated, full-blooded, inflammatory infiltrate of lymphocytic-macrophage nature. Epithelial cover with a slight violation of the row of usual thickness was marked in one case (3.3%). The corneal endothelium was

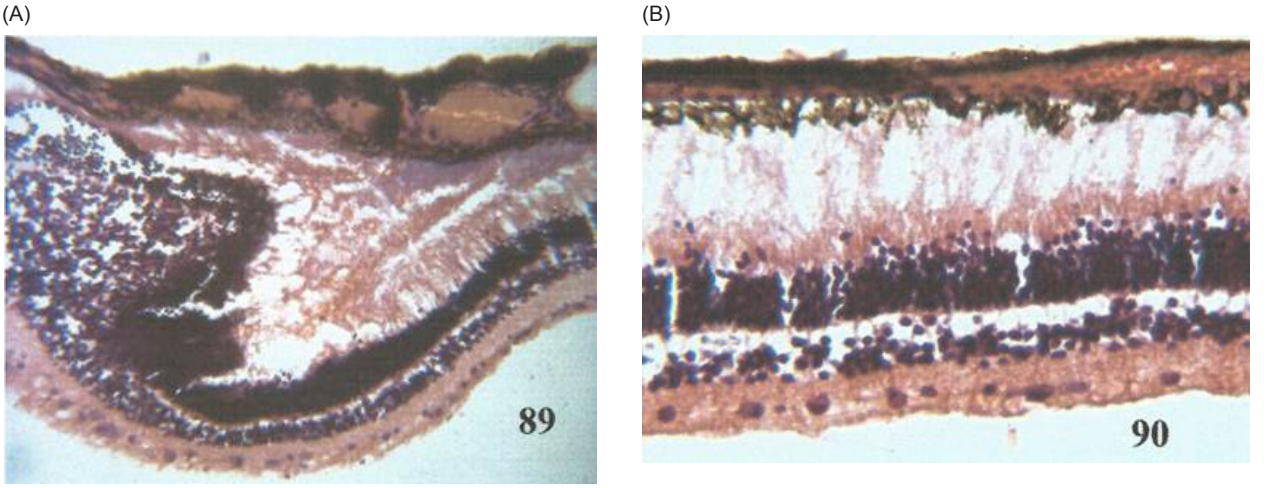

FIGURE 10. Experience with hypertension: (A) Vessels of medium and large caliber in the choroid are expanded; 140-fold increase. (B) In the posterior choroid hypertrophy of pigment epithelial cells and edema in the photoreceptor layer; 200-fold increase.

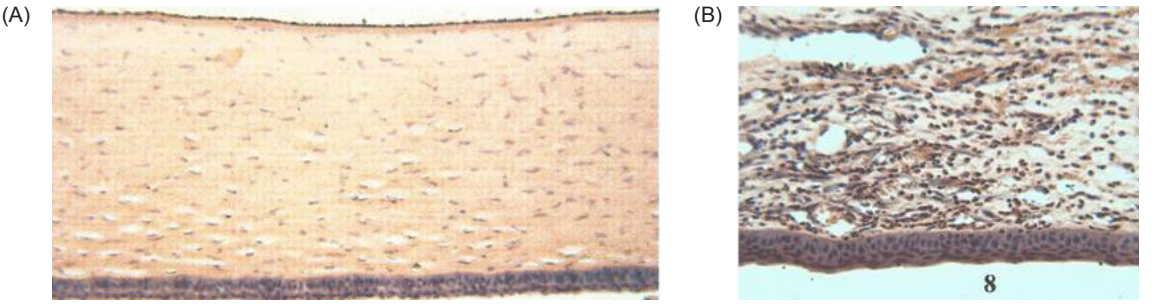

FIGURE 11. Group of rabbits when prescribing drug No. 5: (A) Corneal thickness: 361.58 μm; 140-fold increase. (B) Goblet cells with varying degrees of differentiation; 140-fold increase.

flattened but retained its integrity in two cases (6.7%). The thickness of the cornea was 361.58 μm (Figure 11A). In rest of the eyes, the structure of the cornea was normal.

The average density of conjunctival goblet cells was within the normal range, although there were areas with an increased and decreased number of cells with varying degrees of differentiation (Figure 11B). The epithelium itself was evenly thinned. In other cases, there were no significant changes in the conjunctiva.

The anterior and posterior chambers of the eye were without inflammation and irritation, the iris remained unchanged. The drainage zone was preserved. In the ciliary processes of the ciliary body (part of the middle [vascular] shell of the eye, which serves to suspend the lens and ensure the process of accommodation), normal vascular-tissue ratios were marked.

The choroid was without changes. The retina was normal: It adhered throughout, the photoreceptor layer was also preserved.

Thus, 0.05 mL/kg of drug No. 5 in a dilution of 1:15 SSS instilled in both eyes of animals twice a day for 30 days had an anti-inflammatory, wound-healing, and angioprotective effects. This positively

affected the course of reparative process in the conjunctiva and cornea if not closing the eyelids in the DES model of rabbits.

CONCLUSION

It was demonstrated in the DES model of rabbits that 0.05 mL/kg of drug No. 5 as 1:15 dilution with SSS had a strong anti-inflammatory, wound-healing, and angioprotective effects if instilled into both eyes of animals twice a day for 30 days. The drug positively affected the course of reparative process in the conjunctiva and cornea, complicated by the nonclosure of the eyelids. It was found that drug No. 5 in the tested dose promoted the stimulation of reparative processes in the conjunctiva and cornea, clinically manifesting itself in accelerating the recovery of defects in the anterior epithelium and corneal stroma, in reducing the frequency of formation of deep corneal defects, and reducing the severity of inflammatory response and vascularization. There was a slowdown in the formation of corneal opacities, a decrease in the amount and appearance of a more liquid mucous discharge of the conjunctiva compared to the control. It was established in the DES model of rabbits that drug No. 5 in the test dose had a pronounced pharmacological effect, contributing to a faster recovery of damage caused to the superficial epithelium and stroma of the cornea, anterior and posterior chambers of the eye, the vascular membrane and retina as well as goblet cells of the conjunctiva.

COMPETING INTERESTS

The author declared that he had no competing interests.

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