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Evaluation of Blood-Aqueous Barrier Permeability in Response to Tetracycline Antibiotics under Normal and Pathological Conditions

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ABSTRACT

Under normal physiological conditions, the blood-aqueous barrier plays a vital role in maintaining ocular homeostasis. Research on the blood-aqueous barrier has been conducted through various approaches, with experimental animal studies being a prominent method in this field. This particular study investigated the permeability of the blood-aqueous barrier upon exposure to tetracycline antibiotics, comparing normal and pathological states using three groups of laboratory rabbits. Group 1 consisted of clinically healthy rabbits, while groups 2 and 3 consisted of animals with experimentally induced diseases. Rabbits in groups 1 and 2 were orally administered tetracycline tablets at a dose of 25 mg/kg body weight, three times at 8-hour intervals, while group 3 animals received 3% tetracycline solution topically to the lower eyelid three times daily. Clinical evaluations of the animals included palpation of the affected organs and body temperature measurements. Biological samples, including intraocular fluid and blood, were collected for analysis. This study presents the findings of tetracycline concentrations, hematological, and biochemical blood serum parameters of the subjects. On the ninth day following the Staphylococcus aureus suspension administration, clinical recovery was observed in all animals of groups 2 and 3, as evidenced by a return to normal health status, regular respiratory rate, stable pulse, and normalized body temperature. In the ocular examination, no signs of inflammation were observed in the iris, cornea, or conjunctiva of two animals in group 2.

Keywords: Suspension of Staphylococcus aureus, Tetracycline, Blood-aquatic barrier, Permeability

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Introduction

Blood-tissue barriers play a vital role in controlling metabolic exchanges between blood and organ tissues, thereby maintaining the stability of the biological and physicochemical properties of tissue fluid [1]. These barriers are structurally influenced by the specific characteristics of each organ, and their structure varies based on the morphological and physiological attributes of the tissue. The primary component of these barriers is the blood capillaries, with their endothelial structure exhibiting unique characteristics depending on the organ or tissue in question [2].

In healthy function, the blood-aqueous barrier is essential for preserving ocular homeostasis [3]. This barrier is not a uniform entity along its entire length. The idea of the blood-aqueous barrier, derived from experimental findings, has long been connected to the capillaries and epithelium of the ciliary body, which are involved in aqueous humor production and maintaining the metabolism of non-vascular ocular tissues like the vitreous body, lens, cornea, and trabecular network [4]. This barrier functions as a system that regulates exchanges between the blood and intraocular fluids, ensuring the proper physiological balance within the eye [3].

Research on the blood-aqueous barrier is conducted through several primary approaches, including:

- Animal experiments utilizing isolated electrodes inserted into eye tissues and media to investigate redox processes through polarographic techniques;
- Analysis of substance penetration into ocular tissues using labeled atoms;
- Utilization of light and electron microscopy techniques;
- Clinical investigations employing fluorescence angiography and fluorometry methods;
- Clinical and functional assessments in various ocular diseases.

It has been determined that the vascular-free structures of the eye exhibit a lower intensity of redox processes compared to the tissues with a capillary blood supply [5]. The oxygen utilization coefficient for non-vascular tissues is approximately 0.4, while for capillary-supplied tissues, this coefficient is nearly double, at 0.8 [6].

The permeability of substances into ocular tissues, both vascular and non-vascular, varies significantly. In experimental conditions, the time for oxygen to enter the aqueous humor and lens is about 13-14 seconds, whereas it enters the iris nearly three times faster, within 5 seconds [7]. This marked difference in redox activities reflects the distinct metabolic processes occurring in various parts of the eye. Under pathological conditions, the blood-aqueous barrier may allow the passage of endogenous, exogenous, and other substances that are typically not permitted, leading to abnormal permeability. This may result in clinical symptoms and contribute to the development of ocular diseases. The blood-aqueous barrier functions as a set of three interrelated and interdependent blood-tissue systems: iridociliary, chorioretinal, and papillary [8].

Each of these three systems has unique features tailored to support the trophism of the specific tissues and cells in their respective regions of the eye. Consequently, the ability of drugs to penetrate these barriers varies [9]. For instance, to maintain the trophism of the anterior, vascular-free ocular tissues, the iridociliary system (comprising the iris and ciliary body) allows substances to pass through its barrier that the chorioretinal and papillary systems do not. This is corroborated by the observation of minimal staining of the anterior chamber fluid during fluorescence angiography.

Tetracyclines are one of the earliest classes of antimicrobial agents [10, 11], first isolated in the late 1940s [12]. Their use has become more limited due to the emergence of tetracycline-resistant microorganisms and *Helicobacter pylori* strains that exhibit resistance to these drugs. The tetracycline group includes natural tetracycline as well as semi-synthetic derivatives like doxycycline and minocycline [13].

Tetracyclines exert a bacteriostatic effect by disrupting protein synthesis in microbial cells [14, 15]. Despite their broad-spectrum antimicrobial activity, prolonged use has led to the development of bacterial resistance [16]. When administered orally, tetracyclines are efficiently absorbed, with doxycycline being more bioavailable than tetracycline. The bioavailability of doxycycline remains consistent, whereas food intake reduces tetracycline's bioavailability by half. Peak drug concentrations in the bloodstream occur within 1-3 hours of ingestion [17]. Intravenous administration of tetracyclines results in significantly higher and faster blood concentrations compared to oral administration. These drugs are widely distributed throughout the body's tissues and organs, with doxycycline reaching higher tissue concentrations than tetracycline [18].

This particular study investigates the permeability of the blood-aqueous barrier when exposed to tetracycline antibiotics, comparing normal and pathological states using three groups of laboratory rabbits.

Materials and Methods

To examine the permeability of the blood-aqueous barrier with tetracycline antibiotics in both normal and pathological states, an experiment was carried out involving three groups of adult rabbits (n = 15), organized based on the principle of analogs. Group 1 consisted of healthy animals, while groups 2 and 3 included animals with induced ocular pathology. Rabbits in groups 1 and 2 were given tetracycline tablets orally at a dose of 25 mg/kg body weight, administered three times at 8-hour intervals, whereas group 3 rabbits received a 3% tetracycline solution applied to the lower eyelid three times a day.

Clinical examinations of the animals involved palpation of the affected eye, general observation, and thermometry. Biological samples, including blood and intraocular fluid, were collected for analysis. The eyes of the animals were removed and fixed in 10% neutral formalin for 2-3 weeks to preserve the morphological structures of the ocular system for study under both normal and pathological conditions. The material analyzed included eyeballs from both healthy rabbits and those infected with a Staphylococcus aureus suspension.

Blood biochemical tests were conducted using a Chemwell Combi V 1.03 (USA) analyzer with Cormay test kits. Urea concentration was measured using the urease and glutamine dehydrogenase method [19], albumin levels were assessed via reaction with bromocresol green in an acidic environment [20], and total protein was determined using the biuretic reaction technique [21]. Creatinine concentration was quantified using a modified Jaffe method without protein removal [22]. Cholesterol levels were calculated by the esterase-cholesterol oxidase reaction [23], and α-amylase activity was determined by the change in 2-chloro-4-nitrophenol absorbance at 405 nm [24]. Alkaline phosphatase activity was assessed following the kinetic approach outlined by the International Clinical Federation [25].

Hematological tests were carried out using an Automated Veterinary Hematology Analyzer PCE-90 VET, which can differentiate leukocyte subpopulations and generate histograms. Erythrocytes, leukocytes, and platelets were counted using the Coulter method [26], while hemoglobin was measured by the colorimetric method [27].

Pathological conditions of the eyes in the animals were induced by inserting a sterile 26 G needle into the anterior chamber to extract 0.1 ml of intraocular fluid. After removing the needle, a new syringe was used to inject 0.1 ml of Staphylococcus aureus suspension (containing 1 billion microbial cells per ml) into the eye.

Results and Discussion

After administering the Staphylococcus aureus suspension, distinct clinical signs of ocular pathology emerged. By the fourth day, there was visible conjunctival hyperemia, pericorneal vascular injection, corneal and iris edema, along with dilated blood vessels in the iris. On day five, more pronounced symptoms were observed, including increased lacrimation, conjunctival edema, scleral injection, corneal swelling, and ciliary pain.

Biological fluid samples were collected from the rabbits two hours following the morning antibiotic administration.

On the ninth day after the *Staphylococcus aureus* injection, all animals in groups 2 and 3 exhibited clinical improvement, marked by the normalization of their overall condition, stabilization of body temperature, pulse rate, and respiratory rate, all within normal ranges.

In the visual system, two rabbits from group 2 showed no signs of inflammation in the iris, cornea, or conjunctiva. The tetracycline concentrations in intraocular fluids and blood serum are summarized in **Table 1**.

Table 1. Tetracycline concentration in biological fluids, μg/mg (n = 15; M ± m)

Research Day	Group of laboratory animals		
	1	2	3
Blood serum			
1	4.05 ± 0.2	3.69 ± 0.19	0.005 ± 0.0003
2	4.31 ± 0.22	4.18 ± 0.21	0.004 ± 0.0002
3	4.61 ± 0.23	4.56 ± 0.23	0.005 ± 0.0003
4	4.78 ± 0.24	4.63 ± 0.23	0.004 ± 0.0002
5	4.99 ± 0.25	4.72 ± 0.24	0.005 ± 0.0003
Intraocular fluid			
1	0.71 ± 0.04	0.81 ± 0.14	0.048 ± 0.002
2	0.6 ± 0.03	1.2 ± 0.16*	0.047 ± 0.002
3	0.51 ± 0.03	1.1 ± 0.26*	0.062 ± 0.003
4	0.62 ± 0.03	1.22 ± 0.18*	0.099 ± 0.005
5	0.77 ± 0.04	1.12 ± 0.26*	0.105 ± 0.006

Note: *P ≤ 0.05, the difference is significant concerning healthy animals

In the first experimental group, the blood serum concentration of tetracycline rose by 19% throughout the study. The second group saw a 22% increase in tetracycline levels, while the third group's serum concentration remained unchanged during the entire experiment.

When comparing the blood serum data, the correlation coefficient between the first and second groups was found to be positive (r = 0.961), indicating a strong, direct relationship. However, the correlation between the first and third groups was weak (r = 0.007), suggesting a minimal direct relationship.

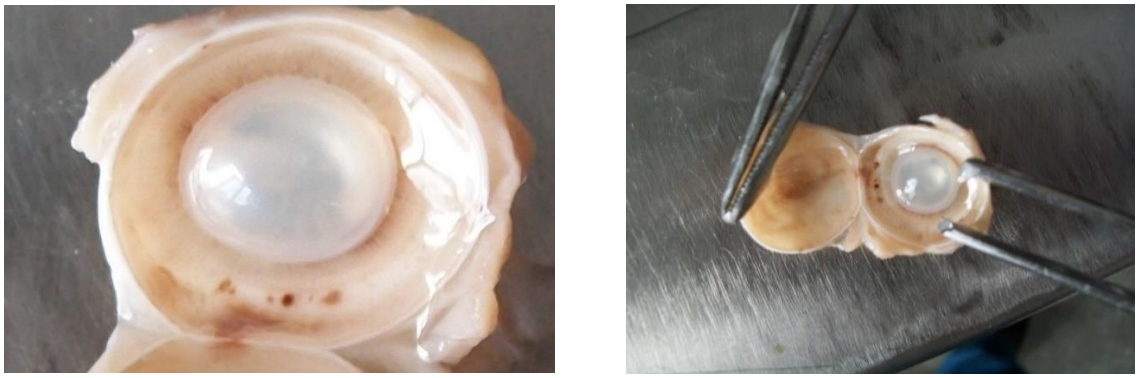
In terms of intraocular fluid, the concentration of tetracycline in the first and second groups was 5.5 times lower than in the blood serum. By day five, the tetracycline level in the clinically healthy group increased slightly, while in the second group, it grew by 1.5 times, and in the third group, it rose by 2.2 times.

The correlation coefficient between intraocular fluid levels in the first and second groups was negative (r= -0.361), indicating a moderate inverse relationship, whereas in the third group, the correlation was positive (r= 0.388), suggesting a moderate direct relationship.

Throughout the experiment, the healthy group exhibited the lowest tetracycline concentration in the intraocular fluid. In the second group, where tetracycline was administered orally, the drug did not achieve the optimal concentration by the study's conclusion. No therapeutic concentration was observed in the third group. The therapeutic tetracycline concentration (0.5-1.5 μg/mg) was effective only against gram-positive bacteria in the first and second groups, with no effect on gram-negative bacteria.

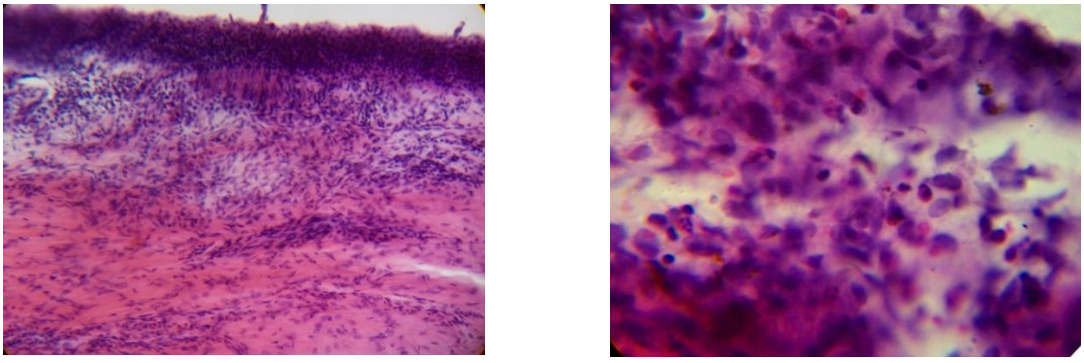
The concentration of tetracycline in the intraocular fluid of infected rabbits was nearly twice as high as in the healthy animals, suggesting that the blood-aqueous barrier's permeability was altered due to the inflammation.

For structural analysis of the visual organ, tissue samples from all groups were examined (see **Figures 1** and **2**). Three days following infection, three rabbits from each group (both healthy and infected) were euthanized, and their eyes were harvested for further examination.



a) b)

Figure 1. Hemorrhages in the scleral region



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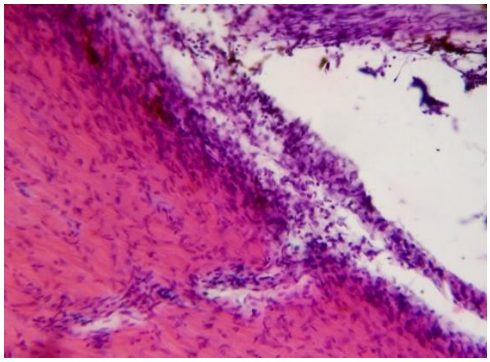

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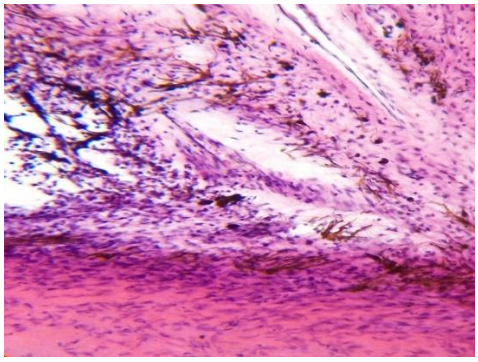

d)

Figure 2. Changes in the structure of the visual analyzer: a) pronounced swelling of the conjunctiva and lymphocytic infiltration of the sclera; b) accumulation of lymphocytes, macrophages, neutrophils, and hemosiderin in the sclera area; c) desquamation of the corneal epithelium, inflammatory infiltration by lymphocytes and macrophages; d) inflammatory infiltration of the cornea, desquamation of the iris epithelium.

The animals in the second group exhibited several changes in the eye structures, including swelling of the conjunctival stroma with lymphocytic infiltration, scleral edema, and altered fiber alignment. The cornea showed signs of edema, and the stroma underwent diffuse polymorphocellular inflammatory infiltration. The iris exhibited leukocyte infiltration and areas of purulent necrosis, while the ciliary body had inflammatory cell clusters. Uveitis signs were noted in the choroid, and lymphocytic infiltration was observed in the retinal layers. Similar changes were observed in the third group. These findings indicate alterations in the micromorphology of the eye's vascular membranes, contributing to the blood-tissue barrier. Mild inflammatory infiltration was also recorded. To assess the overall impact on the animals' health, hematological and biochemical analyses were conducted (**Tables 2** and **3**).

Table 2. Hematological parameters of blood serum of laboratory animals (n = 15; M ± m)

Indicators	Background indicators	Day 1			Day 5		
		Group 1	Group 2	Group 3	Group 1	Group 2	Group 3
White blood cell (*10^9/L)	8.4 ± 0.42	8.7 ± 0.44	13.4 ± 0.67*	14.1 ± 0.41*	8.32 ± 0.42	13.9 ± 0.7*	17.8 ± 0.9*
Lymphocyte percentage (%)	44.7 ± 2.24	47.6 ± 2.38	36.6 ± 1.83*	26.7 ± 1.34*	41.3 ± 2.07	34.7 ± 1.74*	22.8 ± 1.14*
Mid-sized cell percentage (%)	4.7 ± 0.24	4.5 ± 0.23	3.9 ± 0.19 *	4.9 ± 0.25	5.61 ± 0.19*	4.4 ± 0.22	5.12 ± 0.26
Granulocyte percentage (%)	50.6 ± 2.53	48.0 ± 2.4	59.5 ± 2.98*	68.4 ± 3.42*	53.1 ± 2.66	60.9 ± 3.1*	72.1 ± 3.61*
Red blood cell (*10^{12}/L)	5.8 ± 0.3	6.2 ± 0.31	5.6 ± 0.28	5.2 ± 0.26	5.94 ± 0.3	4.9 ± 0.25*	4.3 ± 0.22*
Hemoglobin concentration (g/L)	120.1 ± 6.0	125.7 ± 6.8	116.9 ± 5.9	107.4 ± 5.4*	120.3 ± 5.23	100.9 ± 5.1*	98.7 ± 4.94*
Hematocrit (%)	35.2 ± 1.8	38.7 ± 1.94	34.1 ± 1.71	31.8 ± 1.6	36.0 ± 1.5	29.6 ± 1.5*	27.2 ± 1.4*
Platelet (*10^9/L)	220.5 ± 11.0	154 ± 7.0*	252 ± 13.0	217 ± 10.0	148 ± 6.0*	297 ± 15*	244 ± 12.0

Note: *P ≤ 0.05, the difference is significant concerning the background indicators

Following the final administration of tetracycline, a reduction of 4% in the leukocyte count was observed in clinically healthy animals. Conversely, in sick animals receiving oral tetracycline, leukocyte levels rose by 4%, while in those treated with tetracycline ointment, this parameter showed a notable 21% increase. Throughout the experimental period, leukocyte counts in both groups of sick animals remained above physiological norms, which can be attributed to the organism's defensive response against the introduced pathogenic agent.

Regarding the differential leukocyte composition, lymphocytes, monocytes, and granulocytes remained within normal physiological limits in the first group. However, in the second and third groups, lymphocyte counts dropped below normal values, while granulocyte levels surpassed the reference range — changes consistent with an inflammatory response provoked by pathogen exposure.

The erythrocyte count in the first group exhibited no significant variation by the end of the study. In contrast, a decrease of 12% was recorded in the second group, and a more pronounced 17% reduction was evident in the third group. These decreases are directly linked to the acute inflammatory condition induced experimentally in animals with ophthalmopathology.

Similarly, hemoglobin levels remained relatively stable in healthy animals by day five. However, in sick animals treated with oral tetracycline, hemoglobin concentration declined by 14%, while in those receiving tetracycline ointment, it fell by 8% relative to normal values. The hematocrit values in both sick groups also fell below the standard range. These hematological changes correlate with the observed reduction in erythrocyte numbers.

Platelet dynamics demonstrated a 4% reduction in healthy animals, while sick animals treated orally with tetracycline exhibited an 18% increase, and those treated with ointment showed an 11% increase. Despite these variations, platelet counts remained within normal physiological limits in healthy animals, whereas in the sick groups, the changes reflected the ongoing inflammatory processes.

Altogether, the hematological data support the presence of an acute inflammatory reaction within the organisms of sick animals.

In terms of biochemical markers, the activity of alanine aminotransferase (ALT) rose by 7% in both the healthy group and the sick animals treated orally with tetracycline. No considerable changes in ALT activity were noted in sick animals receiving tetracycline ointment.

Regarding aspartate aminotransferase (AST), healthy animals displayed a modest 3% increase in activity, whereas in sick animals treated orally with tetracycline, ALT activity rose by 9%.

The γ-glutamyltransferase (GGT) activity remained stable in healthy animals throughout the experiment. On the final day, its activity stayed within the physiological range in this group, but in sick animals treated orally with tetracycline, GGT activity rose by 15%, while it remained unchanged in animals treated with the ointment.

Amylase activity showed no significant variation in healthy animals after tetracycline administration. However, in sick animals, its activity increased by 11 percent in the ointment-treated group and by 9 percent in the orally treated group.

By the fifth day, alkaline phosphatase (ALP) activity increased marginally by 2% in healthy animals and by 4% in both groups of sick animals. The elevated ALP activity observed in the sick groups reflects the body's metabolic response to the pathogenic stimulus.

Table 3. Biochemical parameters of blood serum of laboratory animals (n = 15; M ± m)

Indicators	Background indicators	Day 1			Day 5		
		Group 1	Group 2	Group 3	Group 1	Group 2	Group 3
ALAT (Units/l)	57.8 ± 2.9	62.1 ± 3.73	69.6 ± 3.43*	55.7 ± 2.79	67.3 ± 3.1*	75.2 ± 3.7*	64.9 ± 3.5
ASAT (Units/l)	80.9 ± 4.1	89.3 ± 4.5	95.7 ± 4.79*	73.9 ± 3.7	92.4 ± 5.0*	105.3 ± 5.3 *	88.4 ± 4.42
GGT (Units/l)	6.5 ± 0.33	6.0 ± 0.3	8.9 ± 0.45*	6.23 ± 0.26	6.6 ± 0.37	10.5 ± 0.53*	6.2 ± 0.46
Amylase (Units/l)	175.5 ± 8.8	171.6 ± 8.9	223.1 ± 11.2*	199.2 ± 9.9 *	185.3 ± 9.3	244.0 ± 12.2 *	224.2 ± 11.2*
Alkaline phosphatase (Units/l)	7.5 ± 0.38	7.62 ± 0.4	18.1 ± 0.9*	18.8 ± 0.94 *	7.8 ± 0.42	18.9 ± 0.94*	19.6 ± 0.98*
Creatinine (mmol/l)	80.9 ± 4.1	72.7 ± 3.64	112.7 ± 5.64*	93.7 ± 3.19 *	85.3 ± 4.3	113.5 ± 5.7*	95.7 ± 4.79*
Urea (mmol/l)	6.7 ± 0.34	6.91 ± 0.35	9.5 ± 0.48*	9.09 ± 0.46	7.2 ± 0.36	10.2 ± 0.51*	10.4 ± 0.53*
Cholesterol (mmol/l)	0.9 ± 0.1	0.87 ± 0.04	1.5 ± 0.08*	1.1 ± 0.06	1.0 ± 0.1	1.4 ± 0.06*	1.2 ± 0.05*
Glucose (mmol/l)	6.5 ± 0.33	7.2 ± 0.36	5.5 ± 0.28*	5.1 ± 0.2*	6.22 ± 0.31	5.49 ± 0.27*	4.9 ± 0.25*
Total protein (g/l)	60.5 ± 3.0	57.1 ± 2.86	59.4 ± 3.0	60.3 ± 3.02	59.7 ± 2.99	63.1 ± 3.16	62.2 ± 3.11
Albumin (g/l)	30.9 ± 1.55	32.8 ± 1.64	48.2 ± 2.41*	50.9 ± 2.6*	29.4 ± 1.47	53.4 ± 2.67*	55.1 ± 2.76

Note: *P ≤ 0.05, the difference is significant concerning the background indicators

Throughout the experiment, serum creatinine levels in clinically healthy animals exhibited no meaningful fluctuations. A comparable pattern was noted in sick animals treated with oral tetracycline, where creatinine values stayed consistent with those recorded on day one. In contrast, the group of sick animals receiving tetracycline in ointment form showed a marginal elevation in creatinine concentration, increasing by 2%.

Upon completion of tetracycline administration, there was a progressive rise in blood urea levels across all study groups. Specifically, the urea content grew by 4% in the healthy control group, by 7% in the orally treated sick

animals, and by 13% in the group subjected to tetracycline ointment therapy. This elevation is indicative of pathological changes linked to the visual organ disorder.

Cholesterol levels in the healthy animals were maintained within the reference range throughout the experimental timeline. However, in sick animals treated with oral tetracycline, cholesterol concentrations dropped by 7% by day five, whereas in those treated with the ointment preparation, a contrary effect was observed — cholesterol increased by 8%.

Serum glucose levels remained stable in the healthy group until the final day of observation, showing no statistically significant variation. Likewise, glucose content in sick animals receiving oral tetracycline remained unchanged during the study period. On the other hand, a decline of 4% was observed in the group treated with tetracycline ointment.

Following the final administration of tetracycline, the total protein concentration increased across all experimental groups. In healthy animals, a 4% rise was recorded, while sick animals receiving oral antibiotic treatment exhibited a 6% increase, and those treated with the ointment showed a 3% elevation in total protein levels.

Regarding albumin content, no significant differences were detected in the healthy group after the last blood collection. Nevertheless, sick animals treated orally with tetracycline demonstrated a 10% increase in serum albumin, while the ointment-treated group showed an 8% rise. Notably, albumin values in both sick animal groups remained elevated beyond normal limits throughout the experiment, reflecting an inflammatory response of the organism to the invading pathogenic factor.

Conclusion

Following the administration of *Staphylococcus aureus* suspension, characteristic clinical manifestations of ocular pathology were observed. By the fourth day, symptoms included conjunctival hyperemia, pronounced pericorneal vascular injection, swelling of the cornea and iris, with evident dilation of the iris vasculature. On the fifth day, clinical signs progressed, displaying intense lacrimation, conjunctival edema, scleral injection, and pain localized to the ciliary region.

By the ninth day post-infection, complete clinical recovery was noted in all animals belonging to the second and third groups. This recovery was confirmed by the stabilization of systemic parameters such as respiration rate, pulse frequency, and normalization of body temperature. Furthermore, in the visual organ, inflammatory changes affecting the cornea, iris, and conjunctiva had resolved entirely in two animals from the second group.

Throughout the experimental period, serum tetracycline levels in the first group rose by 19%, while in the second group, the increase reached 22%. In contrast, the concentration of tetracycline in the serum of animals from the third group remained largely unchanged throughout the study.

Regarding the intraocular fluid, antibiotic concentrations in the first and second groups were approximately 5.5 times lower than those recorded in blood serum. By the fifth day, a slight increase in tetracycline concentration was recorded in clinically healthy animals, while in the second group, the levels rose by 1.5 times, and in the third group, by 2.2 times. Consequently, therapeutic levels of tetracycline were maintained within the intraocular fluid of healthy animals throughout the experiment. However, in sick animals receiving oral tetracycline, the peak concentration was not achieved by the study's end. In the third group, therapeutic levels were not reached at all.

Notably, therapeutic concentrations of tetracycline within the intraocular environment were effective only against gram-positive bacteria (0.5 - 1.5 micrograms/mg), indicating limited efficacy against gram-negative strains due to insufficient bacteriostatic levels.

Moreover, the antibiotic concentration within the intraocular fluid of infected animals was found to be approximately double that of healthy counterparts, indirectly suggesting that inflammatory processes compromised the integrity of the blood-aqueous barrier.

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Conflict of Interest: None

Financial Support: None

Ethics Statement: All experimental procedures involving laboratory animals were conducted in strict accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes.

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