OnLine Journal of Biological Sciences

Research Article

Morphological Changes in Brain Tissue During Experimental Hypoxia

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Article history
Received: September 05, 2024
Revised: March 06, 2025
Accepted: March 25, 2025

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Abstract: The extensive application of hypoxic training to enhance cerebral circulation highlights its significant role in effectively realizing the therapeutic and health-promoting benefits of this method. The discrepancy between the data on the features of cerebral circulation and cellular metabolism in brain tissues during circulatory hypoxia and significant interest in the physiological patterns of the integrative response of the body determined the feasibility of this study. Hypoxic training is widely used in sports and rehabilitation medicine and it is relevant to study the adaptation mechanisms of this condition. To study the morpho-functional features of cerebral circulation, brain tissue after experimental hypoxic normobaric training. The experiments involved Sprague Dawley rats, which were placed in a hypoxic chamber for 45 minutes daily over periods of 15 and 30 days. Morphological and physiological assessments were performed. Blood flow velocity in the middle cerebral artery of the experimental animals was measured using ultrasound Doppler imaging. A cycle of hypoxic training led to a decrease in the rhythmic (pulse) index and an increase in the functional activity of cells, indicating the expression of cerebral vascular responses and an increase in cerebral blood flow velocity. To safeguard the brain from hypoxia, robust mechanisms regulating cerebral circulation enhance blood flow to compensate for the reduced oxygen levels in arterial blood. The data of this study showed that hypoxic training leads to an increase in brain metabolism and an improvement in cerebral circulation, which is an adaptive response of the cardiorespiratory system of the body after hypoxic training.

Keywords: Hypoxic Training, Blood, Brain, Blood Flow

Introduction

The brain is a crucial organ that requires a continuous and sufficient blood supply to deliver oxygen and glucose according to the metabolic demands of active neurons. Is known that cerebral blood flow to be regulated in response to neuronal activity as well as to various chemical signals (Steinback, 2016).

The brain consumes a significant amount of energy compared to its weight and size. It has a high metabolic activity and is extremely sensitive to hypoxia and hypoperfusion. Cellular damage can begin within minutes and irreversible brain damage will follow if not treated surgically. For this reason, it is essential to understand the clinical presentation, pathophysiology, and treatment options. This topic reviews the causes and manifestations of hypoxic traumatic brain injury and emphasizes the role of the interprofessional team in its management. (Lacerte *et al.,* 2024). In hypoxic-ischemic brain injury, the most critical physiological variable is cerebral oxygen delivery. When the supply of oxygen falls short of what cells require, a cascade of biochemical processes is initiated, ultimately resulting in cell death. Focal and generalized cerebrovascular accidents most often cause severe functional and morphological abnormalities in the central nervous system. (Betz, 1987; Hossmann, 1999).

Under experimental conditions, oxygen delivery to the brain is impaired, primarily by reduced blood flow, such as by blockade of the feeding cerebral artery. As cerebral blood flow values decrease, metabolic and electrophysiological functions gradually disappear in accordance with the threshold concept of cerebral ischemia: first, the most complex functions, such as protein synthesis or spontaneous electrical activity, are suppressed, and then, at significantly lower flow values, the energy state is impaired and cell membranes are depolarized (Shimoda *et al.,* 2011; Lee *et al.,* 2019).

Under hypoxia conditions, not only cerebral blood flow is increased but also energy metabolism is altered (Vestergaard *et al.* 2016, Philip., 2013). Hypoxia causes a complex restructuring of the functioning of various body systems (Yu *et al.,* 2023), delivery the required necessary amount of oxygen to the tissues. The body's adaptation to hypoxia has a significant affects the central nervous system, central hemodynamics, blood microcirculation in various organs, oxygen metabolism, free radical-induced lipid oxidation, key detoxification enzymes, and immune function (Trayhurn *et al.,* 2008).

The mechanisms of the influence adaptation to hypoxia on the brain are considered. It has been established that improved cerebral circulation is one of the important protective effects of adaptation to hypoxia (Chen *et al.,* 2020; Davie *et al.,* 2023). It is of interest to study the effect of oxygen deficiency in inhaled air on brain functions in an experiment. The purpose of this study is to study the morpho-physiological features of brain tissue at various times after experimental hypoxia at an altitude of 2,900–3,000 m.

Materials and Methods

Ethical approval. The study protocols were approved by the Local Ethics Committee at the Institute of Genetics and Physiology CS MSHE RK in Almaty, Kazakhstan (Protocol No. 5 of 25.07.2022). The experiments were performed on animals housed in a vivarium under standard conditions, with natural lighting and a regular laboratory animal diet (GOST R 50258-92). The laboratory animal management in the vivarium met the European Convention for the Protection of Vertebrates animals (Strasbourg, 1986) and the guidelines outlined in the European Union Directive 2010/63/EU of 22 September 2010, titled “On the protection of animals used for scientific purposes”.

Study subject. Forty-five male Sprague Dawley (SD) rats, weighing 265±10 g, were exposed to circulatory hypoxia. The rats were then divided into three groups:

- Group I – normoxic control (n = 14)
- Group II (n = 16) – experimental (circulatory hypoxia 15 days)
- Group III (n = 15) – experimental (circulatory hypoxia 30 days)

The experimental group was subjected to hypoxia for 45 minutes daily over periods of 15 and 30 days (Kilic, 2022). The rats were maintained on a standard vivarium diet.

Hypoxic training. Rats in Groups II and III underwent hypoxic training in a hypoxia isolation chamber for animals (Ox-100, Shanghai TOW Intelligent Technology Co., Ltd.) fitted with sensors for temperature, humidity, oxygen (O_2), and carbon dioxide (CO_2). Each session lasted 45 minutes. The oxygen concentration started at 20.5% and dropped to 14.8% in 4–5 minutes, simulating an altitude of 2,900–3,000 meters.

- Oxygen levels were decreased with the help of a 10L nitrogen concentrator
- Using medical soda lime in open containers resulted in a reduction of CO_2content

Morphological study. After the experiment, the brain was surgically removed and preserved in 10% neutral formalin, embedded in paraffin, and histologically processed. Standard dehydration steps were followed using increasing concentrations of ethanol and xylene. Using a rotary microtome, 3–5 μm thick sections were prepared (Sakura Accu-Cut® SRM™ 200). Sections were stained with hematoxylin and eosin using Bio-Optica staining kits (Milan, Italy).

Blood flow rate study. The rate of arterial and venous blood flow was studied using laser ultrasonic Dopplerography (Sanomed-300, Russia).

Parameters measured:

- Maximum blood flow velocity (Vmax)
- Pulsation Index (PI)
- Resistance Index (RI)
- Statistical formulas were based on Köktener *et al.* (2012); Abdreshov *et al.* (2021)

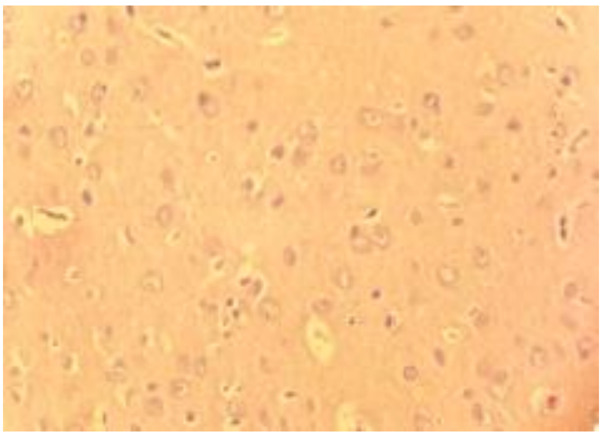
Statistical Analysis

- Fisher criterion was used to test homogeneity
- Kolmogorov-Smirnov criterion for distribution normality
- Data analyzed in StatPlus Pro 2009
- Student’s t-test used
- Values presented as mean±standard error (M±m)
- Significance set at $p<0.05$

Results

The brain lies in the cranial cavity and is covered by three membranes: superficial, hard, adjacent to the brain, soft and arachnoid lying between them. The rat's brain weighs 2.4–2.8 g, which is about 0.9% of the animal's body weight. Conventionally, the boundary between the brain and spinal cord is drawn at the exit the first cervical nerve. The brain in animals is surrounded by the dura mater, arachnoid mater, and pia mater. The dura mater houses venous sinuses that provide blood drainage from the brain. The arachnoid mater is characterized by the absence of vessels brain (Dyce *et al.,* 1987; Budras *et al.,* 2003). Between it and the pia mater of the brain are subarachnoid spaces, which are absent in the area of the convolutions of the brain, where both membranes grow together. The pia mater grows together with the brain tissue, the surface is lined with a single-layer squamous epithelium, under which lie thin collagen fibrils of connective tissue. It contains blood vessels that form the vascular network of the brain (Zelenevsky *et al.,* 2014; Bon *et al.,* 1998). During survey microscopy in control animals, the results obtained by us corresponded to the descriptions of the cytoarchitectural features of the frontal lobe of the brain Figure 1.

When examining the brain of animals in the control group, a typical modular structure of the cortex of the hemispheres of the telencephalon was noted. When stained with hematoxylin and eosin in each module, layers of densely located neurons were determined. In the molecular layer, a few small neurons with spindle-shaped perikaryal cytoplasm were found scattered among the processes of neurocytes from the deeper layers. The outer granular layer is formed by small neurons with a diameter of about 10 μm, having a rounded, angular and pyramidal shape of the cytoplasm of perikarya, as well as stellate neurons with a diameter of about 20 μm. The processes of these cells formed plexuses, located parallel or tangential to the plane of the frontal section.



Hematoxylin and eosin staining. 100x magnification

Fig. 1: Brain morphological index in control rats

When rats were put into a chamber with decreased oxygen concentration, the animals showed motor activity, moved around the cage and made cosmetic movements. And then, after 5-6 minutes, they concentrated together and did not show motor activity. Table 1 shows the effect of hypoxia on the general condition of the animal during hypoxic training. The weight of animals and the weight of the brain, lymph flow, and urine output taken for research after hypoxic training are presented in Table 1. In animals with simulated hypoxia for 30 days, the brain weight averaged 1.98±0.03 grams (in the control groups, 2.01±0.04 grams). During macroscopic examination of the brain of animals in the group after hypoxia, the brain substance after extraction from the cranial cavity was noted to have the correct anatomical shape, and the tissue in the section was gray-pink.

The signaling systems and mechanisms underlying the adaptive mechanisms that remain unclear were considered, which makes this study relevant. The main parameters of arterial and venous blood flow in animals before and after hypoxia were studied. It is known that absolute parameters of blood flow at rest cannot serve as an adequate criterion for assessing the consistency of cerebral blood flow due to the impossibility of assessing its reserve capabilities (Claassen *et al*., 2007; Naqvi *et al*., 2013). Using modern ultrasound Dopplerography during the study, allows to determine the of blood flow velocity in the cerebral circulation. When analyzing the parameters of cerebral hemodynamics in animals of the 2nd experimental group, an increase in PI and a decrease in RI were observed at the extra- and intracranial levels ($p<0.05$), and a statistically significant increase in Vmax in cerebral circulation. The values of the pulsation index in the studied vessels are shown in Table 2. There was a significant decrease in the values of qualitative indicators in the cerebralarteries. Simultaneously, the greatest rate of change occurred during the initial days of the experiment, and the cerebral artery exhibited a notable decline in the pulsation index over the course of the study, indicating a reduction in resistance within the main vessels throughout the experiment.

Table 2 presents the impact of hypoxia on cerebral blood flow hemodynamics. In animals with experimental hypoxia, an increase the blood flow velocity in the brain area of 30.7±0.03 cm/s was found, the control group it was 22.3±0.05 cm/s. The resistance in the main vessel index RI decreased by 19.7%, which is associated with an enlargement of the main vessel's diameter. With hypoxia, a decrease in the rhythmic (pulse) index by 16.8% was recorded (Table 2, Figure 2).

Table 1: Animal and brain weight during hypoxic training at an altitude of 2,900-3,000 above sea level

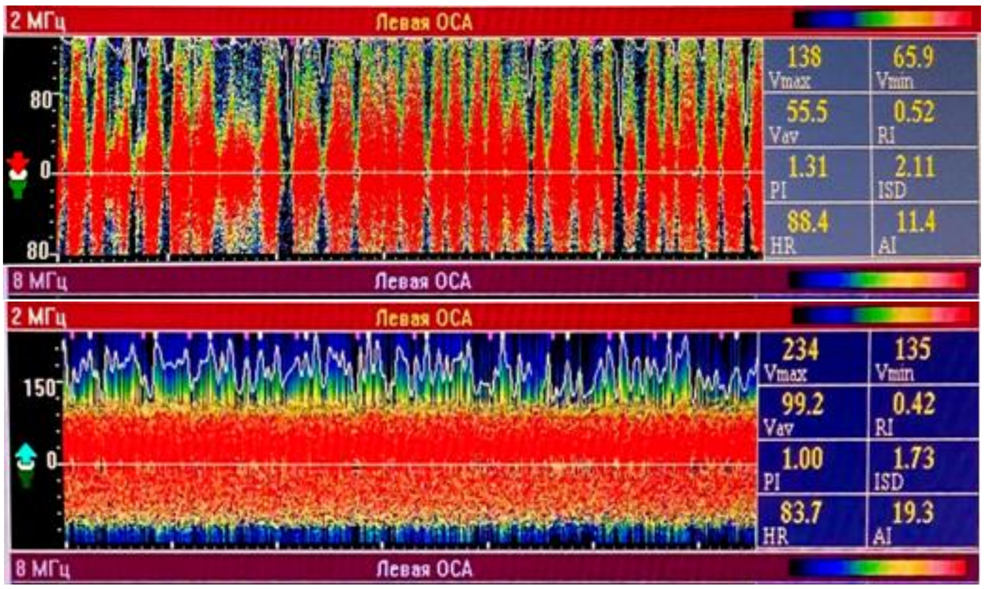
Indicators	Before hypoxic training (control)	After hypoxic training	
		Hypoxia 15 days	Hypoxia 30 days
Weight of rats in g.	265±10	255,7±10,4**	253,2±9,7**
Brainweight in g.	2,01±0,04	1,98±0,03*	1,99±0,01
Urine ml/min	0,05±0,001	0,04±0,006	0,043±0,002
Lymph ml/min	0,1±0,001	0,012±0,002**	0.07±0.01**

Note: A significant change compared to the control group, *p<0.05, **p<0.001

Table 2: Cerebral vessel blood flow characteristics in rats subjected to experimental hypoxia

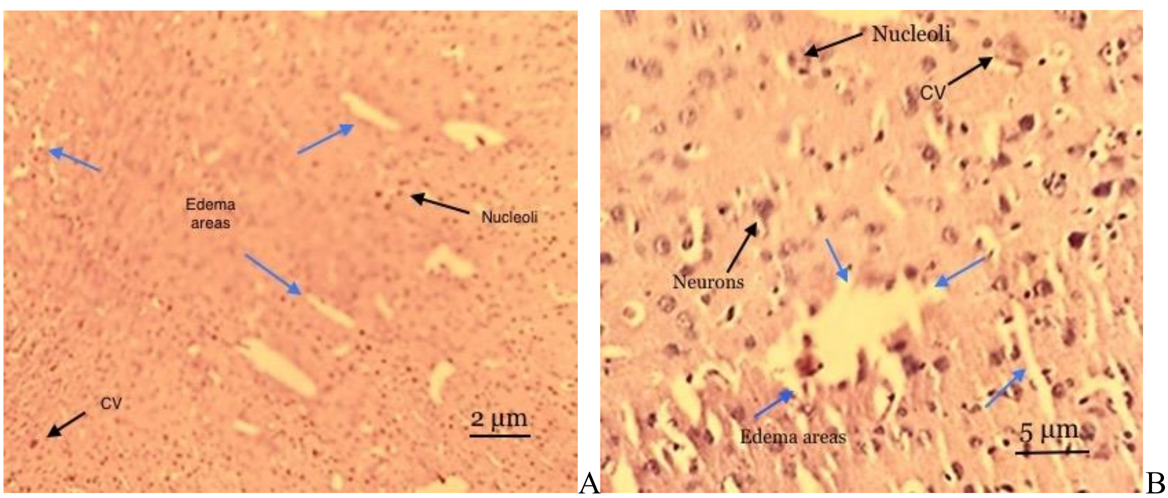
Groups	Flow velocity of blood, measured in centimeters per second (cm/s)	PI (Pulsation index)	RI (Resistance index)
Control group	22,3±0,05	3,09±0,04	0,76±0,03
Group after hypoxia	30,7±0,03**	2,57±0,08*	0,61±0,02*

Note: Significantly different from control, *p<0.05, **p<0.001



Designations: A - control, B - after after hypoxia

Fig. 2: Excerpt from an experiment showing a Dopplerogram of the brain region in rats



A. Hypoxia exposure for 15 days, B – Hypoxia exposure for 30 days. Hematoxylin and eosin staining. 100x magnification

Fig. 3: The brain of rats under hypoxia

Changes in vascular resistance within the brain's blood vessels were examined and analyzed. The systolic-diastolic ratio and pulsation index of blood flow in the main vessels and middle cerebral arteries were evaluated. In the middle cerebral artery, the pulsation index and resistance index decreased by 16.8 and 19.7%, respectively, in the experimental animal groups. The conducted studies demonstrated that ultrasound Doppler examination of major vessels in animals with hypoxia enables the detection of significant improvements in blood circulation. The study revealed that tissue blood supply rates and peripheral vessel elasticity improved compared to the control group. Hypoxic training effectively normalizes hemodynamic parameters. The rate of rapid blood filling and tissue blood supply increased, significantly changed compared to the control group.

When stained with hematoxylin and eosin after 15 days of hypoxia, nucleoli were not found in all neurons. Edema was detected around most neurons, in other cases, manifestations of cytoplasmic vacuolization were detected, which suggests a possible increase in the functional activity of cells Figure 3. It was shown that hemorrhages occurred against the background of small focal and diffuse changes in the brain matter, foci of perivascular edema, necrosis of brain tissue in the perivascular region, edema of the periventricular white matter with the destruction of myelin fibers, swelling and fragmentation of axons with an accompanying macrophage reaction. Hypoxia leads to elevated arterial pressure and enhanced cerebral blood flow, along with variability in arterial pressure. (Kenichi *et al.,* 2007; Willie *et al.,* 2011). Nevertheless, these alterations reached significance only under 14.5% O_2 hypoxia, implying the existence of a potential threshold for these effects.

Shown are changes in the lumen of small blood vessels throughout the entire section of brain matter against the background of edema in the tissue of the perivascular region, small focal and diffuse changes in the brain matter, edema of the periventricular white matter of myelin fibers, swelling and fragmentation of axons with an accompanying macrophage reaction.

At the morphological level, during experimental hypoxia, changes are observed in the structures of the brain, such as edema and the appearance of signs of impaired microcirculation in the vascular systems and changes in blood flow velocity in the arterial and venous vessels. In animals with experimental 15-day hypoxia, pronounced morphofunctional changes in the brain were detected. Histological examination revealed destructive changes in the cortex of the hemispheres and the white matter of the rat's cerebral cortex. A decrease in their total volume RI and an increase in PI in the blood flow of the cerebral vessels in rats were observed. Simultaneously, alterations in blood flow velocity within the vascular system were more evident in the experimental animals during induced hypoxia. In this regard, it can be assumed that with acute exposure to hypoxia, since it causes a violation of cerebral homeostasis in white rats, but after 30 days of hypoxia, adaptation of the body to hypoxic training is observed. Therefore, hypoxia leads to both functional and structural alterations in the brain, which are associated with various morphofunctional modifications. At present, the study of damage and regeneration processes in various parts of the brain, in particular the vessels of the microcirculatory bed, remains relevant.

Discussion

The decrease in the values of qualitative indicators of blood flow in the main arteries is due to a decrease in resistance to blood flow in the cerebral blood flow basin, which is due to physiological changes in the walls of the spiral arteries, which occur during the period due to physiological hypoxic changes in the walls of the arteries, which occur during the experiment. During histological examination focused on the hypothalamic region, a sharp overfilling of the vessels of the choroid plexus of the ventricles was noted. This condition may indicate a violation of venous outflow or an increase in arterial blood flow, which in turn can cause additional disorders in the functioning of the central nervous system. Moreover, a mild rise in cerebrospinal fluid volume within the ventricles is another important factor. This may indicate increased pressure inside the skull or other pathologies affecting cerebrospinal fluid dynamics.

All these observations emphasize the importance of careful histological and morphological analysis for understanding the mechanisms of pathology development in the central nervous system, which can have far-reaching consequences for the health of the animals studied and, potentially, for understanding similar processes in humans. During autopsy, it was observed that transparent or slightly colored fluid flowed out of the cerebral ventricles under pressure. This phenomenon, despite the fact that the ventricular cavities did not show signs of expansion, indicates the presence of certain pathophysiological processes. It is important to emphasize that the lack of focal alterations suggests a systemic reaction rather than localized injury (Yang *et al.,* 2013).

Metabolism in different regions of the brain is an important aspect of neurobiological research. A recent study by Mun and colleagues in 2023 provided an in-depth assessment of the metabolic changes that occur in the brain during hypoxic exposure. Oxygen deficiency, or hypoxia, can profoundly impact brain function, and gaining insight into these mechanisms holds valuable significance for both medicine and neuropsychology (Mun *et al.,* 2021).

As a result of the morphological and morphometric studies carried out on the samples, an important fact was established: an increase in the diameter of capillaries is observed in both the cerebral cortex and the thalamus.

Thus, the obtained results of the experiment after 15 days of experimental hypoxia indicate that the revealed morphofunctional changes in the brain tissue of white rats have not only common patterns, but also their own characteristics. Exposure to hypoxia resulted in more pronounced functional changes within the central nervous system structures. The observed vascular responses in blood flow demonstrate notable alterations in these parameters within the brain during hypoxic training, showing distinct specificity across different brain regions.

After 30 days of cerebral hypoxia, stronger compensatory responses are seen in the vascular plexuses and brain capillaries, highlighting the need for a more detailed investigation. The observed changes in the brain's vascular system may greatly enhance its adaptive capacity.

Conclusion

The most important and rapid adaptive effect of hypoxia is the expansion of capillaries in the brain, heart and lungs, which goes hand in hand with increased blood circulation, due to which the minute volume of blood circulation increases. The body has an extensive arsenal of behavioral, physiological, biochemical tools for restoring adequate oxygen conservation, and this arsenal can be successfully activated and increased with the help of hypoxic training. The oxygen status of the body includes the hypoxic state as an integral regulatory reaction.

Thus, the study of the rate of cerebral blood flow, cerebral circulation in general during hypoxic training is crucial for understanding brain function, but the signaling systems and mechanisms underlying its regulation remain unclear, which makes our study relevant.

The data obtained allow us to conclude that in the study conducted, hypoxic training at altitudes of 2,900-3,000 m stimulates an increase in cerebral blood flow, supporting oxygen delivery to the brain. The most important and rapid adaptive effect to hypoxia is the dilation of capillaries in the brain, which is accompanied by an increase in blood flow, resulting in an increase in circulatory minute volume. The body has an extensive arsenal of behavioural, physiological and biochemical tools to restore adequate oxygen conservation, and this arsenal can be successfully activated and increased by cycles of hypoxic training.

The oxygen status of the body includes the hypoxic state as an integral regulatory response. Cerebral blood flow velocity, cerebral blood flow in general during hypoxic exercise is crucial for understanding brain function, but the signalling systems and mechanisms underlying its regulation require further research. Cerebral blood flow velocity and its state during moments of hypoxic exercise is becoming a relevant task for understanding brain function. However, the signalling systems and mechanisms governing this complex regulation remain unclear, which emphasises the importance of further research.

The study reveals relationships between oxygen status and neurological responses in the body, thereby advancing knowledge of brain processes. Our study contributes to neurophysiology by providing a better understanding of how the brain adapts to conditions of reduced oxygen concentration. The results of the study are an important contribution to the development of sports science, opening new horizons in athlete training practices and rehabilitation methods.

Acknowledgment

We gratefully acknowledge the Committee of Science of the Ministry of Science and Higher Education of the Republic of Kazakhstan for funding this project. Our sincere thanks also go to the publisher for their support in disseminating this research, and to the editorial team for their valuable review and editing, which enabled us to share our work with the wider scientific community.

Funding Information

This study was supported within the framework fthe research project AP26101059 of the Committee of Science of the Ministry of Science and Higher Education of the Republic of Kazakhstan.

Author's Contributions

Serik Abdreshov: Conceptualization, Project Administration, Supervision, and Validation.
Aizhan Mussayeva and Georgii Demchenko: Methodology, Formal Analysis, and Data Curation.
Ulbossin Kozhaniyazova and Laura Koibasova: Investigation (*in vivo*), Resources, and Critical Revision of the manuscript.
Anar Yeshmukhanbet and Makpal Yessenova: Investigation (Histology and Morphology) and Technical Supervision.
Bayan Nurmakhanova and Zarina Orazymbetova: Investigation, Data Interpretation, and Contribution to the Discussion.

Rustembek Karjaubaev, Sholpan Bakhtybekkyzy and Aidana Bekitayeva: Investigation (Sampling and Analysis), Literature Search, and Writing Original Draft.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all co-authors have reviewed and approved the manuscript, and that there are no ethical issues involved.

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