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Abstract

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MORPHOLOGICAL CHANGES IN THE LUNGS IN THE CONTEXT OF QUERCETIN EFFECT AGAINST THE BACKGROUND OF CHRONIC EXPERIMENTAL HYPERGLYCEMIA

The widespread prevalence of diabetes, the early disability of the patients, and their high mortality have allowed WOH experts to determine the situation with diabetes as an epidemic of non-infectious disease, in which, in addition to carbohydrate disorders, there are deviations in all types of metabolism. The issue of etiotropic treatment of diabetes remains open today. The influence on the pathogenetic links of chronic hyperglycaemia often does not solve the problem of metabolic disorders and, as a result, does not prevent the occurrence of complications. In connection with this, there is a need to include treating with antioxidant and angioprotective properties in the treatment of patients with type I diabetes along with hypoglycaemic drugs. Macro- and microangiopathy, which lead to pneumosclerosis with impaired lung elasticity, serve as morphological evidence of affecting pulmonary structures by products of metabolism impaired as a result of chronic hyperglycemia. In view of this, we conducted a study of the structures of pulmonary tissue, blood vessels, and lymphoid follicles in the context of chronic alloxan hyperglycemia against the background of quercetin use. The drug is widely used in medical practice due to its broad-spectrum action..

The research was conducted involving 72 white laboratory rats of both sexes, aged 1 to 7 months, weighing 170.1 ± 0.13 g. Experimental animals were divided into two subgroups: 1) with aloxane hyperglycemia (36 rats), which served as control and 2) of rats who at the same time used quercetin (36 rats). The above subgroups were divided into six subgroups depending on the duration of the study: the first one – with the term of hyperglycemia 30 days, the second – 60 days, the third – 90 days, the fourth – 120 days, the fifth – with 150 days, the sixth – with the term hyperglycemia 180 days In accordance with a series of animals with experimental hyperglycemia with a duration of 30 to 180 days, the slaughter of rats of the corresponding age using the therapeutic agent was performed by thoracic intubation under the thoracic thiopental-sodium anesthesia. For experimental modeling of hyperglycemia, we used chemical compound alloxan.

As a result of the experiment, it was found that quercetin, due to the properties of reducing the permeability of the vascular wall, contributed to the reduction of the stasis; anti-inflammatory and immunomodulatory effect – reduction of size of lymphoid follicles, proliferation of

macrophages in comparison with naive animals. The anti-sclerotic effect of quercetin has been shown to slow down the development of fibrosis in the walls of the pulmonary vessels of the muscular type. On the background of his admission, the process of development of microangiopathy in the capillaries of interalveolar membranes was inhibited. But given that the protective properties of quercetin were demonstrated at the end of the experiment, it can be argued that the effect of accumulation is influenced. The analysis of micromorphometric indices in animals treated with quercetin showed a decrease in the intensity of emphysematic reconstruction of pulmonary structures against chronic hyperglycemia. These findings provide the basis for the use of quercetin for the prevention and treatment of initial complications of hyperglycemia as a corrector.

Keywords: aloxane hyperglycemia, rats, pulmonary arteries of the muscular type, quercetin, thickness of the alveolar barrier, thickness of the interstitium, thickness of the interalveolar septum, broncho-pulmonary lymph nodes.

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Резюме

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МОРФОЛОГІЧНІ ЗМІНИ В ЛЕГЕНЯХ ЗА УМОВ ДІЇ КВЕРЦЕТИНУ НА ТЛІ ХРОНІЧНОЇ ЕКСПЕРИМЕНТАЛЬНОЇ ГІПЕРГЛІКЕМІЇ

Цукровий діабет – це група метаболічних захворювань, які характеризуються хронічною гіперглікемією, що є результатом порушення секреції інсуліну, його властивостей або комбінацією обох факторів. Хронічна гіперглікемія при цукровому діабеті супроводжується ураженням, дисфункцією та недостатністю різних органів, особливо зорового аналізатору, нирок, нервів, серця та кровоносних судин.

Паралельно з вивченням впливу гіперглікемії на організм, ведеться пошук коректора, який міг би максимально нівелювати його. Велика увага приділяється препаратам, які володіють антиоксадантними та антидіабетогеннми властивостями. Незважаючи на це, дослідження направлені на виявлення протективного ефекту лікувальних засобів на організм вцілому, без детального вивчення органів респіраторної системи.

В статті описані зміни в легенях, легеневих артеріях м'язового типу, легеневих лімфоїдних фолікулах тварин, що перебували за умов хронічної гіперглікемії терміном 30 - 180 діб, та щурів, що одночасно вживали Кверцетин на тлі експерименту.

Зважаючи на характер змін у структурах легеневої тканини, та з метою корекції патогенетичних ланок розвитку алоксанової гіперглікемії, був обраний лікувальний засіб Кверцетин у вигляді гранул. Препарат чинить вплив на стінку капілярів, клітинних мембран, в результаті чого зменшується проникність судин та виникає антиоксидантний ефект. Кверцетин, блокуючи ліпооксигеназний шлях метаболізму арахідонової кислоти, зменшує розвиток запальних реакцій. Препарат володіє імуномодулюючою та антисклеротичною властивістю. Експериментально доведено, що Кверцетин стимулює вивільнення інсуліну.

У тварин на тлі прийому препарату, у порівнянні з щурами, що не вживали Кверцетин, стаз у легеневих артеріях розвивався пізніше; імуномодулюючий його ефект сприяв менш активній гіпертрофії

лімфоїдних фолікулів та гіперплазії макрофагів. Антисклеротична дія Кверцетину виявилась у сповільненні розвитку фіброзу у стінках легеневих судин м'язового типу. Зважаючи на те, що свої протективні властивості препарат проявив у кінці експерименту, можна стверджувати про вплив ефекту накопичення.

Ключові слова: алоксанова гіперглікемія, щурі, легеневі артерії м'язового типу, Кверцетин, товщина альвеолярного бар'єру, товщина інтерстицію, товщина міжальвеолярної перетинки, лімфоїдні фолікули.

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Introduction

The widespread prevalence of diabetes mellitus, early disability of patients and high mortality have given WHO experts the reason to identify the diabetes situation as an epidemic of non-infectious disease, in which except for carbohydrate disorders there are problems with all types of metabolism [1].

The issue of etiotropic therapy for diabetes mellitus remains topical. Influencing the pathogenetic mechanisms of chronic hyperglycemia development often does not solve the problem of metabolic disorders and, as a consequence, does not prevent complications. In this regard, there is a need to include therapies with antioxidant and angioprotective properties in the treatment regime of patients with type 1 diabetes, along with the sugar-lowering drugs.

Macro- and microangiopathy, which lead to pneumosclerosis with impaired lung elasticity, serve as morphological evidence of affecting pulmonary structures by products of metabolism impaired as a result of chronic hyperglycemia. Such changes of pulmonary tissue are described in the few works of domestic and foreign scientists Stepanian I. E., Sokolov Ye. I. and Rekha Jagadapillai [2, 3, 4]. In view of this, we conducted a study of the structures of pulmonary tissue, blood vessels, and lymphoid follicles in the context of chronic alloxan hyperglycemia against the background of quercetin use. The drug is widely used in medical practice due to its broad-spectrum action. Due to the capillary-stabilizing properties associated with the antioxidative and membrane-stabilizing effect, the drug reduces capillary permeability. Quercetin has an anti-inflammatory effect due to the blockade of lipoxygenase pathway of arachidonic acid metabolism and reduced synthesis of leukotrienes, serotonin and other mediators of inflammation. The drug has immunomodulatory activity. The antisclerotic properties were experimentally determined. Quercetin is capable of stimulating insulin release and inhibiting thromboxane synthesis [5, 6, 7].

The aim of the study was to define the influence of quercetin on changes in the morphology of muscular type pulmonary vessels, structural components of the lungs at micro- and ultrastructural levels, pulmonary lymphoid follicles in the context of chronic alloxan hyperglycemia.

Materials and methods. The studies were performed involving 72 white laboratory rodents of both sexes, 1 to 7 months old, of 170.1±0.13 g. The experimental animals were divided into two groups: 1) rats with alloxan hyperglycemia (36 rats), which served as the controls and 2) rats that were given quercetin apart from that (36 rats). The above subgroups were divided into six more subgroups, depending on the study period: subgroup I – with 30-days hyperglycemia, subgroup II – with 60-days hyperglycemia, subgroup III – with 90-days hyperglycemia, subgroup IV – with 120-days hyperglycemia, subgroup V – with 150-days hyperglycemia, subgroup VI – with 180-days hyperglycemia. In accordance with the series of animals with experimental 30 to 180 days hyperglycemia, the rats of the appropriate age that were given the drug, were sacrificed by cutting the chest under intra-abdominal thiopental sodium anesthesia.

The animals were kept in the vivarium of the Department of Morphology of Medical Institute, Sumy State University. Before the study, each group of rats had a two-week quarantine. Animals were kept according to standard guidelines, requirements and regulations for laboratory animal care (provisions of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1985); "Regulations for the Procedures Involving Experimental Animals", Annex 4, approved by the Order of the Ministry of Health

No. 755 dated August 12, 1997; "On Measures to Further Improvement of the Organizational Forms of Procedures Involving Experimental Animals"; Declaration of Helsinki of the General Assembly of the World Medical Association (2000); "General Ethical Principles for Animal Experiments" approved by the First National Congress on Bioethics (Kyiv, 2001); regulations approved by the Bioethics Committee of Medical Institute of Sumy State University (Protocol No. 4 dated December 22, 2009). There were no violations of moral and ethical standards during the study.

For experimental modeling of hyperglycemia, we used chemical compound alloxan. After 24-hour fasting on the background of normal blood tests, the animals were subcutaneously injected with a solution of alloxan monohydrate 20 mg per 100 g of rat body weight in 0.1 M citrate buffer (pH 4.0).

The following methods were used: histologic (using van Gieson's stain method with hematoxylin and eosin), ultramicroscopic with dynamic morphometry using the universal certified program "SEO Scan Lab 2.0" and "SEO Image Lab 2.0".

We measured the wall thickness of the muscular type pulmonary arteries, perimeter of the lymphoid follicles, width of the alveoli, depth of the alveoli, width of the alveolar duct entrance, width of the conductive bronchioles, thickness of the interalveolar septa, thickness of interstitial tissue, thickness of blood-air barrier. Blood glucose levels were also determined by means of glucose oxidase method using Filisit reagent kits («Філісіт», Ukraine) and the level of glycated hemoglobin (HbA1c) was measured using BioSystems reagent kits (Spain) before each sacrifice of animals.

All figures obtained were statistically processed using Acer personal computer and a licensed spreadsheet editor Microsoft Office Excel 2013. The arithmetic mean (M), the mean error of the mean (m) were calculated. The significance of the difference (p) was determined using Student's t test (t), with the error being less than 5% ($p < 0.05$).

Study results and discussion. During the first experimental month, the animals had classic symptoms of hyperglycemia: polyuria, polyphagia, polydipsia. Blood glucose levels ranged 13.1 to 19.3 mmol/L, glycated hemoglobin increased starting from the 90-th day of the experiment (8.2 ± 0.4%), indicating chronic hyperglycemia in animals.

No visible changes were detected in the lung tissue during microscopic examination from the 30-th to 150-th day, but on the 180-th day of chronic hyperglycemia accompanied by the administration of quercetin a small intravascular accumulation of blood elements in some vessels was found. There was a slight increase in mature collagen fibers in the walls of the muscular type arteries (t.media et t.externa), as well as interalveolar septa. Some specimens showed signs of focal dystelectasis and minor hypertrophy of lymphoid follicles.

The proliferation of type II alveolocytes was observed from the fourth month of the experiment, but to a lesser extent than in the rats with chronic hyperglycemia that were not given quercetin.The effect of quercetin on the thickness of the blood-air barrier (BAB) was detected as early as on the 60-th day of its administration to animals. The BAB value (418.4±1.1 nm) in the drug-receiving animals was 0.41% ($p < 0.05$) less than in the animals that were not given it (BAB was 420.1±0.4 nm). These phenomena were observed against the background of reduced vacuolization in pneumocytes and lesser sclerosis of the pulmonary stroma than in animals with hyperglycemia (Fig. 1).

The antioxidative properties of quercetin have been shown to slow the development of sclerotic changes in pulmonary interstitium against the background of chronic hyperglycemia since the 60th day of the experiment, the thickness of interstitium (TI) (81.3±0.31 nm) was less by 0.74% ($p < 0.05$) than that in animals that were not given quercetin (TI was 81.9±1.14 nm). The processes of macrophage proliferation and destruction of type II alveolocytes were lesser than those in the control group (Fig. 2).

Quercetin-induced initial changes at microlevel in pulmonary tissue structures began on the 150-th day of alloxan hyperglycemia. Width of the alveoli (81.9±0.18 μm) (WA) was significantly lesser than that in the quercetin-naive animal group (83.1±0.43 μm) by 1.5% ($p < 0.05$) and on the 180-th day, when restrictive changes prevailed, this value increased by 1.9% ($p < 0.05$) as compared to the animals with hyperglycemia. Depth of the alveoli (DA) increased from 150-th day by 0.2% ($p < 0.05$) and increased by 18.8% ($p < 0.05$) by the 180-th day as compared with quercetin-naive animals. Width of the alveolar duct entrance (WAE) varied from 150-th day of experience: after five months of the experiment WAE in quercetin-treated animals was 1.4% less ($p < 0.05$) as compared with rats with hyperglycemia; after six months it was 11.5% less ($p < 0.05$).

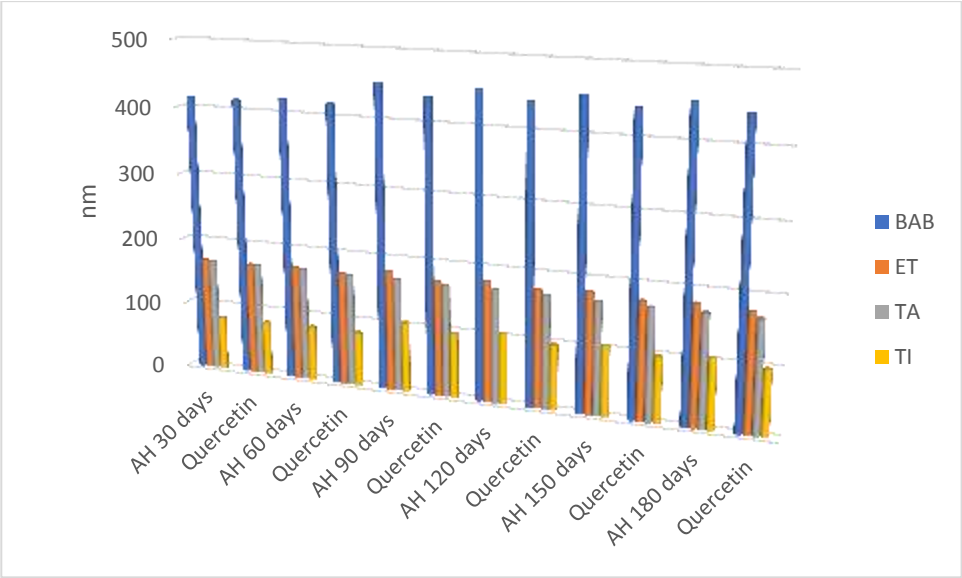

Figure 1 – Effect of quercetin on ultramicroscopic lung structures of rodents with chronic hyperglycemia. AH – alloxan hyperglycemia, BAB – thickness of blood-air barrier, ET – endothelial cell thickness, TA – thickness of alveolocyte, TI – thickness of interstitium

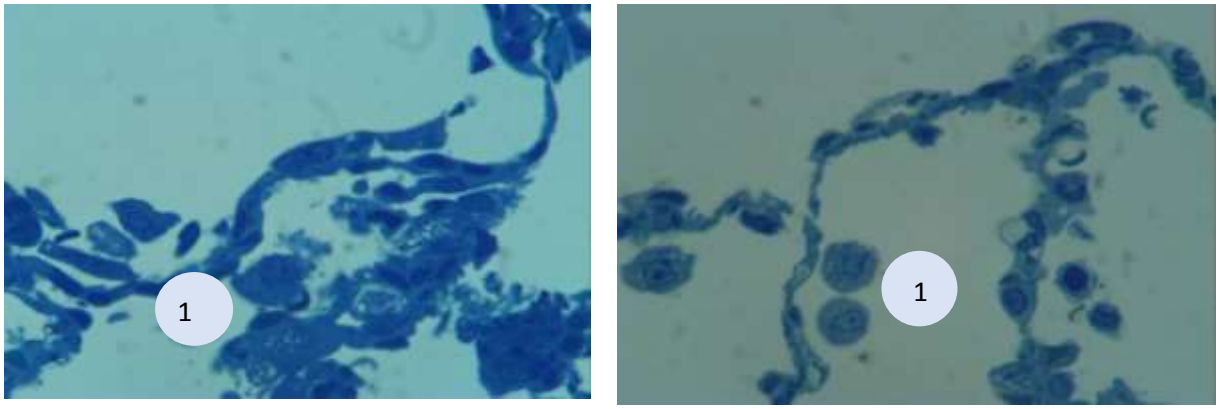

Figure 2 – Cell structure of lung tissue in young rats with experimental hyperglycemia lasting 60 days (2) and against the background of quercetin administration (1). Toluidine blue stain ×1000. 1.1 – a single macrophage in the lumen of the alveoli; 1.2 – macrophage accumulation in the lumen of the alveoli

Width of the conductive bronchioles (WCB) in the background of quercetin administration increased less actively as compared with the quercetin-naive group by the 150-th day of experiment, their size was 0.14% smaller ($p < 0.05$) and up to the 180-th day it increased by 1.3% ($p < 0.05$) (quercetin-naive rats had this index increased by 21.8% ($p < 0.05$)). Thickness of the interalveolar septa (TIS) was less from the 150-th day of the experiment against the background of quercetin administration (in five months by 4.9% ($p < 0.05$), in six months by 61.5% ($p < 0.05$)). Depth of the alveoli (DA) after six months of the experiment was 8.3% more in quercetin-receiving animals ($p < 0.05$) than that in the naive group (Fig. 3).

The walls of the muscular type pulmonary arteries were not significantly thickened since the fifth month of the experiment in the background of quercetin administration. Compared with the 120-th day of the experiment, the thickness of the vessel wall (TVW) decreased by 0.72% ($p < 0.05$ (from 115.7±0.36 μm to 115.5±0.14 μm) (in quercetin-naive group TVW increased by 1.34% ($p < 0.05$) from 115.7±0.36 μm to 116.2±1.4 μm). On the 180-th day of the experiment, TVW (115.5 ± 0.08 μm) was 2.59% ($p < 0.05$) lower than that in the group of quercetin-naive animals (TVW was 118.5 ± 0.44 μm). The reduction of the TVW by 120-th day was due to a significant 1.1% decrease in the thickness of the vessel outer coat ($p < 0.05$).

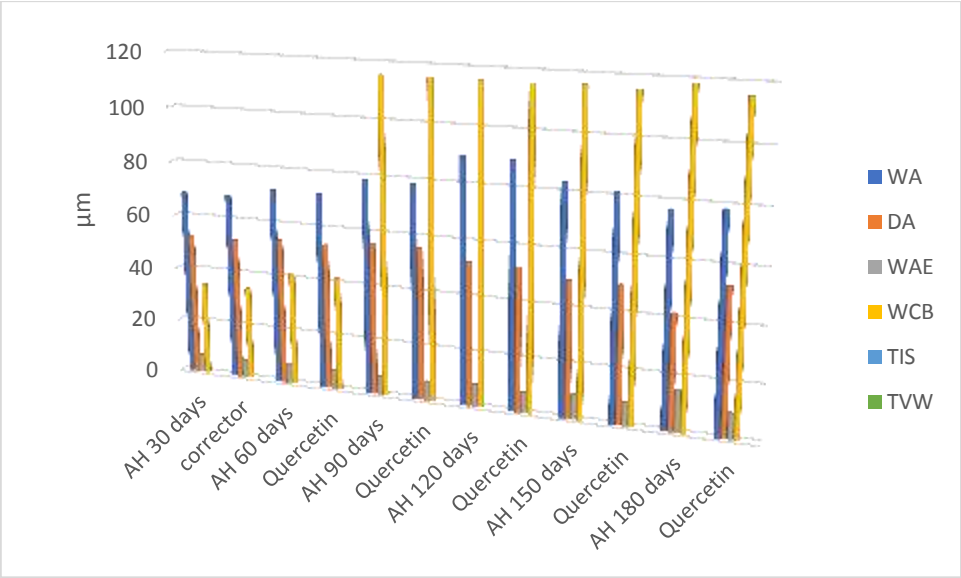

Figure 3 – Effect of quercetin on texture of lungs, muscular type pulmonary arteries, lymphoid follicles in rodents with chronic hyperglycemia. AH – alloxan hyperglycemia, LFP – lymphoid follicles perimeter, TVW – thickness of vessel wall, WA – width of alveoli, DA – depth of alveoli, WAE – width of the alveolar duct entrance, WCB – width of the conductive bronchioles, TIS – thickness of the interalveolar septa

Reaction of peripheral immune system organs started on the 60-th day on the background of quercetin administration. Size of lymphoid follicles on the 90th day of the experiment was 1.2 ($p < 0.05$) times smaller than that in the quercetin-naive group. On the 120-th, 150-th, and 180-th days of chronic hyperglycemia, lymphoid follicles perimeters were 1.14, 1.73, and 1.5 ($p < 0.05$) times lesser, respectively, than in quercetin-naive groups, confirming data on quercetin high protective effectiveness (Table 1).

Conclusions

The antisclerotic effect of quercetin has been found to slow the hyperplasia of mature collagen fibers in the outer coat and myocytes in the middle membrane of muscular type pulmonary vessels. However, due to the fact that quercetin showed its protective properties at the end of the experiment, accumulation effect can be assumed. Analysis of micromorphometric parameters in quercetin-receiving animals from 30 to 180 days of the experiment showed an increase in WA by 15.4% ($p < 0.05$), in quercetin-naive rats – by 12.7% ($p < 0.05$); WAE – by 55.7% ($p < 0.05$), in quercetin-naive rats – 73.7% ($p < 0.05$); WCB increased by 43.9% ($p < 0.05$) in experimental rats, in the control rats – by 55.6% ($p < 0.05$), indicating lower intensity of emphysematous remodeling of the pulmonary structures, which occurred along with restrictive changes. Fibrosis development in the interalveolar septa and blood-air barrier structures was less active in experimental rats receiving quercetin (BAB increased by 6.7% ($p < 0.05$) in the background of quercetin use from the 30-th to 180-th days of the experiment), whereas in the animals with hyperglycemia it was 9.3% ($p < 0.05$). The size of lymphoid follicles in experimental rats increased 5.4 times ($p < 0.05$) by the end of the experiment, and 8.2 times ($p < 0.05$) in the control rats. These findings provide the basis for quercetin being used as a corrector for prevention and treatment of initial hyperglycemia complications.

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Table 1 - Results of the study of pulmonary tissue in young rats with alloxan hyperglycemia with and without quercetin (M ± m), n = 6

Value	30 days		60 days		90 days		120 days		150 days		180 days	
	Que	No que	Que	No que	Que	No que	Que	No que	Que	No que	Que	No que
TVW, µm	34.2	34.2	41.8	41.8	115.6	115.6	115.7	115.7	115.5*	116.2	115.5*	118.5
	±0.58	±0.04	±0.63	±0.02	±0.49	±0.1	±0.14	±0.36	±0.14	±1.4	±0.08	±0.44
LFP, µm	449.3	449.3	1049.0*	1049.6	1156.2*	1330.0	1254.7*	1430.1	1305.3*	2260.1	2447.3*	3690.1
	±0.8	±0.7	±0.82	±0.6	±1.3	±0.8	±0.51	±0.2	±0.4	±0.5	±0.3	±0.5
Glucose, mmol/L	19.3	19.3	13.81	13.8	13.3	13.3	13.5	13.5	13.69	13.7	8.09	13.1
	±0.21	±0.22	±0.8	±0.4	±1.3	±0.1	±0.11	±0.17	±0.15	±0.9	±0.11	±0.2
HbA1C, %	7.11	7.1	7.16	7.18	8.2	8.2	8.6	8.59	9.31	9.3	9.4	9.39
	±0.4	±0.15	±0.9	±0.4	±0.4	±0.26	±0.4	±0.8	±0.24	±0.41	±0.58	±0.28
BAB, nm	414.8	414.8	418.4*	420.1	436.7*	452.8	440.54*	453.31	441.2*	453.4	442.6*	453.4
	±0.8	±0.13	±1.1	±0.4	±0.2	±0.53	±0.27	±0.04	±0.92	±0.08	±0.2	±0.63
TI, nm	80.2	80.2	81.3*	81.9	97.6*	105.1	97.63*	105.3	98.1*	105.3	98.4*	105.3
	±0.8	±0.13	±0.31	±1.14	±0.42	±0.1	±0.18	±0.4	±0.12	±0.27	±0.3	±0.15
DA, µm	52.2	52.18	53.6	53.6	55.8	55.8	52.33*	52.3	49.69*	46.9	53.2*	44.8
	±0.67	±0.37	±0.01	±0.4	±0.05	±0.12	±0.71	±0.08	±0.14	±0.03	±0.3	±0.06
WAE, µm	31.14	31.14	31.14	31.14	31.2	31.2	43.18*	43.2	48.65*	49.4	48, *5	54.1
	±0.91	±0.12	±0.5	±0.01	±0.03	±0.4	±0.5	±0.12	±0.2	±0.33	±0.2	±0.6
WCB, µm	61.05	61.05	62.003	62.003	63.5	63.5	70.3	70.3	77.9*	78.02	87.9*	95.02
	±0.5	±0.71	±0.9	±0.1	±0.4	±0.11	±0.2	±0.9	±0.82	±0.51	±0.8	±0.61
TIS, µm	6.3	6.3	6.9	6.9	7.0	7.03	7.5	8.0	8.6*	9.02	9.3*	15.02
	±0.4	±0.04	±0.05	±1.3	±0.2	±0.5	±0.41	±0.17	±0.1	±0.4	±0.1	±0.04
WA, µm	68.31	68.3	72.1	72.1	78.3	78.3	89.4	89.4	81.9*	83.1	78.5*	77.0
	±0.1	±0.5	±0.09	±0.5	±0.21	±0.19	±0.17	±0.31	±0.18	±0.43	±0.6	±0.21

Note: * – deviation was significant for the quercetin-naive group ($p < 0.05$)

References

1. Smirnov LD, Inchina VI, Kostin YaV. [Possibilities of pharmacological correction of metabolic disorders in experimental diabetes with antioxidant drugs]. *Biomeditsinskaya himiya.* 2004; 50(3):502-508.
2. Stepanyan IE. [Features of lung diseases in diabetes]. *AtmosferA. Pulmonologiya i allergologiya*. 2009;1(4):8-10.
3. Sokolov EI, Demidov YuI. [Gas exchange function of the lungs in patients with diabetes mellitus]. *Terapevticheskiy arhiv*. 2008; 80(1):63-66.
4. Rekha J, Madhavi J, Rane. Diabetic Microvascular Disease and Pulmonary Fibrosis: The Contribution of Platelets and Systemic Inflammation *International Journal of Molecular Sciences*. 2016;17(1):1-13.
5. Nuraliev YuN. [The efficacy of quercetin in alloxan diabetes]. *Eksperimentalnaya i klinicheskaya farmakologiya*. 1992;55(1):42-44.
6. Zaychenko AV, Bryuhanova TA. [Morphological study of the lungs of guinea pigs after the prophylactic administration of homeopathic granules of cloclorin against the background of bronchial obstruction syndrome]. *Nauchnyie vedomosti*. 2013;25(168):202-205.
7. Stechishin IP, Posohova KA. [An effective drug with quercetin for experimental cerebral diabetes]. *FarmakologIya ta lIkarska toksikologIya.* 2014;6(41):66-71.

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