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Modeling Mediated Crossover Acute and Subacute Damage to the Pulmonary System During Systemic (Subcutaneous) Administration of Bleomycin in Mice to Test the Potential Therapeutic Effect of Polyphenols

Modelado del Daño Agudo y Subagudo Cruzado Mediado al Sistema Pulmonar Durante la Infección Sistémica (Subcutánea) de Administración de Bleomicina a Ratones para Probar el Posible Efecto Terapéutico de los Polifenoles

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SUMMARY: The potential anti-inflammatory and antifibrotic activity of polyphenolic extracts of blueberry and grape was evaluated in a mouse model of lung damage induced by subcutaneous administration of bleomycin. The results of testing the polyphenolic extracts on two different systemic administration variants of bleomycin (intraperitoneal and subcutaneous) were compared. It was found that regardless of the method of bleomycin administration, indirect cross-acute and subacute damage to the pulmonary system was observed. Both patterns exhibited the same prevalence and severity. The administration of polyphenolic extracts of blueberry and grape to mice resulted in a significant decrease in theseverity of acute and subacute patterns of lung damage, suggesting their protective properties for the microcirculatory bed and a pronounced anti-inflammatory effect.

KEY WORDS: Fibrosis; Bleomycin; Polyphenols; Blueberry extract; Grape extract.

INTRODUCTION

It is necessary to agree with the opinion of Tashiro *et al*. (2017), that no animal model completely replicates the histological picture of human pulmonary fibrosis or shows signs of progressive disease. However, this does not diminish the value of animal models for testing therapeutic interventions.

The model of pulmonary fibrosis involving intratracheal bleomycin administration to laboratory mice and rats is widely regarded as the most suitable animal model for preclinical testing (Jenkins *et al*., 2017). Nevertheless, the intratracheal administration of bleomycin requires a surgical incision at the tracheal level, which is associated with significant perioperative mortality in experimental animals. Furthermore, it is known that bleomycin can be delivered not only topically to the lungs but also through systemic administration (such as intraperitoneal or subcutaneous). Regardless of the route of administration,

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bleomycin causes direct damage to cells by inducing DNA strand breaks, generating free radicals, and inducing oxidative stress (Moeller *et al*., 2008). This is followed by cell necrosis and/or apoptosis, leading to inflammation and the development of fibrosis (Moore & Hogaboam, 2008). With systemic administration (intraperitoneal or subcutaneous), the initial site of damage is the endothelium of the pulmonary vessels, which is believed to reflect similar processes occurring in humans affected by bleomycin-induced pneumonitis. Following this initial damage to endothelial cells, bleomycin can then access the alveolar epithelium and cause damage resembling fibrosis (Adamson & Bowden, 1974).

We previously evaluated the possibility of using the intraperitoneal method of administration as a simpler approach to induce bleomycin-induced pulmonary fibrosis (Shulgau *et al*., 2023). In this study, we aimed to conduct an experimental morphological and morphometric investigation of the antifibrotic function of polyphenolic extracts from blueberries and grapes using a mouse model of lung damage induced by subcutaneous bleomycin administration. Similar to intraperitoneal administration, subcutaneous administration of bleomycin is simpler compared to intratracheal administration and can be considered more humane for experimental animals. Therefore, by also studying the subcutaneous route of administration, we aim to enhance the evidence base for the systemic use of bleomycin in modeling pulmonary fibrosis to evaluate the potential of antifibrotic therapy.

MATERIAL AND METHOD

Grape extract. Cabernet Sauvignon – old French grape variety of medium-term maturity, currently located in Almaty and Zhambyl regions of Kazakhstan, were used in the study. The concentrate polyphenols obtained from seed ridges and grape skin – secondary wine products using water-alcohol feedstock extraction (40 % aqueous-alcoholic solution of ethyl alcohol in the ratio 1:5), followed by concentrating the extract on a rotary evaporator to a dry matter content of 25 %. The total concentration of phenolic derivatives in the concentrate of grape polyphenols used in this experiment is 5,000 mg/L.

Blueberry extract. Polyphenols were sourced from wild blueberries that were collected in summer-autumn period of 2018 in Surgut district of Khanty-Mansiysk County of the Tyumen region of the Russia. The concentrate polyphenols obtained from berry skin using water-alcohol feedstock extraction (40 % aqueous-alcoholic solution of ethyl alcohol in the ratio 1:5), followed by concentrating the extract on a rotary evaporator to a dry matter content of 25 %. The final concentration of phenolic derivatives of berry is of 5,000 mg/L.

Concentration of polyphenols in samples determined using a commercially available kit “Polyphenols Folin-Ciocalteu” (ENOLOGY line by BioSytems. Spain) according to the manufacturing guidelines. Principle of the method: polyphenols in the sample react with Folin-Ciocalteu reagent in basic media. The increase of coloration is proportional to polyphenols concentration in the sample. Gallic acid solution (2000 mg/L) was used as a standard.

Animals. The study was approved by the Institutional Animal Care and Use Committee (IACUC) of the National center for biotechnology (decision 04/AP09260159 dated 8 September 2020). The reporting in this study is in accordance with guidelines published by the National Centre for the Replacement, Refinement and Reduction of Animals in Research (ARRIVE guidelines). All research work with laboratory animals was performed in accordance with generally accepted ethical standards for the treatment of animals based on standard operating procedures that comply with the rules adopted by the European Convention for the Protection of Vertebrate Animals Used for Research and Other Scientific Purposes.

Adult female outbred CD-1 mice 8–10 weeks of age, weighing 22 ± 2 g were used in experiments. These lines of rodents are bred in the animal facility of the National Center for Biotechnology, Nur-Sultan, Kazakhstan. The animals were housed in a room with a controlled temperature and a 12 h light-dark cycle with unlimited access to standard food (SSNIFF V1534-300, HTLab AG, Heideck, Germany) and drinking water ad libitum.

Two weeks before experiments, animals were randomly distributed in cages by ten mice per cage. The animals were used after a 14 day adaptation period. The animals were preserved in the same groups and same cages to reduce the stress. As required by the IACUC for survival experiments, moribund animals meeting the euthanasia criteria were euthanized using CO2. The euthanized animals were counted as dead.

Experimental Designs. Mice (n=40) were randomly assigned to one of 4 groups of 10 mice in each study group.

Group I (Intact animals, n=10) received 0.1 ml of sterile saline subcutaneously (manipulation control), similar to BLM, three times a week for 4 weeks, total number of BLM injections – 12. Animals in group I received 0.2 ml of drinking water intragastrically daily (manipulation control) for 4 weeks (28 days).

Group II (Control, n=10) – received 0.05 mg BLM per mouse in 0.1 ml sterile saline subcutaneously, three times a week, for 4 weeks, total number of BLM injections – 12. Animals in group II received 0.2 ml of drinking water as treatment (placebo) intragastrically daily for 4 weeks (28 days).

Group III (Blueberry extract, n=10) – received 0.05 mg BLM per mouse in 0.1 ml of sterile saline subcutaneously, three times a week, for 4 weeks, a total number of BLM injections – 12. Animals in group III received blueberry extract as treatment intragastrically daily (0.2 ml per mouse) for 4 weeks (28 days).

Group IV (Grape extract, n=10) – received 0.05 mg BLM per mouse in 0.1 ml sterile saline subcutaneously, three times a week, for 4 weeks, total number of BLM injections – 12. Animals in group IV received grape extract as treatment intragastrically daily (0.2 ml per mouse) for 4 weeks (28 days).

Euthanasia of laboratory animals was carried out 28 days after the start of the experiment by carbon dioxide overdose.

Histopathological examination. The quantitative assessment of the lung structure was carried out in accordance with the recommendations of the American Thoracic Society/European Respiratory Society (Hsia *et al*., 2010).

After removing the animals from the experiment, the lungs were removed in a single block, fixed with 4 % buffered paraformaldehyde at a constant hydrostatic pressure of 25 cm for 4 h and then the trachea was bandaged and immersed in a fixator for 48 h. Each of the tissue samples taken from the lungs was fixed by immersion in 10 % neutral buffered formalin at room temperature. The samples were left in the fixators for 24 h. After fixation, the tissues were washed in running water, dehydrated by immersion in ethyl alcohols of increasing concentration, purified in two shifts of xylene, poured into paraffin blocks at room temperature.

Tissue sections with 5 µm thick were made on a “Leica SM 2000R” rotary microtome. Tapes containing 8-10 sections were collected and placed in a flotation bath for tissues at 40 °C, and then placed on slides for microscopy. Before staining, the tissue sections were dewaxed with xylene and hydrated with ethanol.

Sections (5 µm) were stained with: (1) hematoxylin and eosin were used to determine the general morphological pattern of the lungs, inflammatory pattern and interstitial edema; (2) Masson trichrome was used to demonstrate fibrosis, identify collagen fibers and newly synthesized collagen, determine its predominant localization and degree of fibrosis (Titford, 2009); Gomori silvering was used for histological evaluation of reticulin fibers (ab236473 Reticulum Stain Kit (Modified Gomori's), 2018). When stained with silver, reticulin fibers look like thin, dark fibrils, and violations in the structure of reticulin can be detected, which occur, for example, with pulmonary fibrosis (Titford, 2009).

The sections were examined by light microscopy. Microscopy of preparations was performed on a Zeiss AxioLab 4.0 microscope with magnifications of ×100, ×200 and × 400. AxioVision 7.2 for Windows was used to photograph the images.

Damage pattern criteria. The acute injury pattern is a histopathological pattern of lung injury in animals in an experimental study, which is a stress-induced (BLM) disorder of the histoarchitectonics of the respiratory regions with the development of acute interstitial injury (edema) and granulocytic infiltration.

The pattern of subacute damage is a histopathological pattern of damage to the lungs of animals in an experimental study, which is a type of damage with a predominance of immature collagen fibers without the formation of diffuse fibrotic fields and a disorder of the obvious histoarchitectonics of the respiratory compartment with lymphocytic infiltration.

Morphometric study. Histomorphometric evaluation of cellular infiltrate and acute interstitial injury (edema).

Morphometric analysis was carried out by two independent researchers who had experience working with animal models, without information about the animal’s belonging to the group and the intervention performed.

Morphometric analysis of the lungs was performed using a grid with 100 dots and 50 lines under a light microscope (Zeiss AxioLab 4.0).

Polymorphonuclear granulocytes and lymphocytes giant multinucleated cells and active macrophages with intracytoplasmic inclusions (foam cells) were counted in alveolar spaces, alveolar walls and interstitial in 20 random fields of view at 400 magnification. The visual fields were formed by successive random displacements of at least one visual field length from the current position, provided that the pulmonary alveoli made up at least 50 % of the cut area. The final value for the histological section was expressed as the average of all visual fields at ×200 and the average value of all sections per animal was recorded.

Interstitial perivascular edema was detected in 20 random fields of vision at ×200 magnification. On the cross sections of intraparenchymatous arteries and veins, the number of points falling on the areas of perivascular edema and the number of intersections between the basal membrane of the vessel and the surrounding lung parenchyma were counted. The index of interstitial perivascular edema was calculated using the formula: number of points 1/2 / number of intersections (Hizume *et al*., 2007).

The fraction of the lung occupied by atelectatic alveoli was determined by counting points in 20 random fields of view at x200 magnification. The points falling on the atelectatic alveoli were counted and divided by the total number of points in each microscopic field.

Histomorphometric evaluation of collagen fibers. Pulmonary fibrosis was assessed by a semi-quantitative method on the Ashcroft scale (Ashcroft *et al*., 1988) modified by Hübner *et al* (2008). Five sections for each lung sample stained with Masson trichrome were evaluated on a scale from 0 to 8. The final score was expressed as the average of individual scores observed in all fields of view of the microscope at magnification ×100 and the average of such indicators per animal was recorded.

In histopathological analysis of reticulin fibers, the assessment was carried out in the central (perfusion) compartment (respiratory bronchioles, arteries and veins), and the peripheral (diffusion) respiratory compartment (microvessels, alveoli, respiratory bronchioles).

Histomorphometric evaluation of reticulin fibers of the central compartment included an analysis of the structure of reticulin fibers: thin filamentous fibers with a clear margin or unfolded fibers with a blurred margin.

Histomorphometric assessment of reticulin fibers of the peripheral compartment included an analysis of the localization of reticulin fibers: localization is predominantly focal in the wall of thin alveolar septa or localization is predominantly diffuse in the wall of thickened alveolar septa.

For structural assessment of reticulin fibers of the peripheral respiratory compartment, the pattern of the native structure of reticulin fibers was assessed, including the structure of reticulin fibers and the distribution of reticulin fibers (uniform / chaotic).

Statistical Comparisons. Statistical data processing was carried out using a spreadsheet Microsoft Excel (from the Microsoft Office 2010 package) and the software package for statistical analysis IBM SPSS Statistics 20.0. The Mann-Whitney test and c-square were used for pairwise comparison between the two groups. The Kruskal-Wallis test was used to compare several groups. Differences were considered significant if $p < 0.05$.

RESULTS AND DISCUSSION

We conducted an experimental morphological and morphometric study of the antifibrotic function of blueberry and grape extracts in a model of lung injury in mice induced by subcutaneous administration of bleomycin.

The comparative histomorphometric characteristics of interstitial edema and cellular infiltration (hematoxylin and eosin staining) are presented in Table I.

In the intact group, lung tissue corresponded to the histological norm with preservation of histoarchitectonics, thin alveolar walls without signs of accumulation of extracellular matrix or infiltration of inflammatory cells.

Histological examination using hematoxylin and eosin staining of the lungs of mice from the group receiving BLM revealed progressive changes in the structure of the lungs. These changes included excessive infiltration of inflammatory cells, perivascular interstitial edema, and a large number of dormant alveoli.

In the control group, a pattern of severe acute interstitial injury was detected in 10 (100 %) cases, with an increase in the number of polymorphonuclear granulocytes (1.80 ± 1.14), lymphocytes (32.90 ± 15.44) and giant multinucleated cells (5.80 ± 3.26), diffuse edema of the interalveolar septa (0.83 ± 0.11), alveolar atelectasis (16.18 ± 3.74).

In the group with extracts of blueberry and grape, severe acute interstitial damage was not detected in all cases. The index of perivascular interstitial edema was 0.52 ± 0.27 and 0.63 ± 0.25, respectively. Chronic nonspecific inflammation was observed in the group with blueberry and grape extract with lymphocytic infiltration (11.50 ± 6.29 and 14.50 ± 5.97, respectively) and mild infiltration

Table I. Histomorphometric characteristics of cellular infiltration, interstitial edema and atelectasis

Indicator name	Intact animals (n=10) (M±SD)	Control (n=10) (M±SD)	Blueberry extract (n=10) (M±SD)	Grape extract (n=10) (M±SD)
PMN[1]	0.04 ± 0.13	1.80 ± 1.14 #	0.18 ± 0.23 *	0.16 ± 0.37 *
		p_1=0.0008	p_2=0.1509	p_3=0.4727
			p_4=0.0013	p_5=0.0022
				p_6=0.4727
Lymphocytes[1]	3.30 ± 2.31	32.90 ± 15.44 #	11.50 ± 6.29 #*	14.50 ± 5.97 #*
		p_1=0.0002	p_2=0.0025	p_3=0.0002
			p_4=0.0013	p_5=0.0046
				p_6=0.2899
Multi-core giant cells[1]	0.10 ± 0.32	5.80 ± 3.26 #	0.53 ± 0.54 #*	0.78 ± 0.42 #*
		p_1=0.0002	p_2=0.0588	p_3=0.0010
			p_4=0.0002	p_5=0.0002
				p_6=0.2568
Macrophages[1]	0.50 ± 0.71	1.65 ± 0.97 #	2.38 ± 0.86 #	2.68 ± 0.42 #*
		p_1=0.0113	p_2=0.0007	p_3=0.0003
			p_4=0.1405	p_5=0.0343
				p_6=0.4728
Interstinal edema[2]	0.34 ± 0.17	0.83 ± 0.11 #	0.52 ± 0.27 *	0.63 ± 0.25 #*
		p_1=0.0003	p_2=0.1405	p_3=0.0091
			p_4=0.0126	p_5= 0.0413
				p_6=0.4492
Interstinal congestion[3]	2.52 ± 0.97	16.18 ± 3.74 #	10.82 ± 1.80 #*	12.91 ± 2.90 #
		p_1=0.0001	p_2=0.0002	p_3=0.0002
			p_4=0.0025	p_5=0.0696
				p_6=0.1304

by polymorphonuclear leukocytes (0.18 ± 0.23 and 0.16 ± 0.37, respectively). In all these cases, polymorphonuclear granulocytes were represented by single cells or were absent.

Giant multinucleated cells in groups with extracts of blueberry and grape were observed in single histological sections (0.53 ± 0.54 and 0.78 ± 0.42, respectively). Macrophages with cytoplasmic inclusions (foam cells) were detected in all histological sections of animals in groups with extracts of blueberry (2.38 ± 0.86) and grape extract (2.68 ± 0.42).

The comparative histomorphometric characteristics of the subacute damage pattern in the studied groups (Masson trichrome and Homori silvering) are presented in F**igure 1 and in Table II.**

In the intact group, the structure of the lung corresponded to the histological norm with an average score on the Ashcroft scale of 0.3=0.52, the distribution of available collagen is minimal (<5 %), the native structure is discontinuous, filamentous, ordered.

In the control group of mice treated with bleomycin, mild fibrous changes in the alveolar septa were observed with an average score on the Ashcroft scale of 2.35 ± 0.34, which corresponds to minimal fibrous changes having mainly subpleural and perivascular location. The native structure of collagen is discontinuous, filamentous and ordered. In 2 (20 %) cases in the respiratory compartment, loose fibers with a blurred margin were observed, and in 2 (20 %) animals diffuse fibers were observed in the wall of thickened alveolar septa, distributed mainly randomly.

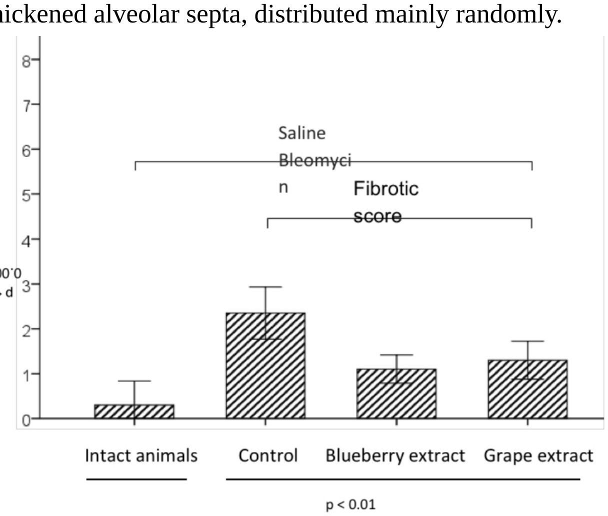

Fig. 1. Histomorphometric characteristics of pulmonary fibrosis according to Ashcroft *et al.* (1988).

Table II. Morphometric assessment of the central (perfusion) and peripheral (diffusion) compartments of the lung.

Name of indicator	Intact animals (n=10)	Control (n=10)	Blueberry extract (n=10)	Grape extract (n=10)
Central (perfusion) compartment				
Characteristics				
- thin filamentous fiber with clear margin	10/100	10/100	10/100	10/100
- loose fibers with blurred margin	-	-	-	-
Peripheral (respiratory) compartment				
Characteristics				
- thin filamentous fiber with clear margin	10/100	8/80	10/100	10/100
- loose fibers with blurred margin	-	2/20	-	-
Localization				
- predominantly focal in the wall of thin alveolar septa	10/100	8/80	10/100	10/100
- predominantly diffuse in the wall of thickened alveolar septa	-	2/20	-	-
Distribution				
- Uniform	10/100	2/80	9/90	9/90
- Chaotic	-	2/20	1/10	1/10

Isolated alveolar septa with single profibrous changes were observed in the groups with blueberry and grape extracts in separate sections (the average score on the Ashcroft scale was 1.10 ± 0.32 and 1.30 ± 0.42, respectively). The histological pattern was characterized by the predominance of resorbed immature type III collagen fibers (reticulin) in 100 % of cases, localized mainly focally in the wall of thin alveolar septa and distributed evenly.

A comparative histopathological assessment of the pattern of bleomycin-induced lung injury in mice with subcutaneous bleomycin administration showed that histopathological signs of acute and subacute interstitial lung injury were observed in the lungs of mice of experimental groups. Micrographs of the lungs of mice of the studied groups are shown in Figure 2.

We found that a cross pattern of damage was detected in the lungs of the control group mice: both the pattern of acute injury and subacute injury. The pattern of acute injury was characterized by multifocal lymphomacrophagal vasculitis and bronchiolitis, diffuse edema of the interalveolar septa, violation of the histoarchitectonics of the respiratory departments with the development of acute interstitial edema and granulocytic infiltration. Histopathological evaluation of the lungs of the control group mice showed a slight increase in the number of alveolar macrophages and an increase in the cellularity of the alveolar septa. Perivascular clutches and tissue infiltration of neutrophils and macrophages were also observed, as well as an increase in the number of perivascular and peribronchiolar cell infiltrates. These changes were absent in the lungs of mice treated with saline, and therefore are considered specific early changes in response to bleomycin.

The pattern of subacute injury was characterized by single peripheral short filamentous strands of collagen fibers in the control group. Subcutaneous exposure to bleomycin mice led to the development of subpleural foci of inflammation (clusters of macrophages, lymphocytes and fibroblasts), which spread into the lung parenchyma and involved the bronchi and vascular network.

When using extracts of blueberries and grapes, collagen fibers on the periphery and in the central part are not detected, histochemical signs of profibrous collagen synthesis were not detected in the stromal component of peripheral respiratory tissue, unlike in the control group ($p<0.05$). The interstitial component of the respiratory zone of the lung groups with extracts of blueberries and grapes is represented by a thin-fibrous continuous connective tissue component with a fiber thickness that does not differ statistically significantly from the intact group. During histochemical examination for reticulin in the control group, the lung parenchyma had a lighter tone of staining of reticulin fibers in contrast to the group of intact mice and the group with blueberries and grapes. Reticulin fibers were distributed evenly, but with diffuse character and discontinuity in the intralveolar and intravascular spaces. Reticulin fibers are characterized by an intermittent arrangement with uneven fiber thickness, multiple sections of microfiber spreading to the perivascular space and with a tendency to protrude into the lumen of the vessels. In the groups with blueberry and grape extract, reticulin fibers were mainly represented by thin filamentous fibers with a clear margin, localized mainly in the wall of thin alveolar partitions and distributed evenly, statistically not significantly different from the intact group ($p<0.05$).

Lymphohistiocytic inflammatory infiltrate was

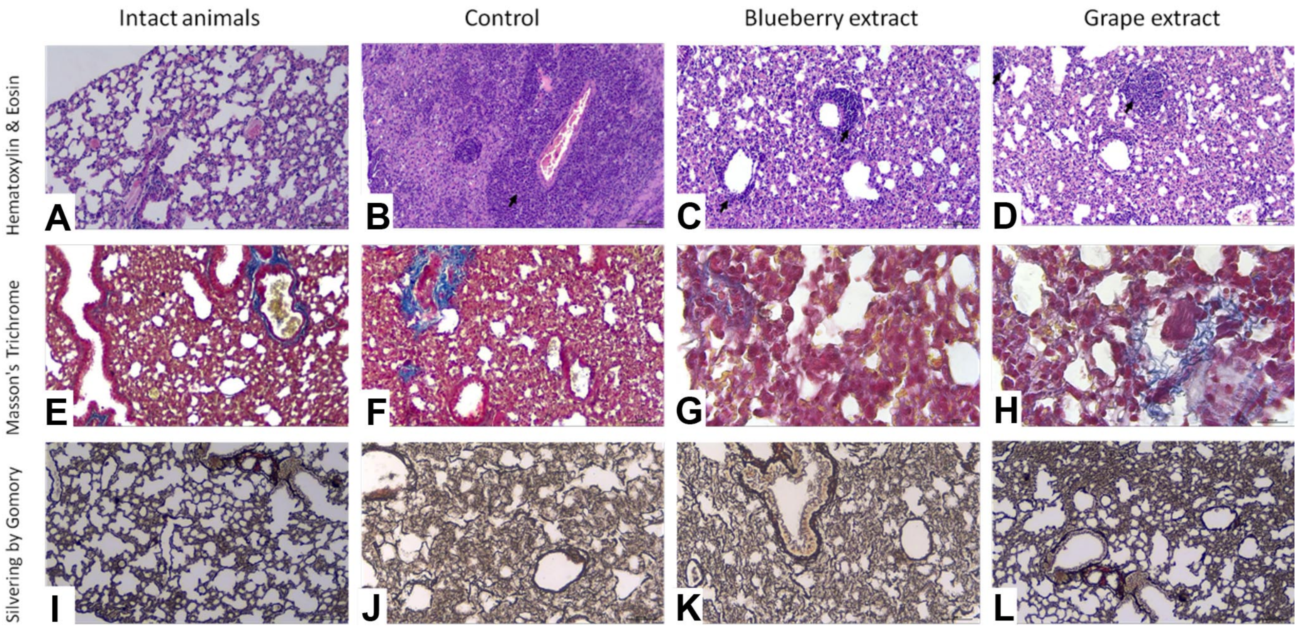

Fig. 2. Microphotographs of the lungs of mice. **A.** Intact mice. The morphological structure of the lung corresponds to the histological norm: thin interalveolar septa without signs of edema or inflammatory infiltration (hematoxylin and eosin stain, × 100); **B.** Control. Multifocal lymphomacrophage vasculitis and bronchiolitis, diffuse edema of the interalveolar septa, multi-row lymphohistiocytic infiltrate in the perivascular and peribronchial zones (hematoxylin and eosin stain, × 100); **C.** Blueberry extract. Focal lymphomacrophage vasculitis and bronchiolitis, lymphohistiocytic infiltrate in the perivascular and peribronchial zones with the formation of couplings with rows (hematoxylin and eosin stain, × 100); **D.** Grape extract. Focal lymphohistiocytic infiltrate in the interalveolar septa (hematoxylin and eosin stain, × 100); **E.** Intact mice. Single peripheral short filamentous filaments of collagen fibers located in the respiratory compartment and collagen fibers in the wall of large vessels, corresponding to the histological norm (Masson's trichrome stain, × 100); **F.** Control. Pronounced edema and defibration of the vessel wall, single peripheral short filamentous filaments of collagen fibers (Masson's trichrome stain, × 200); **G.** Blueberry extract. Single peripheral short filamentous filaments of collagen fibers (Masson's trichrome stain, × 200); **H.** Grape extract. Single peripheral short filamentous filaments of collagen fibers (Masson's trichrome stain, × 200); **I.** Intact mice. Thin filamentous structures diffusely distributed in alveoli and respiratory bronchioles; **J.** Control. Uniform distribution of reticulin fibers, but with a diffuse character and discontinuity in intra-alveolar and intravascular spaces. Reticulin fibers are characterized by a discontinuous arrangement with uneven fiber thickness, multiple areas of defibration on microfibers that extend into the perivascular space and with a tendency to protrude into the lumen of the vessels; **K.** Blueberry extract. Uniform distribution of reticulin fibers; **L.** Grape extract. Uniform distribution of reticulin fibers, but with a diffuse character and discontinuity in intra-alveolar and intravascular spaces.

mainly observed in the peripheral zones, that is, in the respiratory zones of lung tissue. When using blueberry and grape, the lymphohistiocytic infiltrate was microfocal in nature with the formation of one to three rows with moderate lymphohistiocytic infiltration, with a statistically significant difference from the control group ($p<0.05$), the interalveolar septa are thin, without visual signs of edema. Lymphohistiocytic infiltration was characterized by the formation of 1 to 3-layer cell couplings around vessels and bronchioles.

We believe that the pattern of lung damage has a cross-character of prolonged, constantly progressive damage. Previous studies have shown that bleomycin causes lung damage, leads to hypoxia, reactive inflammatory processes and fibrotic processes, damage to the microcirculatory bed of the respiratory system (Harrison Jr. & Lazo, 1987; Ortiz *et al*., 1998; Roman, 1998). At the same time, more than one of these patterns may be present in each case (Hay *et al*., 1987; Silva & Müller, 2006).

In this study, we have shown that extracts of blueberry and grape reduce lung damage in mice in a model of subcutaneous bleomycin administration, acting on the microcirculatory bed, enhancing phagocytic function, reducing lymphohistiocytic infiltrates, locoregional foci of residual long-term inflammatory effects, reducing the acute phase of damage to the microcirculatory bed. The results obtained are consistent with the results previously published by us with the intraperitoneal method of bleomycin administration (Shulgau *et al*., 2023), testifying that the protective effect of berry extracts persists regardless of the method of administration of bleomycin (subcutaneous or intraperitoneal).

We tend to associate the detected anti-inflammatory effect of the studied extracts with the presence of a high concentration of polyphenols in these extracts, based on the earlier description of the multifaceted anti-inflammatory effects of plant polyphenols (Accomando *et al*., 2010; Hussain *et al*., 2016; Roy *et al*., 2022). The results of the morphometric study demonstrate that extracts of blueberry and grape preserve the structural histoarchitectonics of the lungs, which is manifested by the absence of acute inflammatory damage and subacute pattern. The detected microfocal lymphomacrophagal infiltration in groups with blueberry extract and grape extract is nonspecific. In previously presented studies, it was shown that the proliferation of lymphocytes in the lungs can be a non-specific sign of long-term consequences in the pathogenesis of bleomycin-induced pneumonitis (Seitzman *et al*., 1998; Elewa *et al*., 2021).

We also showed that at the time of the study, there were no active processes in the lungs of mice; the immune cells were chaotic, with a monotonous lymphohistiocytic infiltrate located micro-focally. These histopathological signs may be a non-specific sign of long-term consequences, however, these lymphohistiocytic infiltrates may also be a trigger mechanism of a locoregional focus of hypersensitivity with subsequent administration of the drug and cause uncontrolled progressive lung lesions.

CONCLUSION

Thus, we have shown that, regardless of the method of systemic administration of bleomycin (subcutaneous or intraperitoneal), indirect cross-acute and subacute damage to the pulmonary system appears in mice. Both patterns had the same pattern in terms of prevalence and severity. We believe that the stages or patterns of damage are not transitional, but are of a cross-constantly progressive nature. In our experiments, the administration of polyphenolic extract of blueberry or grape to mice against the background of subcutaneous or intraperitoneal bleomycin induction of pulmonary injury showed a significant decrease in the severity of acute and subacute patterns of lung damage, which may reflect their protective properties for the microcirculatory bed and a pronounced anti-inflammatory effect. The detected lymphohistiocytic infiltrates, we believe, belong to locoregional lymphohistiocytic zones of distant lung tissue damage, the biological role of which remains unspecified in this study. On the one hand, the detected microfocal infiltrates can be considered a sign of the damage suffered, on the other hand, these lymphohistiocytic zones can be sentinel and again, under unfavorable conditions, cause hypersensitivity and sensitization (locoregional) with the progression of lung damage.

Thus, polyphenolic extracts of blueberries and grapes can be considered as promising anti-inflammatory and antifibrotic substances for further research.

The uniformity of the lesion of the lung tissue during bleomycin induction by intraperitoneal or subcutaneous administration and the uniformity of the reaction of the lung tissue to the introduction of the tested polyphenol extracts in both cases allow us to conclude that systemic administration of bleomycin is acceptable as an alternative to intratracheal administration when performing the tasks of selecting candidate compounds for the therapy of pulmonary fibrosis.

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RESUMEN: La potencial actividad antiinflamatoria y antifibrótica de los extractos polifenólicos de arándano y uva se evaluó en un modelo de daño pulmonar en ratón inducido por la administración subcutánea de bleomicina. Se compararon los resultados de las pruebas de los extractos polifenólicos en dos variantes diferentes de administración sistémica de bleomicina (intraperitoneal y subcutánea). Se encontró que, independientemente del método de administración de bleomicina, se observaba daño indirecto cruzado, agudo y subagudo al sistema pulmonar. Ambos patrones exhibieron la misma prevalencia y gravedad. La administración de extractos polifenólicos de arándano y uva a ratones dio como resultado una disminución significativa en la gravedad de los patrones agudos y subagudos de daño pulmonar, lo que sugiere sus propiedades protectoras del lecho micro-circulatorio y un efecto antiinflamatorio pronunciado.

PALABRAS CLAVE: Fibrosis; Bleomicina; Polifenoles; Extracto de arándano; Extracto de uva.

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