Polish Annals of Medicine

Journal homepage: https://www.paom.pl

Research paper

# Study of organ-protective effect of blueberry leaves decoction (*Vaccinium myrtillus*) in experimental application of dexamethasone

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Article info

Article history
Received: December 5, 2021
Accepted: November 9, 2022
Available online: May 5, 2023

Keywords
Liver
Kidneys
Heart
Retina
Dexamethasone
Blueberry leaf decoction

Doi
https://doi.org/10.29089/paom/156418



Abstract

Introduction: The World Health Organization (WHO) recommended dexamethasone as the only effective drug for treatment severe cases of COVID-19.

Aim: To study the organoprotective effect of blueberry leaves decoction during the experimental use of dexamethasone.

Material and methods: Experimental studies were performed on 30 white outbred laboratory rats of white males (initial weight 200 g), which were kept on the standard diet of the vivarium of National Pirogov Memorial Medical University, Vinnytsya, Ukraine, which were divided into three groups: (1) intact rats, (2) animals treated intramuscularly with 6 mg of dexamethasone for 10 days, (3) rats which were intramuscular injected with dexamethasone in the recommended dose and simultaneously intragastrically using 2 mL of the decoction, which was prepared as follows: steamed 1 g of dry blueberry leaves (*Vaccinium myrtillus*) with 5 mL of boiling water, infused for 1 h.

Results and discussion: Intramuscular administration of dexamethasone at a dose of 6 mg for 10 days (according to WHO recommendations for patients with COVID-19) causes severe degenerative changes in the type of dystrophy and atrophy of rat's liver, heart, kidneys, periodontium and retinas. Prophylactic intragastric administration of a decoction of blueberry leaves prevents the development of dystrophic changes in cells and tissues in studing organs caused by the action of dexamethasone.

Conclusions: Therapeutic use of dexamethasone leads to severe morphological changes in the most important organ systems and preventive introduction of a decoction of blueberry leaves protects the development of adverse effects.

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## 1. INTRODUCTION

The World Health Organization (WHO) called dexamethasone the only effective treatment for severe cases of COVID-19. As early as June 2020, it called for increased production of dexamethasone for the treatment of critically ill patients. On September 2, 2020, the WHO issued interim recommendations for the use of dexamethasone based on data from seven clinical trials. The document contains two recommendations: (1) WHO strongly recommends oral or intramuscular administration of corticosteroids (dexamethasone) for the treatment of patients with severe and critical forms of COVID-19; (2) mode and duration of the course: once a day for 7–10 days at a dose of 6 mg.[1]

Dexamethasone is often used experimentally by doctors and biologists to simulate metabolic disorders (steroid diabetes). It is believed that the effect of dexamethasone on carbohydrate metabolism is manifested by increased glucose-6-phosphatase activity and inhibition of hepatic glucosokinase activity. The anti-insulin effect of the steroid on the periphery is manifested by a decrease in tissue glucose utilization. Along with the stimulation of gluconeogenesis in the liver, dexamethasone inhibits glucose oxidation, enhances protein breakdown and inhibits their synthesis. With increased release of amino acids from tissues and their entry into the liver significantly accelerates the process of transamination and deamination of amino acids used for gluconeogenesis. The combination of these effects leads to the development of hyperglycemia and other manifestations of so-called steroid diabetes.

During the experimental administration of dexamethasone to animals, a significant decrease in body weight, increase in blood glucose levels, dystrophic changes in the target organs – heart, liver, kidneys, brain, periodontium and retina – are noted. Decreased insulin tolerance was also noted. Scientists believe that the dexamethasone model is of practical importance for studying the physiological and molecular mechanisms of metabolic diseases caused by long-term use of glucocorticosteroids.[2,3]

Monitoring of patients with moderate to severe COVID-19 revealed symptoms that persisted after infection.[4] They affect most systems of the human body: cardiac, respiratory, neuromuscular, circulatory, immune, digestive, urinary,[5,6] prolonged hyperglycemia and visual impairment.[7] Understanding the possibility of cumulative adverse effects of dexamethasone in the proposed dose on the structure of the main target organs and the action of the virus, it is necessary to predict and prevent their development.

A decoction of blueberry leaves (*Vaccinium myrtillus*), as a generally available, inexpensive and practically harmless remedy has been chosen. Blueberry leaf has many useful properties.[8,9] A decoction of blueberry leaves reduces blood sugar and stimulates the pancreas, normalizes digestion, is useful for biliary tract diseases, treats anemia, dissolves kidney stones. Such tea is also recommended to be taken for the prevention of viral diseases, as well as to strengthen the immune system.[10] These properties of blueberry leaves are due to the unique chemical composition: the composition of blueberry leaves includes: vitamins A, B, C, trace elements including potassium, phosphorus, magnesium, sulfur, iron, calcium, organic acids, including citric, apple, oxalic, tartaric, anthocyanins – plant glycosides, tannins (about 20%), arbutin, which normalizes kidney function, flavonoids and carotenoids – plant pigments responsible for the percentage of glucose in the blood, essential oils that strengthen the cardiovascular system.[11]

We can hypothesize that the normalization of metabolism due to a decoction of blueberry leaves may help preserve the structure and function of the organs most affected by both coronavirus infection and dexamethasone load.

## 2. AIM

To study the organ-protective effect of blueberry leaf decoction in the experimental use of dexamethasone in the WHO-recommended therapeutic dose for treatment of patients with COVID-19.

## 3. MATERIALS AND METHODS

Experimental studies were performed on 30 white outbred laboratory rats of white males (initial weight 200 g), which were kept on the standard diet of the vivarium of National Pirogov Memorial Medical University, Vinnytsya, Ukraine, which were divided into three groups: (1) intact rats, (2) animals treated intramuscularly with 6 mg of dexamethasone for 10 days, (3) rats which were intramuscular injected with dexamethasone in the recommended dose and simultaneously intragastrically using 2 mL of the decoction, which was prepared as follows: steamed 1 g of dry blueberry leaves with 5 mL of boiling water, infused for 1 h.

Histological examinations and biochemical research were performed according to the generally accepted method. Rats were kept in standard vivarium conditions under 12-h lighting, air temperature 20°C–25°C, humidity 50%–55%. All animal manipulations were carried out in accordance with the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Scientific Purposes (Strasbourg, 1986)[12, 13] and the provisions of the IV National Congress on Bioethics (Kyiv, 2010).[14]

## 4. RESULTS AND DISCUSSION

Animals were killed under light ether anesthesia. Biochemical blood count of rats showed that according to the main indicators we were able to reproduce metabolic disorders. Thus, under experimental administration of dexamethasone, the blood glucose level of rats increased by 23% compared to the animals of the intact group, and the therapeutic administration of the phytopreparation even led to a decrease in the blood glucose level by 17% compared to rats

**Table 1. Biochemical studies of blood serum of rats (in mM/L).**

| Group of animals | Intact rats | Dexamethasone injection | Dexamethasone injection + blueberry leaf decoction |
|---|---|---|---|
| index | ($n$ = 10) | ($n$ = 10) | ($n$ = 10) |
| Glucose | 4.41 ± 0.14 | 5.42 ± 0.19* | 3.95 ± 0.65*# |
| Total cholesterol | 1.90 ± 0.01 | 2.85 ± 0.02* | 1.6 ± 0.04 *# |
| Triglycerides | 0.32 ± 0.01 | 1.38 ± 0.05* | 1.13 ± 0.01*# |
| Total lipids | 7.44 ± 0.06 | 11.66 ± 0.03* | 10.59 ± 0.04*# |

Comments: * the difference is significant in comparison with the group of intact animals ($P \leq 0.05$), # the difference is significant in comparison with the group of animals with experimental pathology ($P \leq 0.05$).

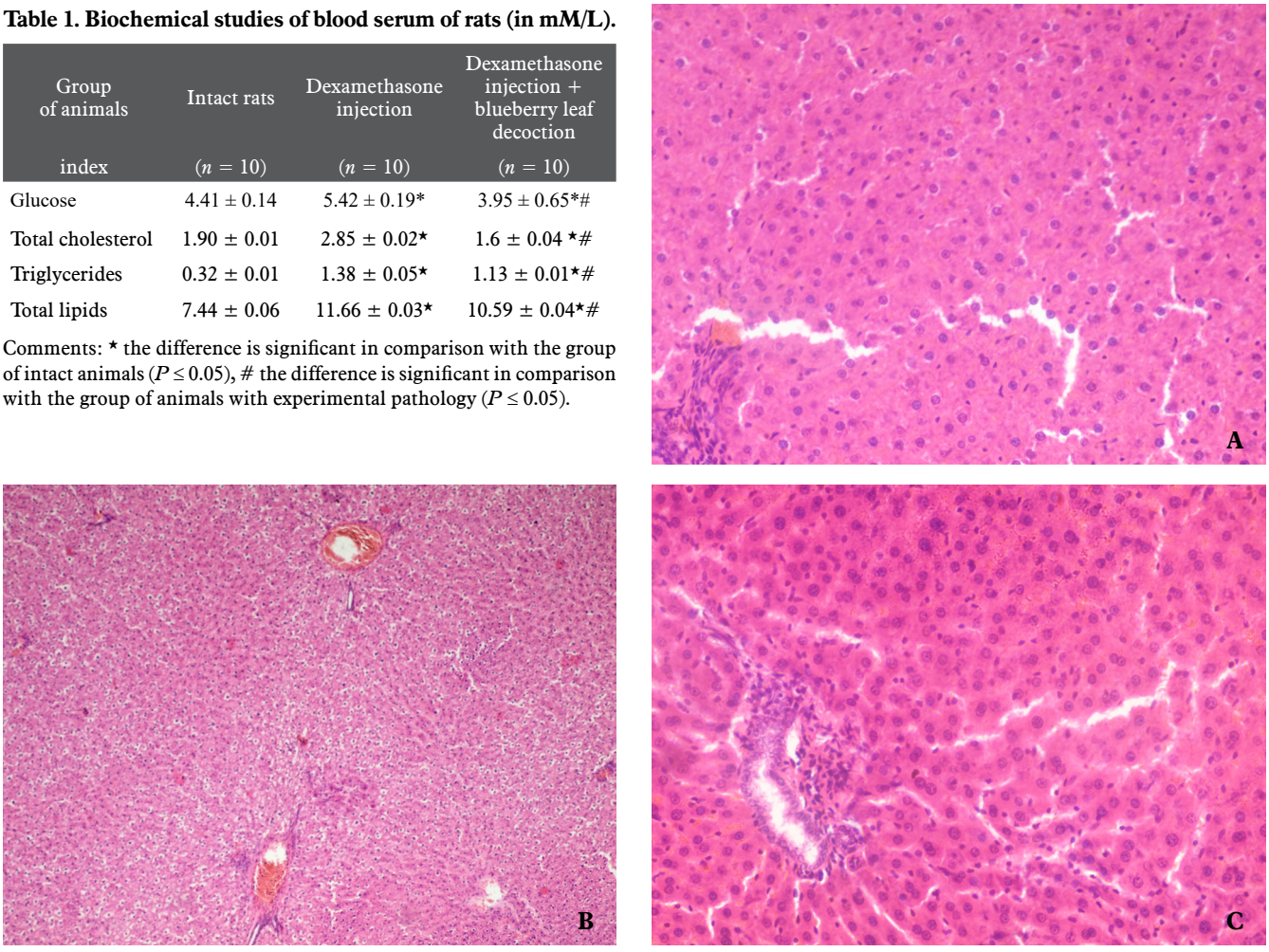


**Figure 1. Histological examination of the liver tissue of: (A) the rat of intact group; (B) dexamethasone group; (C) dexamethasone group and prevention. Magnification ×200.**

of the intact group. Accordingly, the study of blood lipids showed that in animals with experimental dexamethasone administration, the level of total cholesterol increased by 1.5 times, and with preventive administration of the herbal preparation, this indicator decreased by 20% compared with intact animals. The level of triglycerides increased in the second group of animals by 4.5 times and was lower in the third group. The level of total lipids with dexamethasone administration was 57% higher, and with the additional introduction of a decoction of blueberry leaves, it was higher by only 40% compared to the intact group (Table 1).

For morphological examination, the right lateral lobe of the liver was constantly taken. Experimental administration of dexamethasone led to the fact that in the liver tissue with the preserved lobular structure in the centrolobular zone there were areas where the radial arrangement of the liver plates was disturbed, sinusoidal capillaries significantly expanded. Hepatocytes in such areas were often non-nuclear, optically vacuolated. In hepatocytes with preserved nuclei, their edema and hyperchromia were noted. Some cells had a swollen cytoplasm with a large number of optically empty vacuoles. In the centrolobular zone there were also isolated foci of hepatocyte necrosis with infiltration of a large number of lymphocytes. Blood clots were observed in the dilated lumens of the sinusoidal capillaries and central veins. Endothelial cells of sinusoidal capillaries were inhomogeneously stained. Hepatocyte dystrophy and necrosis were noted in the periportal area. Dystrophy was a fatty degeneration of hepatocytes (Figures 1A and 1B).

Prophylactic intake of blueberry leaf decoction contributed to the fact that the negative changes were significantly compensated. Microscopic light-optical examination of the liver tissue of rats of this group showed signs of disruption of the structure of liver beams, dilation of sinusoidal capillaries, a decrease in the number of macrophages, lymphocytes. Hepatocytes had a homogeneous cytoplasm, only some had optically empty vacuoles (Figure 1C).

The left kidney was always taken for research. In experimental animals, a decrease in the size of renal corpuscles with dystrophically and atrophically altered cells of the outer and inner walls of the glomerular capsule was determined. There were renal corpuscles with a sharply reduced capsule cavity, up to its absence. The formation of synechiae of the glomerular capillaries with the parietal leaf of the glomerulus, focal thickening of the basement membrane of the glomerular capillaries, expansion of the mesangium

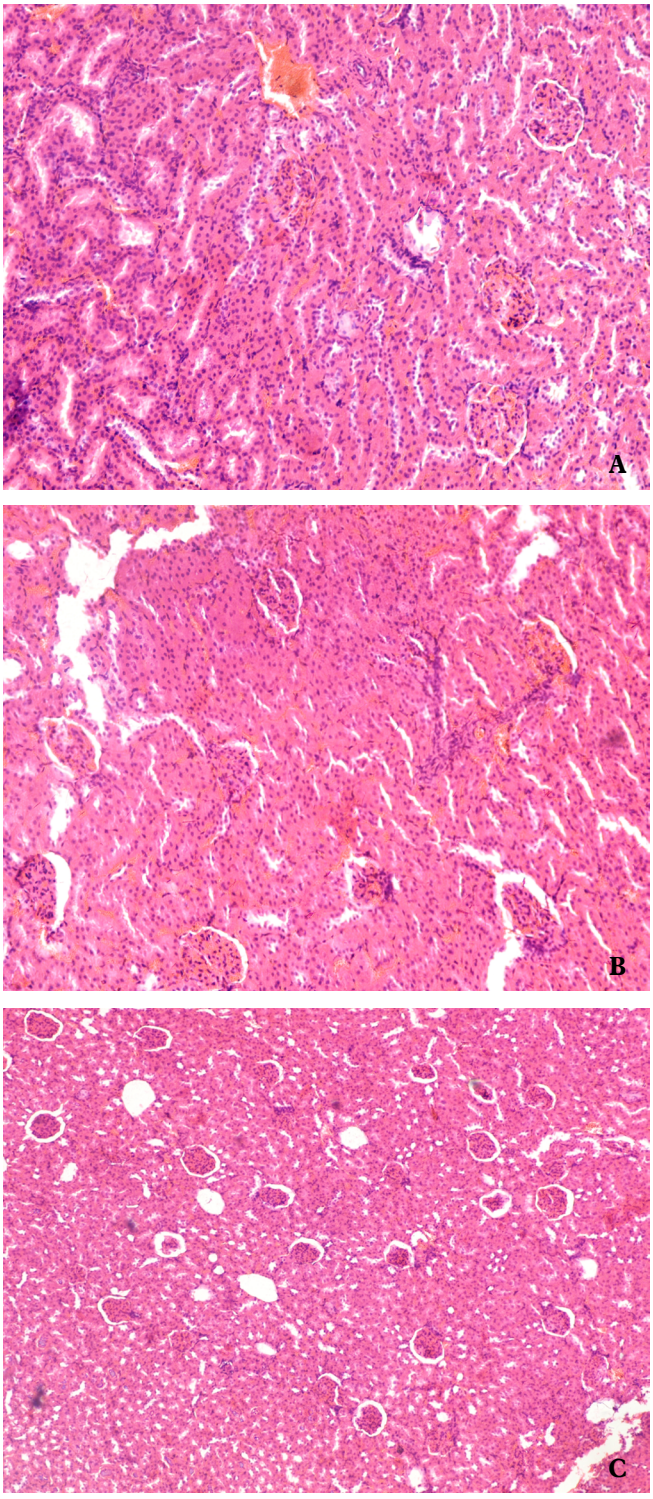


**Figure 2. Histological examination of the kidney tissue of: (A) the rat of intact group; (B) dexamethasone group; (C) dexamethasone group and prevention. Magnification ×200.**

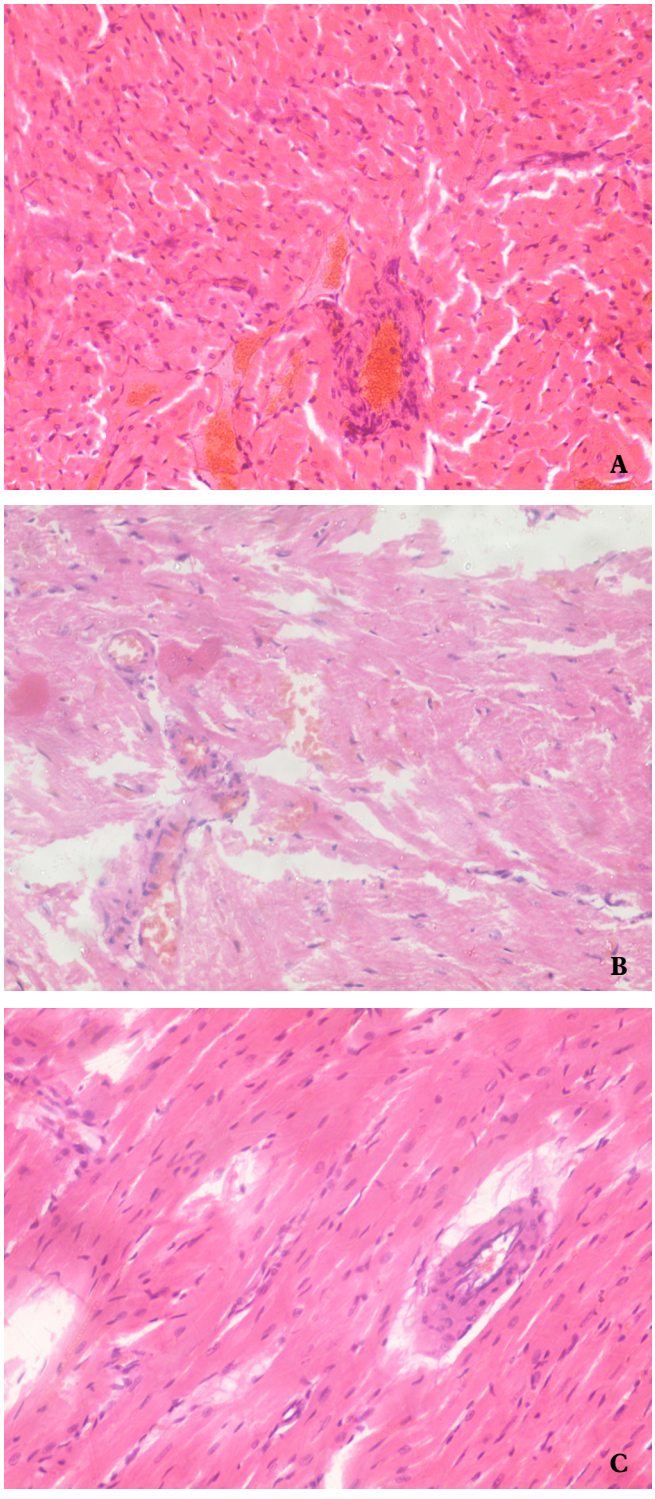


**Figure 3. Histological examination of the heart tissue of: (A) the rat of intact group; (B) the rat of dexamethasone group; (C) dexamethasone group and prevention. Magnification ×400.**

were noted. In the fenestrated capillaries of the glomeruli, circulatory disorders in the form of plethora, stasis and diapedesis of erythrocytes were noted. The proximal tubules are characterized by a membrane represented by a single-layer cubic epithelium. Tubular lumen is rounded. The nuclei of epithelial cells were round in shape, located in the central part of the cells (Figures 2A and 2B). The cytoplasm was characterized by moderate acidophilia. The distal tubules were formed by monolayer cubic and low prismatic epithelium. The tubular lumen was rounded. The nuclei of

epithelial cells were characterized by a rounded shape, and the cytoplasm was characterized by moderate acidophilia. The collecting ducts were formed by a single layer of prismatic epithelial cells. Intrarenal blood vessels are unevenly congested and sometimes filled with plasma. Endothelial cells are uniform, elastic membranes are not thickened, evenly spiralized. The veins are full-blooded.

Thus, the introduction of dexamethasone provoked structural damage in the kidneys of rats, the most pronounced changes were found in the renal corpuscles in the form of dystrophic and atrophic changes of the glomerular capsule epithelium, expansion of mesanigia and an increase in the area of connective tissue.

The introduction of a decoction of a traditional medicinal plant restored the histological picture of the kidney (Figure 2C).

The left ventricle of the heart was taken for research. Polymorphism of the cardiomyocyte population was detected on histological preparations of the myocardium of animals loaded with dexamethasone. Along with typical cardiomyocytes, there were hypertrophied, partially or completely destructive cells in which the transverse striation (at the optical level) was not noticeable. Significant vacuolation of the sarcoplasm was observed. The nuclei had an irregular shape and were located both in the center of cardiomyocytes and on the periphery. The fibrillar apparatus has undergone significant changes. In cardiomyocytes with contractile lesions of myofibrils, there were the shrunken, displaced to the periphery nucleus cells. Examination of the myocardium revealed changes in cardiomyocytes of three types: in the form of contractures, primary lumpy decay of myofibrils and intracellular myocytolysis (Figures 3A and 3B).

Prophylactic administration of the phytopreparation under simultaneous loading with dexamethasone restored the transverse striation of myofibrils, reduced perivascular edema of connective tissue (Figure 3C).

Microscopic examination of semi-thin sections of the lungs of animals of the experimental group on the 10th day of dexamethasone administration didn't detect significant structural differences from intact animals, except for slight hypertrophy of peribrochial and perivascular lymph nodes with pronounced vascularization. (Figures 4A and 4B).

It is noteworthy, the prophylactic intake of blueberry leaf decoction on the background of almost unchanged lung tissue leads to a sharp increase in the number of alveolar macrophages (Figures 4C and 4D). This shows the positive

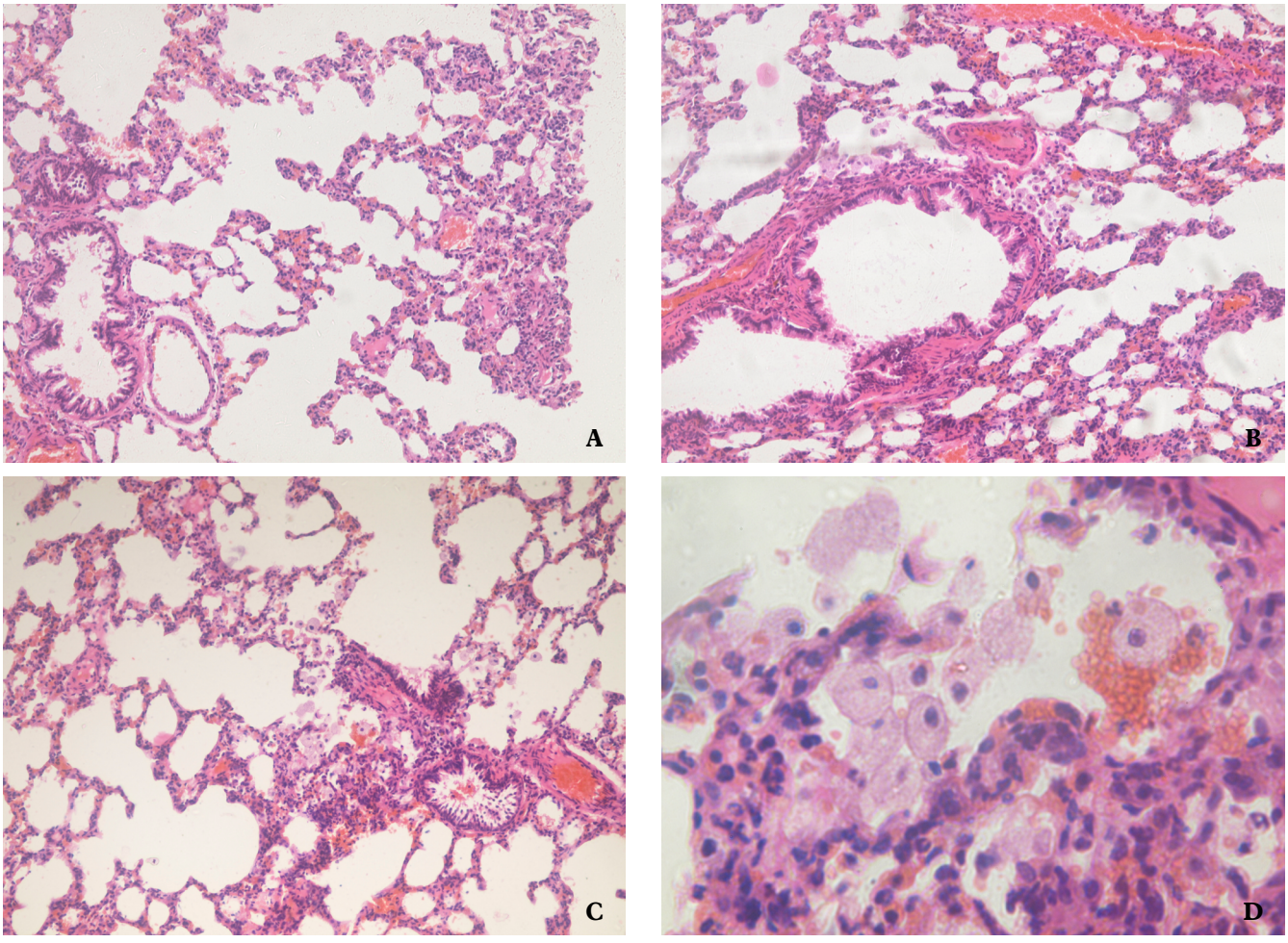


**Figure 4. Histological examination of the lung tissue of: (A) the rat of intact group (magnification ×200); (B) the rat of dexamethasone group (magnification ×200); (C) dexamethasone group and prevention (magnification ×200); (D) dexamethasone group and prevention (magnification ×1000).**

effect of blueberry leaf decoction on lung tissue, after all, pulmonary macrophages, airway epithelium and dendritic cells are key cellular elements of the host's innate defense against respiratory infections.

Confirming the rational need for this drug in severe acute respiratory syndrome (SARS) caused by coronavirus.

The rat's right eye was always taken for examination. Histological examination showed thickening of the cornea. Swelling of the outer and inner plexiform layers of the retina was detected in the dexamethasone group. The intercellular spaces of the inner and outer nuclear layers are expanded and deformed. There are individual plasmorrhagias and hemorrhages in the inner nuclear and inner reticular layers. There is also a decrease in the thickness of the outer nuclear layer with photoreceptor segments. The total thickness of the retina is much greater due to edema. Heterochromatization of nuclei, condensation of chromatin, which is a precursor and early manifestation of apoptosis, were also detected in ganglion nerve cells, cells of inner (bipolar cells and Mueller cells) and outer nuclear (rods and cones) layers. Thus, retinal changes in rats of this group should be characterized as initial neurodegenerative on the background of vasculopathy (Figures 5A and 5B).

There is a residual edema of the inner and outer reticular layer, as evidenced by an increase in its thickness in the retina of animals, which on the background of dexamethasone was injected with a decoction of blueberry leave. There is also less thickening of the outer nuclear layer with photoreceptor segments, which may be associated with the proliferation of photoreceptor cells. One of the reasons for the change in retinal thickness compared to the control group is the atrophy of the inner nuclear layer, which can be caused by the lack of retinal trophism (Figure 5C).

Histological examination of the mandibular mucosa of animals treated intramuscularly with dexamethasone at a dose of 6 mg per day for 10 days, showed that compared to intact animals (Figure 6A) there was a reorganization of all structural components of gum tissue: thickening of the epithelial layer of the free edge, increasing width of the gingival cleft, thickening of the attached part (Figure 6B).

Epithelial hyperkeratosis, parakeratosis, edema of the own plate of the mucous membrane with vascular hyperemia, dilation of intercellular spaces, growth of lymphocytic infiltration were noted.

Morphological study of the gums of rats, which on the background of the introduction of dexamethasone was injected with a decoction of blueberry leaves showed that compared with animals without treatment decreases thickening of the attached part, the width of the gingival cleft approaches the size of intact animals, the thickness of keratosis decreases, the width of the own plate of the mucous membrane decreases. At the same time there is a decrease in vascular hyperemia, single infiltration (Figure 6C).

## 5. CONCLUSIONS

(1) Intramuscular administration of dexamethasone at a dose of 6 mg / day for 10 days (as recommended by the WHO in the treatment of severe forms of COVID-19) causes severe degenerative changes in rat's liver, heart, kidneys, periodontium and retina.

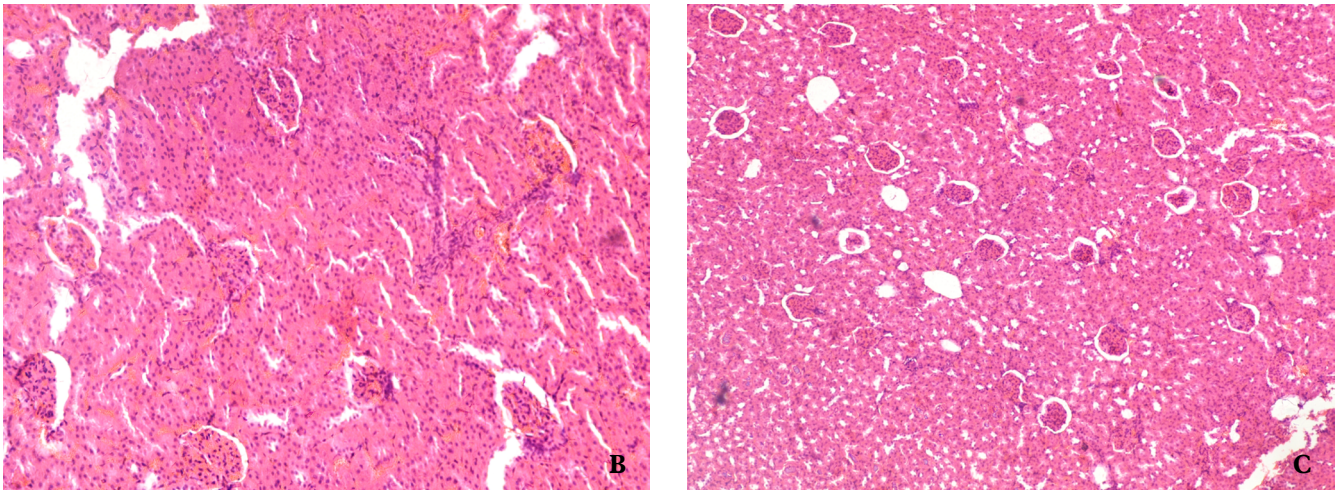


**Figure 5. Histological examination of the retina of: (A) an intact rat x100; (B) the rat of dexamethasone group; (C) the rat of dexamethasone group and prevention. Magnification ×200.**

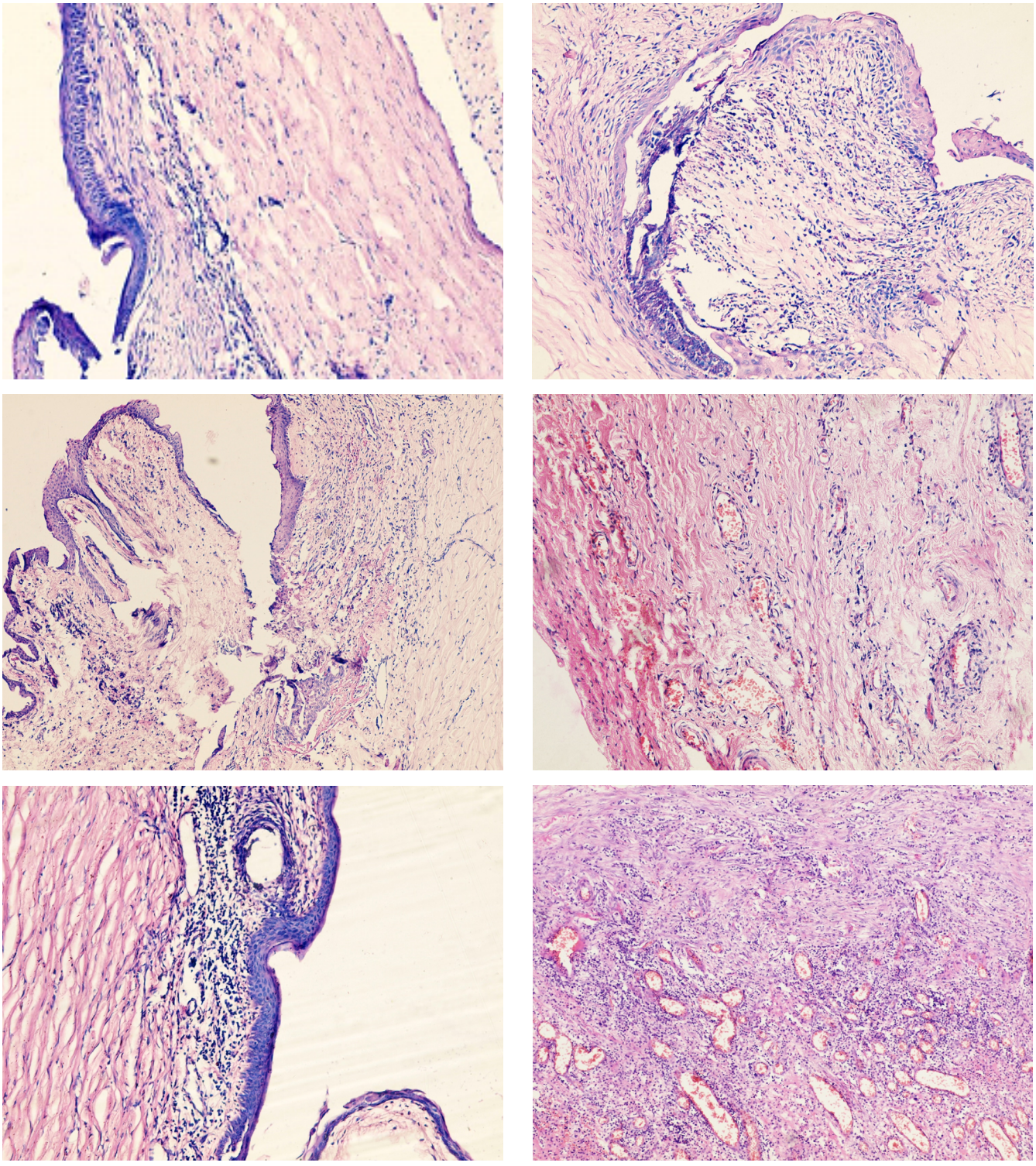

**Figure 6. Microstructure of (A) rat's periodontium; (B,C,D) rat's periodontium administered dexamethasone; (E,F) rat's periodontium injected with dexamethasone and decoction of blueberry leaves. Magnification ×100.**

(2) Taking a decoction of blueberry leaves has a pronounced organoprotective effect.
(3) Prophylactic decoction of blueberry leaves on the background of almost unchanged lung tissue leads to a sharp increase in the number of alveolar macrophages.

## Conflict of interest

The authors declare no conflict of interest.

## Funding

None declared.

## Acknowledgments
We thank our colleagues from Departments of Medical Biology, Biochemistry and Pediatric Stomatology of National Pirogov Memorial Medical University, Vinnytsya, Ukraine.

## Ethics
The minutes for this study was approved by the institutional ethics committee.

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