



Research Article

Antioxidant, Anti-infective and Anticoagulant Properties of *Ganoderma Lucidum* and Alkhadaya in the Comprehensive Correction of Coronavirus Infection COVID-19

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Abstract

Background: COVID-19 is characterized not only by active SARS-CoV-2 replication but also by systemic inflammation, oxidative stress, endothelial dysfunction, and coagulation abnormalities, which contribute to severe disease and multiorgan injury. Therefore, agents capable of simultaneously modulating viral replication, oxidative imbalance, inflammatory responses, and hemostatic disturbances may represent a promising approach to comprehensive correction of coronavirus infection. **Objective:** This study aimed to evaluate the antioxidant, anti-infective, and anticoagulant properties of a *Ganoderma lucidum*- and Alkhadaya-based intervention in an experimental model of SARS-CoV-2 infection. **Methods:** One hundred experimental animals were intranasally infected with SARS-CoV-2 and divided into four groups (n=25 each) receiving a *Ganoderma lucidum*-based intervention, remdesivir, molnupiravir, or baicalin. The 30-day study included assessment of viral load by RT-PCR, SARS-CoV-2-specific antibody responses, cytokine and chemokine profiles, biochemical and coagulation parameters, antioxidant enzyme activity, histopathological changes, body-weight dynamics, and survival. Statistical evaluation included the Mann–Whitney U test, Wilcoxon test, Pearson χ^2 test, ROC analysis, logistic regression, and Kaplan–Meier survival analysis. **Results:** Animals receiving the *Ganoderma lucidum*-based intervention demonstrated a substantially lower pulmonary viral RNA level on day 3 (3.5 log copies/mL) compared with the remdesivir, molnupiravir, and baicalin groups (6.65, 6.40, and 6.78 log copies/mL, respectively). No deaths were recorded in this group, corresponding to 100% survival, whereas mortality reached 23% in the remdesivir and molnupiravir groups and 100% in the baicalin group. Treatment was also associated with enhanced SARS-CoV-2-specific humoral responses; antibody levels against the spike protein and RBD were significantly higher than in the comparator groups ($p=0.0043$ and $p<0.0001$, respectively). The reported efficacy coefficient reached 92.7%. In addition, treatment was associated with modulation of oxidative-stress-related parameters, including increased glutathione peroxidase activity after prolonged administration. **Conclusion:** The experimental findings indicate that the *Ganoderma lucidum*- and Alkhadaya-based approach has potential multimodal activity against SARS-CoV-2 infection, combining antiviral, immunomodulatory, antioxidant, and hemostasis-related effects. Its association with reduced viral load, enhanced antibody responses, and improved survival supports further controlled experimental studies to clarify its mechanisms, safety, and therapeutic potential.

Keywords

COVID-19, SARS-CoV-2, Coronavirus Infection, *Ganoderma Lucidum* (Reishi mushroom), Alkhadaya, Antioxidant Properties, Anticoagulant Properties, Antiviral Activity

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1. Introduction

Coronaviruses (family Coronaviridae) are common pathogens of humans and animals. Four coronaviruses are endemic to humans (human coronavirus NL63 (HCoV-NL63), HCoV-229E, HCoV-OC43, and HCoV-HKU1) and typically infect the upper respiratory tract, causing symptoms of the common cold [33, 54, 66]. Over the past two decades, three zoonotic coronaviruses (severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome coronavirus (MERS-CoV), and SARS-CoV-2) have infected humans after transmission from animal hosts. SARS-CoV originated in China and caused an epidemic in 2003, while MERS-CoV currently causes periodic outbreaks in the Middle East [12, 24, 46]. SARS-CoV-2, the causative agent of COVID-19, was first identified in Wuhan, China, in late 2019 in a cluster of patients with pneumonia. These three viruses can replicate in the lower respiratory tract and cause potentially fatal acute respiratory distress syndrome (ARDS) [3, 41, 55].

In a review article by Mart M. Lamers (2024) [71], the main factor of coronavirus tropism is the spike glycoprotein, which forms trimers on the surface of virions. The spike protein consists of two subunits: the S1 subunit, which binds to the host cell entry receptor angiotensin-converting enzyme 2 (ACE2) [11], and the S2 subunit, which mediates membrane fusion. These two subunits are separated by the S1–S2 region, which contains a furin cleavage motif and is cleaved in the virus-producing cell [28, 63, 74]. After binding to ACE2 on the target cell, the spike protein is cleaved by the transmembrane serine protease TMPRSS2 at the S2' region. This cleavage activates S2 subunit trimers to fuse the viral and cellular lipid bilayers, releasing the viral ribonucleoprotein complex into the cell. Another entry pathway that may be used by the virus is the endosome, in which cathepsins can cleave the spike protein, but this pathway is inefficiently used in primary epithelial cells [7, 13, 31]. Other coreceptors (e.g., neuropilin 1) and proteases (e.g., cathepsin L, TMPRSS11D, and TMPRSS13) are also proposed to be involved in SARS-CoV-2 entry, but their respective contributions to SARS-CoV-2 pathogenesis remain unclear [2, 16, 35].

The concept of "oxidative stress" was first formulated by Zies in 1985, according to which "oxidative damage caused by reactive oxygen species is called "oxidative stress"" [5, 38]. Since then and to this day, "oxidative stress" has attracted considerable interest in the scientific community and is a prerequisite for basic research in various biomedical fields [34, 53, 61]. Oxidative stress is a process of disturbance of redox homeostasis caused by psychological, physiological or environmental stressors. Redox stress is an imbalance between antioxidants and prooxidants in favor of oxidants. Redox stress is a phenomenon that includes abnormally high levels of reactive oxygen species and reactive nitrogen species (ROS and RNF), and a violation of antioxidant capacity [10, 19, 26].

The term "free radicals" can be broadly defined as any molecular species containing an unpaired electron and capable of independent existence [20, 37, 49]. In the last decade, many researchers have confirmed that RONS are not only mitochondrial and enzymatically generated by-products but also important signaling molecules required for regulating the immune response [27, 48, 56]. Within physiological limits, free radicals play a major role in various biological processes such as cellular signaling and regulation of redox homeostasis [22, 42, 60]. Reactive oxygen and nitrogen species (RONS) play a role in the pathophysiology of infectious processes, including viral infections. RONS are produced by immune cells (macrophages and neutrophils) as part of host defense against viral infections. They act as cytotoxic molecules that damage viral proteins, nucleic acids and lipid membranes of the virus [17, 30, 47].

Thus, they inhibit viral replication and dissemination and serve as signaling molecules in the activation of the immune response [23, 43, 64]. Despite their important physiological role, reactive oxygen and nitrogen species (RONS) are major inducers of oxidative stress in the body. Although RONS may be beneficial in limiting viral replication, excessive or uncontrolled production can lead to oxidative stress and damage to host cells. This oxidative stress can contribute to tissue damage and inflammation observed in many viral infections [4, 9, 14].

According to Ekaterina Georgieva (2023) [50] defined the term "redox status" as a general characteristic of an interconnected set of redox reactions and a comprehensive description of the "redox environment" in cells and tissues. It describes the redox state of complex biological systems and multiple redox processes, summarizing the reducing potential and capacity of the organism as a whole. A remarkable discovery in redox biology is the understanding that redox reactions are used in living cells in the fundamental processes of redox regulation, which are collectively referred to as "redox signaling" and "redox control" [11, 57, 69]. The intracellular redox state is a dynamic system that can change under the influence of various external and internal factors. A healthy organism is in a state of equilibrium, characterized by the maintenance of physiological levels of ROS by intracellular reductants [1, 32, 68]. Several studies demonstrate persistently high levels of ROS and their indirect formation by xenobiotics, pollutants, or other factors [36, 52, 59]. Toxicants initiate oxidative damage to various macromolecules and cellular components, thereby contributing to the development and progression of many neurodegenerative, cardiovascular, and endocrine diseases, inflammation, aging, and cancer [8, 44, 65]. For example, in patients with COVID-19, the virus can cause increased ROS production and decreased antioxidant levels, leading to redox imbalance and contributing to the development of severe symptoms such as acute respiratory distress syndrome

(ARDS) [6, 18, 25].

According to the concept of Mohammad Shah Alam (2022) a particularly dangerous medical situation in some patients with COVID-19, called “cytokine storm syndrome”, is a hyperinflammatory response associated with elevated levels of neutrophils and proinflammatory cytokines, often leading to respiratory failure [72]. Specifically, an increase in neutrophils and a significant decrease in lymphocytes, as well as increased levels of D-dimer, interleukin-6 (IL6), and C-reactive protein (CRP), correlate with the worst outcomes and highest mortality in patients with COVID-19 [15, 40, 62]. A recent prospective A cohort study showed that the risk of mortality increases by 10% for every 10% increase in IL6 or D-dimer levels [39, 58, 67]. Interestingly, these critically ill patients were also found to have higher levels of ROS, which contribute to a cascade of biological effects leading to pathophysiological responses such as endothelial cell dysfunction, hypercoagulability, thrombosis, and platelet aggregation, which contribute to the severity of COVID-19 [21, 45, 70]. Thus, oxidative stress and associated inflammation are now recognized as important factors in the pathogenesis and severity of COVID-19 [29, 51, 73].

The aim of the study was to evaluate the antioxidant, anti-infective, and anticoagulant properties of *Ganoderma lucidum* and *Alkhadaya* in the comprehensive correction of coronavirus infection COVID-19.

2. Materials and Methods of the Study

To achieve the stated objectives, the results of treatment with *Ganoderma* were analyzed. *Lucidum* and *Alkhadaya*'s coronavirus infection tests were performed on 100 mice weighing 15-20 grams. The mice were of both sexes.

The virus was isolated using viro cell culture, from a virus-containing clinical sample (nasopharyngeal swab). The efficiency of SARS-CoV-2 virus replication in cell culture was assessed based on the dynamics of the cytopathic effect and the presence of viral RNA in the culture fluid using polymerase chain reaction (PCR).

Rats were intranasally infected with the SARS-CoV-2 strain at 50% of the tissue culture median infectious dose (TCID₅₀) per 50 µl of inoculum (live culture biopreparation) after intraperitoneal anesthesia using 2.5% sodium thiopental solution.

All animals were divided into 4 groups:

Group 1 – 25 animals that were administered *Ganoderma* oil solution as a treatment *Lucidum* at a dose of 100 mg/kg body weight. *Ganoderma* oil solution *Lucidum* prepared by dissolving *Reishi* capsules Gold with vitamin E in a 1:1 ratio, i.e. 1 capsule of *Reishi* to 1 ampoule of vitamin E.

II group – rats infected with coronavirus infection, treated with remdesivir (n = 25).

III – rats infected with coronavirus infection, treated with molnupiravir (n = 25)

IV – rats infected with coronavirus infection, treated with

baicalin (n = 25)

Molnupiravir is an experimental oral formulation of a potent ribonucleoside analogue that inhibits the replication of SARS-CoV-2, the causative agent of COVID-19. In this study, Molnupiravir was administered at a dose of 25 mg/kg body weight.

Baicalin was obtained from *Scutellaria baicalensis* with a particle size of 0.1-0.5 mm. The extraction method used is simple maceration for a specified period of time, at a raw material: extractant ratio of 1:10 w/v and a temperature of 24 ± 1 °C. Baicalin contains the active enzyme beta-glucuronidase, contained in the cells of the root of *Scutellaria baicalensis*, which, when the raw material is wetted with an extractant containing water, begins to actively hydrolyze. Baicalin is converted to its aglycone (baicalein) and glucuronic acid. Baicalin stimulates the glutathione pathway of the NADPH-GSH-dependent antioxidant system: it increases the level of reduced glutathione in the liver of healthy animals and the activity of glutathione synthetase and glucose-6-phosphate dehydrogenase in the liver and kidneys under conditions of oxidative stress caused by various toxicants. Baicalin was administered to rats intraperitoneally at a dose of 15 mg/kg body weight.

To study the morphofunctional zones of the hepatic lymph node, an MBI-3 microscope was used with an objective magnification of 90x under oil immersion and a 25-node grid in the eyepiece (x10). The structural organization of the organ was assessed using the MBI-3 microscope with objective magnifications of x10 and x40x and an eyepiece magnification of x10.

Animals were euthanized under ether anesthesia, after which they were decapitated on days 3, 10, 20, and 30, and biochemical indicators of lipid peroxidation and the activity of antioxidant enzymes were determined in the blood serum.

Cardiac tissue samples: endomyocardial biopsies, autopsy material, surgical material - fixed in 10% neutral formalin solution with phosphate buffer, dehydrated in the apparatus for histological processing of tissues of the company "Pool" Scientific Instruments (Switzerland) were embedded in paraffin. Paraffin blocks were then cut, and 4-µm-thick sections from each block were placed on standard slides. One specimen from each case was then stained with hematoxylin and eosin using the standard method, and with picrofuchsin using Van Gieson. Microscopic examination was performed using a Leica microscope. Standard. The myocardium, endocardium, and small branches of the coronary arteries were assessed in the biopsy material. The pericardium was also assessed in the autopsy and surgical material. Myocardial analysis included an assessment of the condition of cardiomyocytes and their nuclei, their size, the presence of inflammatory infiltration, the presence of fibroblastic foci, and foci of lipomatosis. Endocardial and pericardial preparations were analyzed for pericardial thickness, the extent of connective tissue, and the presence/absence of infiltrates. Coronary arteries were examined for the condition of the vascular endothelium, the presence of inflammatory infiltration, and wall sclerosis.

Serum samples from all experimental animals were analyzed to determine various parameters: electrolytes (K, Na, Ca), troponin T, creatinine, urea, uric acid, total protein, albumin, bilirubin, transaminases (ALT, AST, ALP), procalcitonin, ferritin, glucose, cholesterol fraction, high-sensitivity C-reactive protein, ethanol test, blood type, and Rh factor. D-dimer levels, prothrombin time, fibrinogen, APTT, and platelet count were also determined in the blood, with subsequent regular monitoring of these parameters.

Clinical blood test parameters were examined using an automatic hematology analyzer DxH 800 from Beckman Coulter (USA). Blood biochemistry was performed using the AU-480 Beckman biochemical analyzer. Coulter (USA). The coagulogram was performed using an automatic hemostasis analyzer (coagulometer) ACL TOP 300 from Instrumentation Laboratory Co. (USA).

In order to assess the cytokine status, all animals ($n = 100$) were assessed for levels of proinflammatory cytokines and chemokines, such as IFN- α , IFN- γ , IL-1, IL-6, IL-10, MIP-1 α , MIP-1 β , IP-10, in the blood serum using solid-phase ELISA.

Cytokine levels were determined using Bioscience kits with automated microplate processing. reader Anthos 2020 (Anthos Labtec Instruments GmbH, Austria) at a wavelength of 450 nm with correction of 620 nm with the construction of a standard curve of optical density and determination of the concentration of the studied cytokines.

The assay methodology is based on a sandwich solid-phase ELISA using mAbs to two different antigen epitopes. The assay was performed according to the manufacturers' instructions. In the first step, calibration samples with known concentrations of the analyte, as well as the test samples, were incubated in the wells of a strip plate with highly specific mAbs to the analyte immobilized on the well surface. The wells were then washed three times with wash buffer and decanted. Residual liquid was removed by tapping the inverted strip frame on filter paper.

In the second step, the factor to be determined, bound to the first mAbs in the wells, was treated with second mAbs labeled with the biotype. The mixture was incubated for 1.5 hours with continuous shaking. After incubation, the washing process was repeated. In the third step, the conjugate was added to all wells of the plate. Streptavidin with horseradish peroxi-

dase. Incubated for 1 hour with continuous shaking. After removing excess conjugate, a substrate mixture containing hydrogen peroxide and o-phenylenediamine was added to the wells. In this process, o-phenylenediamine is oxidized to form a dye, the color intensity of which is proportional to the concentration of the determined factor in the analyzed sample. The enzymatic reaction was stopped by adding a stop reagent, and the color intensity was measured on an ELISA photometer at a wavelength of 450 nm.

The experiment lasted 30 days and was conducted in compliance with the regulations of the European Commission for the Supervision of Laboratory and Other Experiments Involving Experimental Animals. All mice were maintained under standard conditions with natural lighting and a standard viviparous diet. All animals had free access to food and water.

For statistical data processing, we used the Python programming language v.3.8.12 (Anaconda distribution) and its modules for analysis, in particular: Sklearn, Pandas, Nona, Numpy, Sweetviz, Seaborn for data and Matplotlib and Plotly for visualization. Statistica and SPSS programs were also used. The database was created in Microsoft Excel 2019. The Mann-Whitney U test was used to compare quantitative parameters in independent patient groups, and the Wilcoxon signed-rank test was used to compare related samples. Percentage comparisons in multi-field contingency tables were performed using the Pearson chi-square test. Since the outcome was initially unknown, survival was estimated using the Kaplan-Meier estimator. Using ROC analysis and AUC, the most accurate threshold values for these parameters were determined. The odds ratios for achieving the selected endpoints were then calculated.

3. Study Results

Lymphocytic myocarditis was detected in all rats (100%). Dystrophic changes in cardiomyocytes were also detected in 57.4% of cases, apoptotic bodies in 29.41% of cases, uneven cardiomyocyte hypertrophy in 85.29% of cases, and lipofuscinosis in 45.59% of cases. Subendocardial lipomatosis in the cardiac conduction system was observed in 51.47% of cases. The average percentage of lipomatosis area from the micrograph area was $2.47 \pm 3.12\%$.

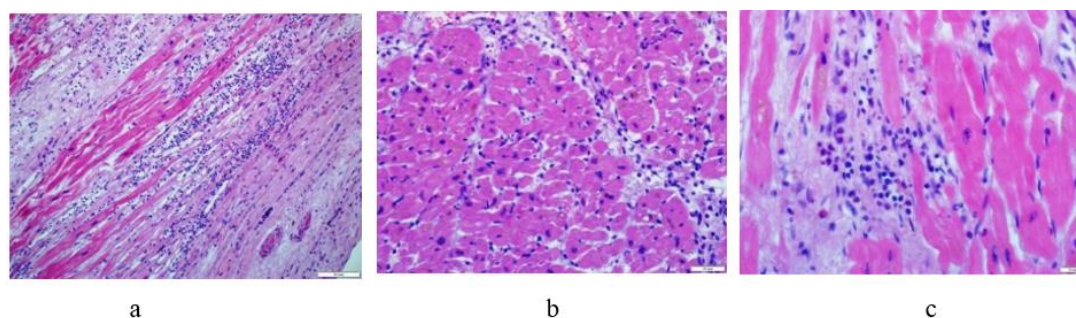


Figure 1. Lymphocytic myocarditis.

When compared with I In experimental animals of group II, dystrophic changes in cardiomyocytes were significantly less common: fatty degeneration occurred in 11 cases (34.37%). However, a high frequency of apoptosis was detected. cardiomyocytes in group II (68.75%), which may be associated with the mechanism of action of the SARS-CoV-2 virus on cardiomyocytes. Subendocardial lipomatosis was detected in 7 (21.88%) cases. The average percentage of lipomatosis area from the micrograph area was $1.21 \pm 1.66\%$, which is significantly lower than in group III. In group IV, lymphohistiocytic infiltrate in the endocardium was detected significantly more

often than in group II, in 11 (34.37%) cases, in the pericardium - in 8 (25.00%). Mural thrombi consisting of fibrin, platelets, erythrocytes and a large number of lymphocytes - in 7 (21.88%) cases.

Also, dystrophic changes in cardiomyocytes were detected in 39 cases (57.4%), apoptotic bodies – in 20 cases (29.41%), uneven hypertrophy of cardiomyocytes – in 58 cases (85.29%), lipofuscinosis – in 31 cases (45.59%). Subendocardial lipomatosis in the area of the cardiac conduction system was observed in 35 cases (51.47%). The average percentage of lipomatosis area from the micrograph area was $2.47 \pm 3.12\%$.

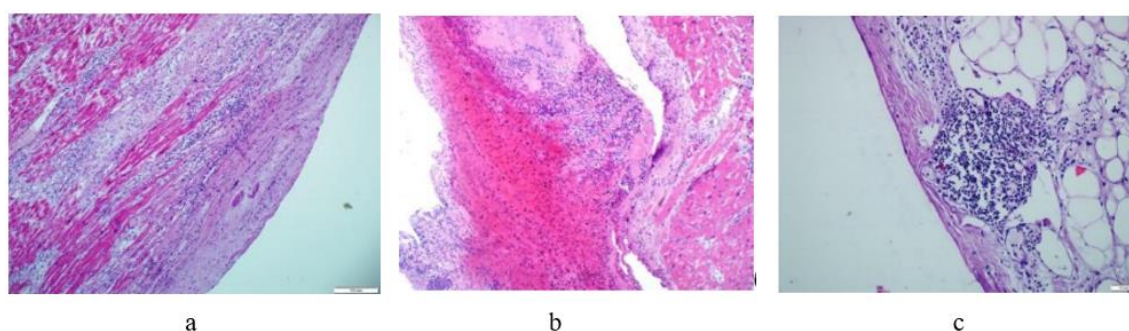


Figure 2. Endocardium and pericardium in the main group.

Inflammatory markers were higher in the rats of Group 1: IL-6 was greater than 18% of the norm, ferritin was greater than 3% of the norm, LDH was greater than 0.5% of the norm, and procalcitonin (10.4% versus 3.9%) was significantly

higher than in the other groups. Table 1 provides a comparative analysis of blood coagulation parameters in the study groups.

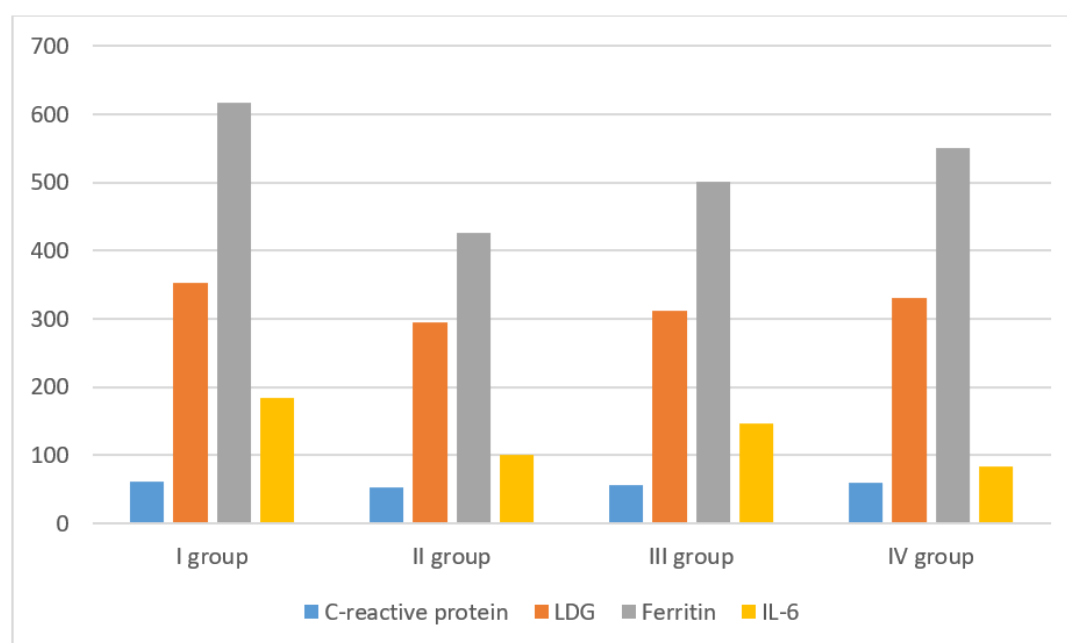


Figure 3. Laboratory parameters in the study groups.

Table 1. Blood clotting parameters in the study groups.

Indicators	I	II	III	IV	p
Prothrombin index (PTI)	93.6±2.5	94.5±4.8	92.7±1.2	90.8±3.2	<0.05
International Normalized Ratio (INR)	1.2±0.03	2.7±1.1	3.5±1.4	2.0±0.4	< 0.05
Activated partial thromboplastin time (APTT)	27.4±0.7	24.8±0.6	23.9±0.5	26.5±0.7	
D- dimer	341.3±45.8	80.8±26.1	110.3±30.5	200.7±34.9	< 0.05

Significant differences in coagulation parameters in the study groups were noted in relation to D- dimer. An increase in the INR level was revealed in Group III, caused by anticoagulant therapy used in experimental animals with COVID - 19.

ferritin) as markers of coronavirus disease progression were

analyzed. infection by constructing a receiver operating characteristic (ROC) curve and calculating the area under the curve (AUC). Changes in CRP, LDH, and ferritin levels were characterized by low sensitivity and specificity as markers of infection progression. ROC curves with AUC for these parameters are presented in [Figure 4](#).

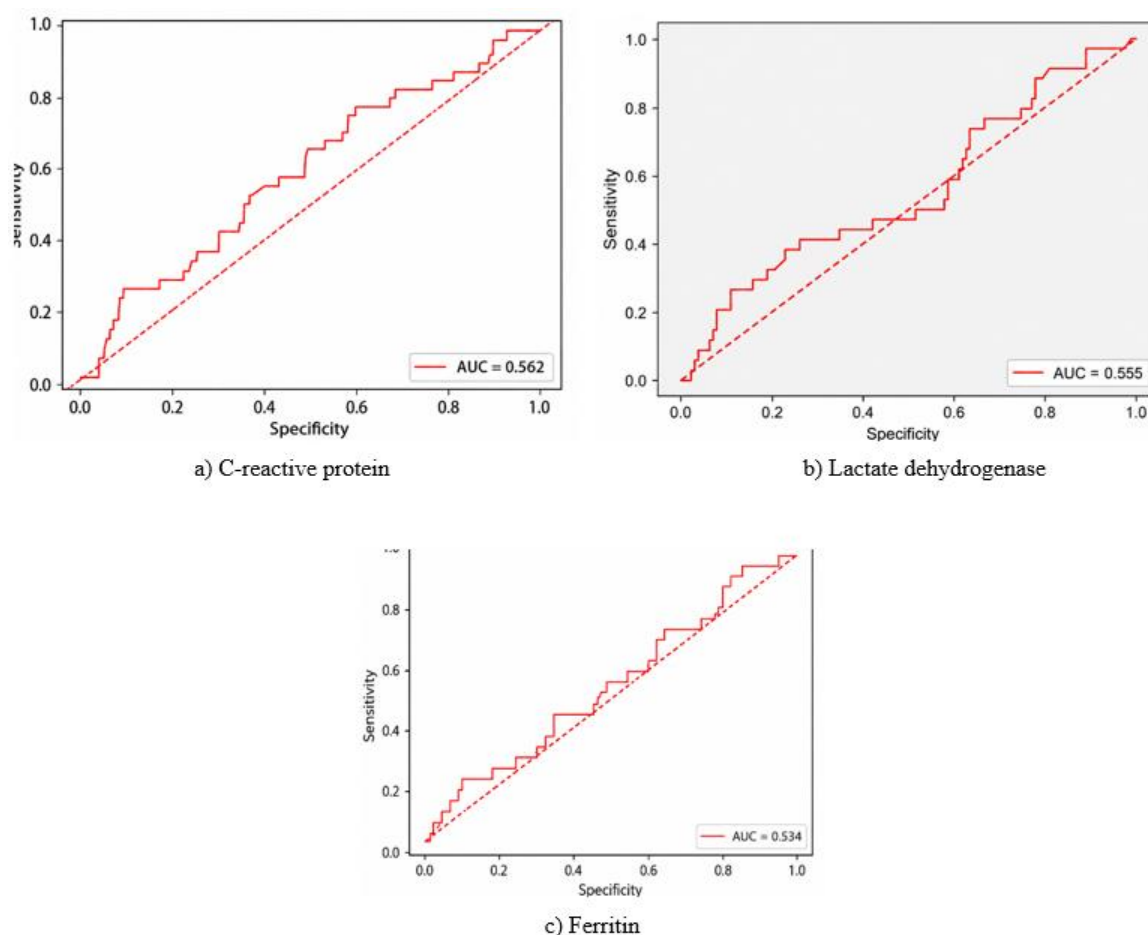


Figure 4. ROC analysis of the logistic regression model based on the dynamics of CRP, LDH and ferritin levels and the progression of COVID-19.

When analyzing D - dimer as a marker of thrombosis using ROC analysis with AUC calculation, high sensitivity and high specificity of this indicator as a diagnostic marker for the presence of thrombosis were noted ([Figure 5](#)).

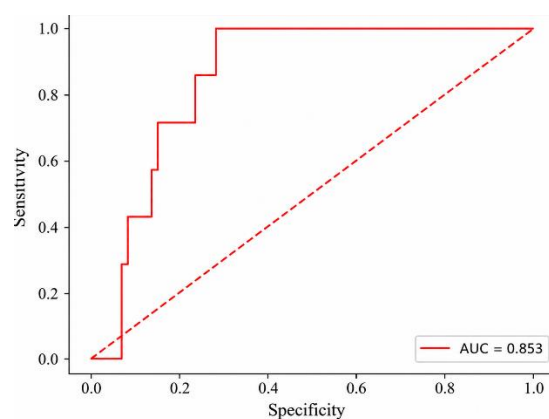
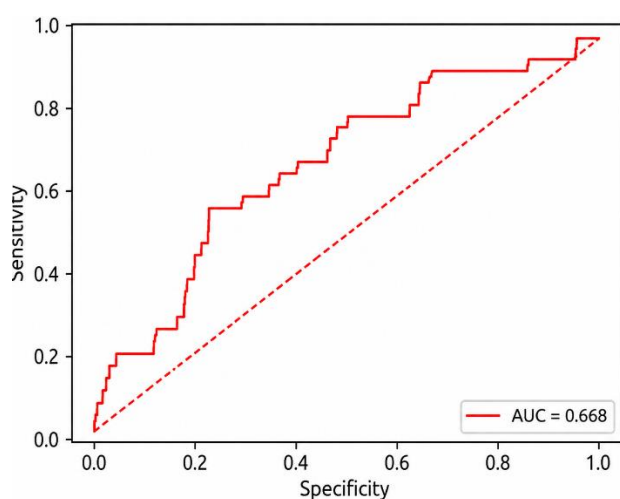
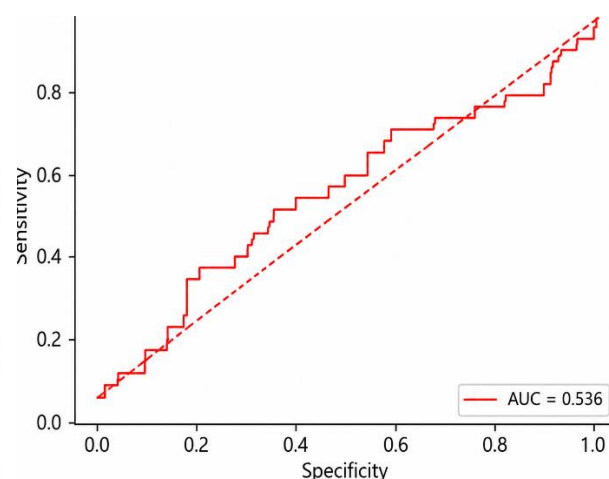


Figure 5. ROC analysis of the logistic regression model based on the D- dimer level and the presence of thrombosis.

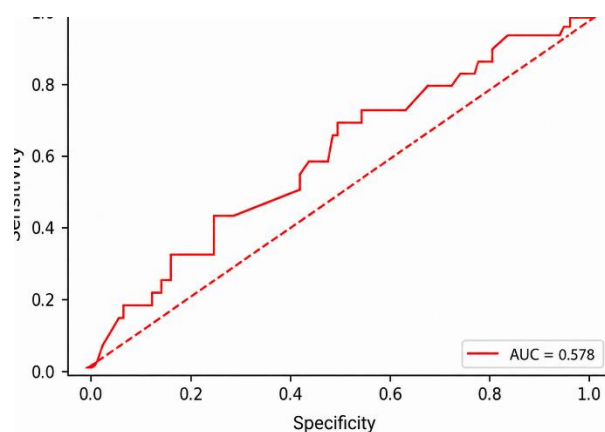
Leukocyte, neutrophil, and procalcitonin levels demonstrated low sensitivity and specificity as markers of bacterial infection using ROC analysis (Figure 6).



a) White blood cell count



b) Neutrophil level



c) Procalcitonin test

Figure 6. ROC analysis of the logistic regression model based on the dynamics of leukocyte levels, procalcitonin test, neutrophils and the presence of bacterial infection.

When assessing the cytokine status of experimental animals with COVID-19, the levels of IFN- α , IFN- γ , IL-1, IL-6, IL-10, MIP-1 α , MIP-1 β and IP-10 were examined.

Table 2. Levels of cytokines and chemokines in the study groups.

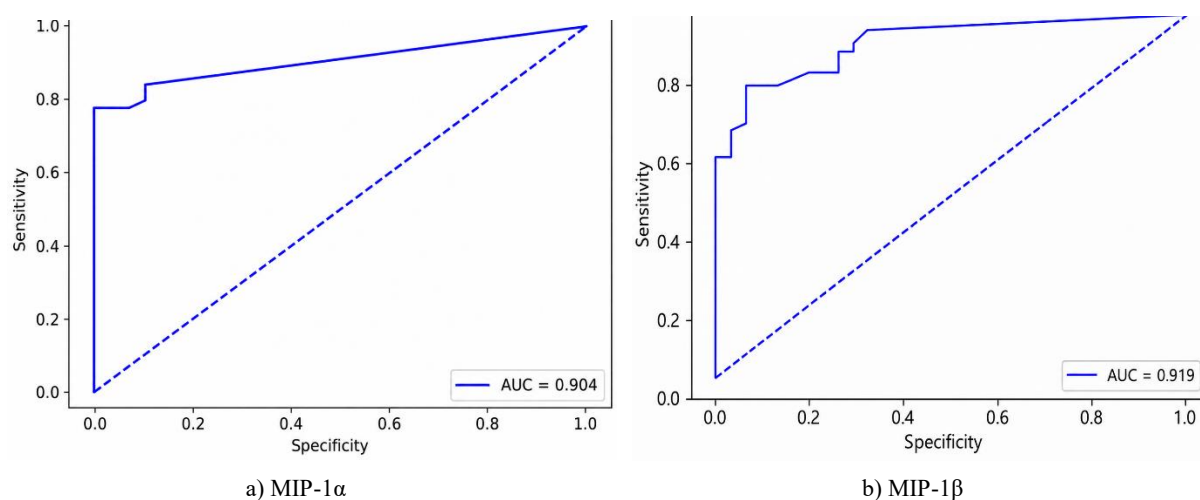
Indicators. pg / ml	I	II	III	IV	Reference values
IFN- α	0.78 (95% CI: 0; 2.25)	0.66 (95% CI: 0; 2.01)	0.89 (95% CI 0; 2.12)	0	Up to 10
IFN- γ	2.24 (95% CI: 0.71; 2.96)	0.55 (95% CI: 0; 1.09)	1.12 (95% CI: 0.12; 2.01)	0.98 (95% CI: 0.22; 1.34)	Up to 50
IL-1	9.41 (95% CI: 3.99; 14.83)	5.54 (95% CI: 1.52; 11.95)	7.03 (95% CI: 1.96; 12.03)	6.73 (95% CI: 2.31; 13.47)	Up to 5
IL-6*	159.53 (95% CI: 94.14; 224.91)	29.08 (95% CI: 10.65; 47.52)	50.92 (95% CI: 30.84; 78.77)	98.26 (95% CI: 42.45; 147.03)	Up to 7
IL-10 *	74.27 (95% CI: 40.27; 108.26)	65.83 (95% CI: 31.55; 97.84)	0	4.26 (95% CI: 0.64; 7.89)	Up to 9.1
MIP-1 α *	103.64 (95% CI: 66.86; 140.43)	3.71 (95% CI: 0.56; 6.85)	69.44 (95% CI: 42.62; 97.33)	45.13 (95% CI: 22.06; 77.83)	Up to 15.6
MIP-1 β *	131.12 (95% CI: 86.34; 175.91)	55.28 (38.64; 101.72)	7.53 (95% CI: 4.64; 10.43)	97.61 (95% CI: 56.94; 154.37)	Up to 7.8
IP-10*	176.21 (95% CI: 138.2; 214.22)	55.67 (95% CI: 48.09; 63.25)	90.44 (95% CI: 66.71; 138.49)	122.86 (95% CI: 100.45; 188.63)	Up to 3.1

*- Mann-Winney U -test, $p < 0.05$

A positive correlation was found between the levels of MIP-1 α and MIP-1 β ($r = 0.76$).

The conducted ROC analysis revealed the high potential of MIP-1 α and MIP-1 β as prognostic markers for severe COVID-19 (Figure 7). Thus, the sensitivity of MIP-1 α as a

marker of severe disease was 81.6%, and the specificity was 90%. MIP-1 β as a marker of severe disease had a sensitivity of 89.8% and a specificity of 73.3%. When MIP-1 α and MIP-1 β were determined together, the sensitivity was 86.1%, and the specificity was 90%.



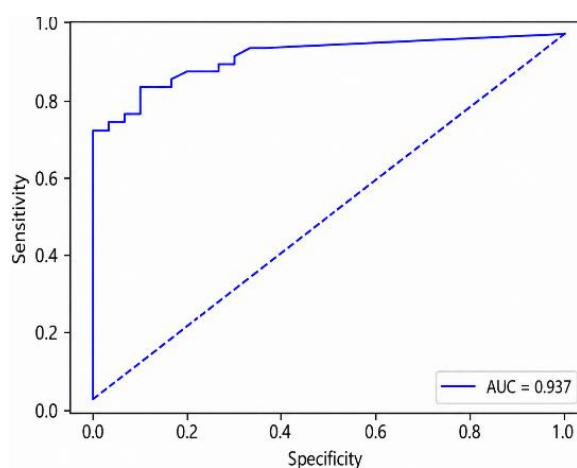
c) MIP -1 α and MIP -1 β

Figure 7. ROC analysis of logistic regression models based on levels MIP-1 α and MIP-1 β in blood serum as a marker of disease severity.

When analyzing IL-10 as a potential marker of lethal outcome, low sensitivity (56.9%) with a fairly high specificity (88%) was revealed (Figure 8).

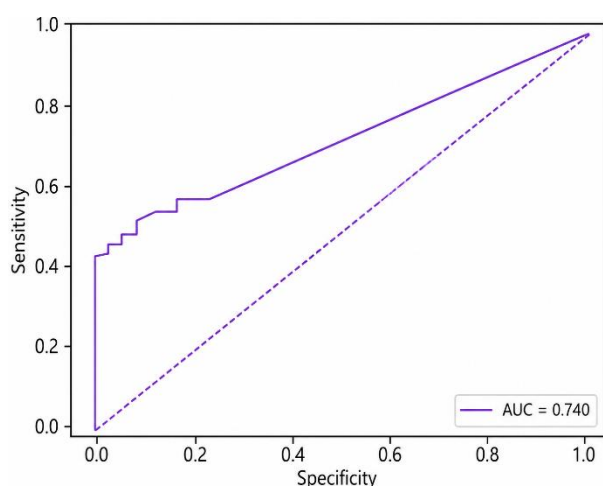


Figure 8. ROC analysis of logistic regression models based on serum IL-10 concentration and mortality.

The sensitivity and specificity of MIP-1 α as a marker of mortality were retrospectively analyzed using ROC analysis (Figure 9). This indicator was highly significant for predicting mortality: the sensitivity of MIP-1 α was 76.5%, and the specificity was 93.8%.

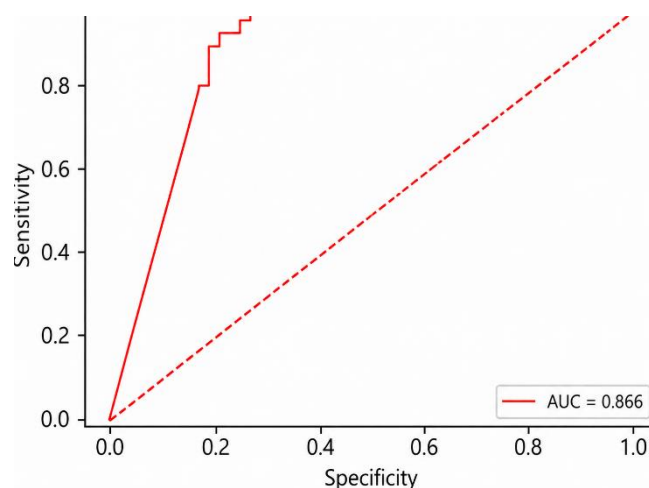


Figure 9. ROC analysis of the logistic regression model based on the dynamics of MIP-1 α levels and mortality in experimental animals with COVID-19.

The sensitivity and specificity of MIP-1 β as a mortality marker were retrospectively analyzed using ROC analysis (Figure 10). This indicator was highly significant for predicting mortality: sensitivity was 70.6%, specificity 70%.

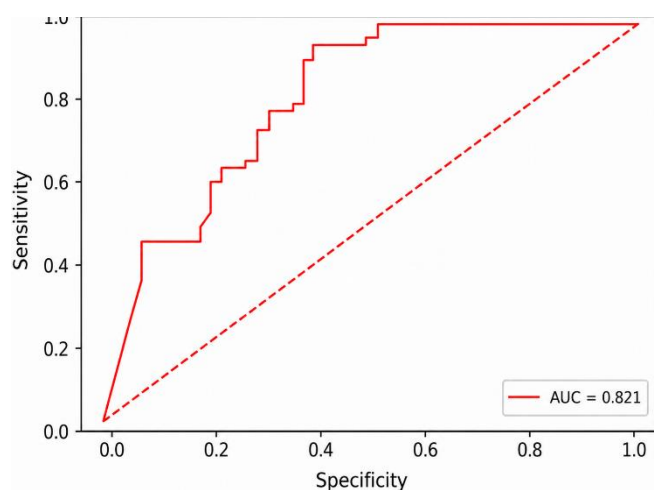


Figure 10. ROC analysis of the logistic regression model based on dynamics MIP-1 β levels and mortality in experimental animals with COVID-19.

The sensitivity and specificity of IP-10 as a mortality marker were retrospectively analyzed using ROC analysis (Figure 11). This indicator was characterized by low sensitivity (45.1%) and high specificity (77.6%).

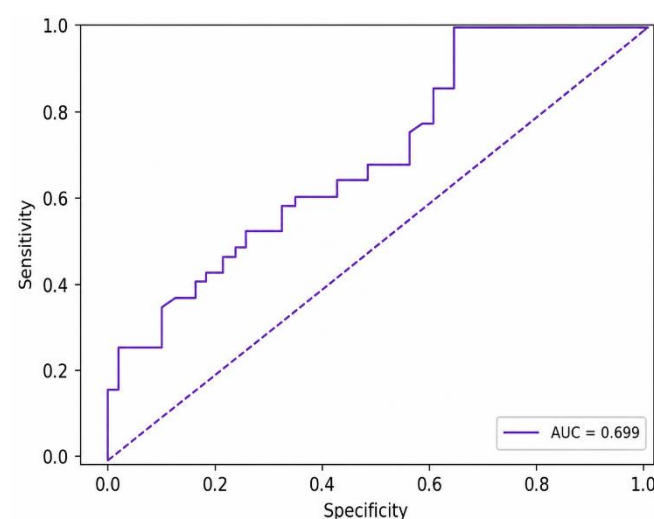


Figure 11. ROC analysis of the logistic regression model based on dynamics IP-10 levels and mortality in experimental animals with COVID-19.

Levels of MIP-1 α and MIP-1 β were statistically higher in experimental animals with a lethal outcome (Mann–Whitney test, $p < 0.05$), suggesting a significant role of macrophage activation syndrome in the development of lethal outcomes. High levels of IL-10 likely indicate that macrophage activation occurs via the alternative pathway (M2).

When analyzing blood biochemistry parameters in animals with mild, moderate, and severe forms of COVID-19, high levels of CRP (40.34 mg/L with a norm of 0.0–5.0 mg/L) were

detected on the first day of the experiment with severe forms of the disease. A significant increase in D-dimer was observed in animals with severe COVID-19 (949.40 $\mu\text{g/L}$ FEU). With mild COVID-19, the D-dimer level in the first days was normal – 256.76 $\mu\text{g/L}$ FEU, and with moderate forms of the disease, it was also normal – 314.66 $\mu\text{g/L}$ FEU. A decrease in serum protein was noted in severe forms of the disease.

The resulting regression model is statistically significant ($p=0.001$). Using ROC analysis, the optimal value of the prognostic function p was determined. The area under the ROC curve (AUC) was 0.844. The value of the logistic function p in the cut-off cutoff value was 0.1305. Experimental animals with p values of 0.1305 or higher were predicted to have a high probability of developing a severe course of this infection; at $p < 0.1305$, a low probability of developing severe forms of COVID-19 was predicted. The sensitivity of this model at the chosen cutoff value point " was 84.1%, specificity – 74.5%.

Epithelial cells of the mucous membranes of the upper respiratory tract are capable of intense expression of the viral receptors ACE2 and TMPRSS2, which are targets for SARS-CoV-2. Moreover, a high viral load in respiratory tract tissues is a risk factor for severe COVID-19 and death [37]. However, respiratory epithelial cells are surrounded and colonized by complex microbial communities, which can obviously influence the adhesion of infectious disease pathogens, including coronaviruses, and their accumulation in tissues during the infectious process. In turn, SARS-CoV-2 virions, upon entering the body interact with local immunity factors and with resident microbiota, which performs a protective function.

Thus, elevated levels of C-reactive protein (CRP), interleukin-6, and D-dimer in the blood are key to diagnosing systemic hyperinflammation, cytokine storm, and severe cases of COVID-19.

Preventive efficacy of the drug based on Ganoderma Lucidum and Alkhadaya were significantly more effective than baicalin ($\chi^2=4.09$, $p=0.043$) and remdesivir ($\chi^2=7.31$, $p=0.00683$). There were no significant differences in prophylactic efficacy between the Ganoderma-based drug No significant differences were found between Lucidum and Alkhadaya and molpupiravir ($\chi^2 = 0.04$, $p = 0.82$). No significant differences were found in the prophylactic efficacy of baicalin and remdesivir ($\chi^2 = 0.028$, $p = 0.96$).

The median level of virus-neutralizing IgG antibodies to the SARS-CoV-2 S protein among animals in group III was 608 BAU/ml. In group II, it was 362.2 BAU/ml (CI 95% [104.4–510.5]; asymptotic significance 0.002) and was lower than in In group I, 1133 BAU/ml (CI 95% [623.7–2953.0]; asymptotic significance 0.001). However, it did not differ from the median level of virus-neutralizing IgG antibodies to the SARS-CoV-2 S protein in group IV, 196.45 BAU/ml (CI 95% [40.1–505.6]; asymptotic significance 0.001).

We have found that infectivity In group I it was 194.4 versus 548.3 per 1000 in group II (RR = 2.8; CI 95% [1.59–5.01]; ($\chi^2=20.9$; $p=0.001$). The effectiveness coefficient of the drug based on Ganoderma Lucidum and Alkhadaya accounted for

92.7%.

When comparing animals in group II, the incidence of COVID-19 was 586.2 per 1000 and was 2.6 times higher than the incidence in group III (RR=2.6; CI 95% [1.8-4.8]; ($\chi^2=10.7$; $p=0.001$). A similar situation was observed in group IV (RR=6.4; CI 95% [1.6-25.3]; (F-test = 0.0014).

The level of antibodies formed after the correction of the new drug based on Ganoderma Lucidum and Alkhadaya proved to be an important criterion for the risk of developing the disease. Based on the ROC curve analysis method (AUC = 0.688; 95% CI: 0.581-0.795, $p = 0.002$), a cutoff point of 287.00 BAU/ml was identified, i.e., an antibody value below 287.00 BAU/ml predicts the risk of COVID-19 infection.

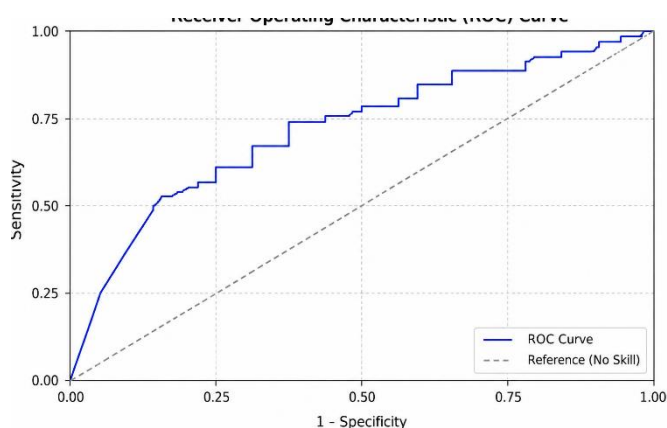


Figure 12. ROC curve characterizing the discriminatory ability of the antibody level in predicting the incidence of the new coronavirus infection.

The Kaplan-Meier curve was used to evaluate animal survival data after coronavirus infection.

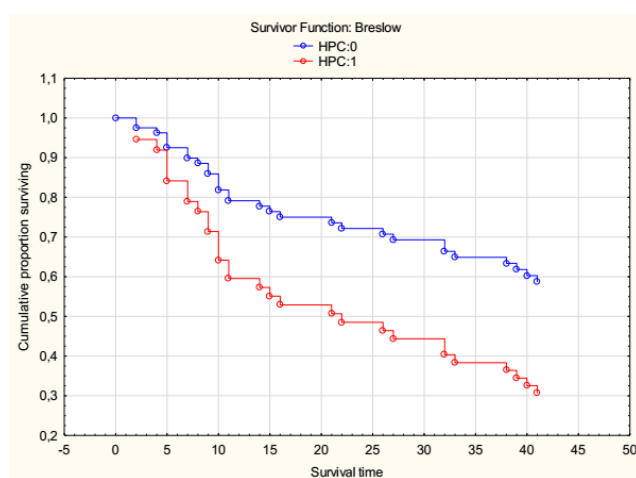


Figure 13. Kaplan-Meier curve in groups: 1-main group, 0-control group.

The vertical lines on the graph represent 95% confidence

intervals.

According to the Kaplan-Meier curve, the survival probability for animals in the study group is 65%, while for animals in the control group it is 75%. The data obtained indicate that the risk of death after coronavirus infection in Group IV remains quite high.

Moreover, in the γ -correlation model, the degree of dependence of an unfavorable outcome on the presence or absence of coronavirus infection was not close ($r = 0.20$ at $p = 0.32$), but according to the Pearson criterion, this difference was not significant ($p = 0.32$, $\chi^2 = 2.145$).

The model for the main group is statistically significant (Wald Chi-square $p=0.002$), consistent with the initial data (Hosmer-Lemeshow test $p=0.470$), has satisfactory information content (Nagelkirk criterion $R^2=55\%$), sufficient sensitivity (62%) and high specificity (95%)

The model for the control group is statistically significant (Wald Chi-square $p=0.003$), is in good agreement with the initial data (Hosmer-Lemeshow test $p=0.515$), has satisfactory information content (Nagelkirk criterion $R^2=40\%$), sufficient sensitivity (62.5%) and high specificity (87.5%).

Using long The rank test results showed that the model of coronavirus infection proposed by us is statistically significant (Wald Chi-square $p=0.003$), is in good agreement with the initial data (Hosmer-Lemeshow test $p=0.790$), has satisfactory information content (Nagelkirk criterion $R^2=56\%$), sufficient sensitivity (63.2%) and high specificity (87.8%).

7-day Ganoderma treatment Lucidum and Alkhadaya did not demonstrate statistically significant changes in serum glutathione peroxidase (GPO) activity. In particular, a 9% increase in enzyme activity with the administration of Ganoderma Lucidum and Alkhadaya at a dose of 15 mg/kg/day was not statistically significant. However, intake at a dose of 15 mg/kg/day Ganoderma Lucidum and Alkhadaya administered for 14 days resulted in a statistically significant increase in serum GPO activity by 9% and 8%, respectively, compared to the corresponding values in Groups 2 and 3. The overall trend toward increased serum GPO activity was close to statistically significant. It is important to note that intra-gastric administration of the drug at various doses and over different periods did not result in any significant modification of serum SOD activity in the blood of the experimental animals.

In laboratory animals, during both 7- ($r = 0.651$; $p = 0.006$) and 14- day ($r = 0.554$; $p = 0.017$) experimental observations, correlation analysis showed a statistically significant relationship between the concentration of molnupinavir and the activity of GPO in the blood serum. Moreover, the level of Ganoderma Lucidum and Alkhadaya and molnupinavir in other tissues and organs of laboratory animals were not characterized by a statistically significant relationship with the activity of serum GPO and SOD.

Therefore, the obtained data indicate a positive effect of molnupinavir administration into the body on serum GPO activity.

The results of the study indicate a statistically significant decrease in the serum creatinine concentration in rats receiving Ganoderma Lucidum and Alkhadaya in the amount of 15 mg/kg/ day (Group I), by 23% relative to the values of Group II). Despite the increase in the lactate content in the serum of rats receiving Ganoderma Lucidum and Alkhadaya at a dose of 15 mg/kg/ day), by 25% was statistically significant.

The results of multivariate analysis of variance revealed a significant effect of Ganoderma administration Lucidum and Alkhadaya on serum creatinine levels. However, no statistically significant factorial interaction was found. Interaction with Ganoderma administration Lucidum and Alkhadaya produced statistically significant effects on lactate levels, as well

as a significant effect on creatine kinase activity. It is also worth noting that the level of statistical significance of the effect of Ganoderma administration Lucidum and Alkhadaya on the body of experimental animals on the level of lactate approached the value of $p < 0.05$.

lactate level was highest on day 7 in Group II and on day 14 in Group I, and lowest on days 7 and 14 in Group II I. Thus, the following assumptions can be made. First, the dosage of Ganoderma Lucidum and Alkhadaya 5 mg/kg/ day is more physiological than 15 mg/kg/ day, secondly, the increase in lactate levels compared to the control group (Group IV) is better demonstrated in the groups with moderate Ganoderma administration Lucidum and Alkhadaya.

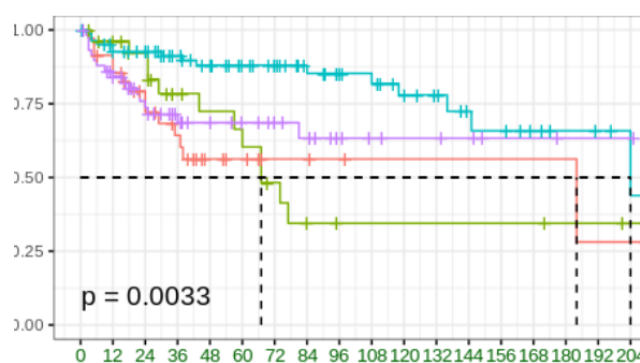


Figure 14. Kaplan-Meier curve for all 4 groups.

Thus, the relative risk of developing a fatal outcome increased by 2.3 times in Group IV ($p = 0.03$), and the γ -correlation model also showed a high degree of dependence of an unfavorable outcome on the presence of this fact ($r = 0.63$ at $p = 0.028$). Plotting the Kaplan-Meier curve illustrates the prognostic significance of this factor in the development of mortality and in the prognosis as a whole (Figure 14).

As a result of the experiments, 60% of the mice in the control group died. Characteristic signs of impaired respiratory function were noted: increased respiratory rate and shallow

breathing, pronounced exophthalmos, and iris discoloration. Hindlimb paresis was also observed: the mice did not move even in response to prodding and were unable to withdraw their hind legs when pressed firmly. Tetanic seizures were visually observed, and the animals adopted a characteristic posture in which their hind legs were straightened and drawn back.

To determine the informativeness of the changes, ROC analysis of cytokines was performed in rats with coronavirus infection.

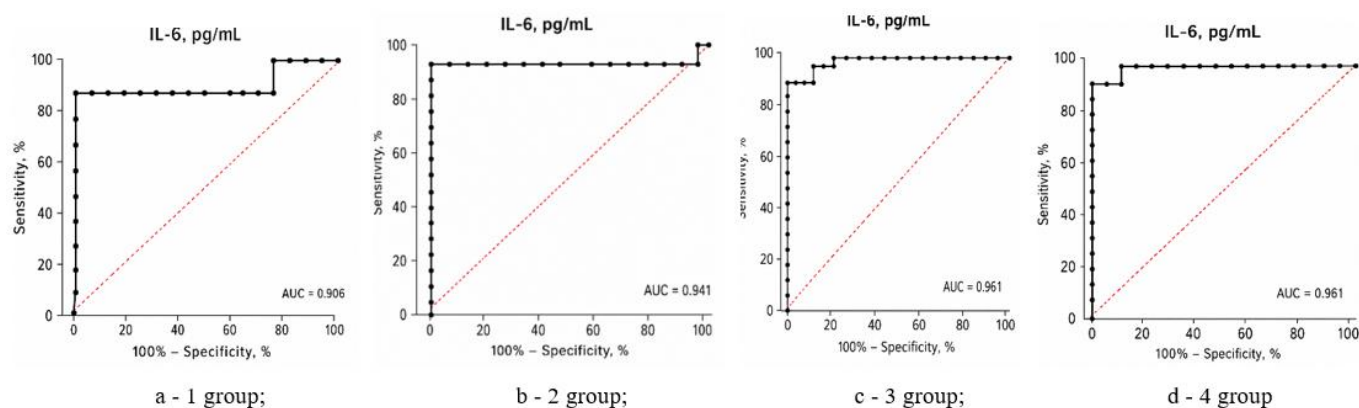


Figure 15. ROC curves characterizing the sensitivity and specificity of determining IL-6 in the blood of rats with coronavirus infection after correction.

Among them: sensitivity - 87.5%; specificity - 100%; AUC = 0.906; criterion > 11.4 pg / ml at $p = 0.0117$ for the 2nd group; sensitivity - 94%; specificity - 100%; AUC = 0.941; criterion > 17.33 pg / ml at $p < 0.0001$ for the 3rd group; sensitivity - 100%; specificity - 87.5%; AUC = 0.961; criterion > 241 pg / ml at $p < 0.0001$ for the 4th group.

The resulting model is statistically significant (Wald Chi-square $p=0.037$), is in good agreement with the original data (Hosmer-Lemeshow test $p=0.967$), has high information content (Nagelkirk criterion $R^2=84\%$), sensitivity (89.5%) and specificity (94.1%).

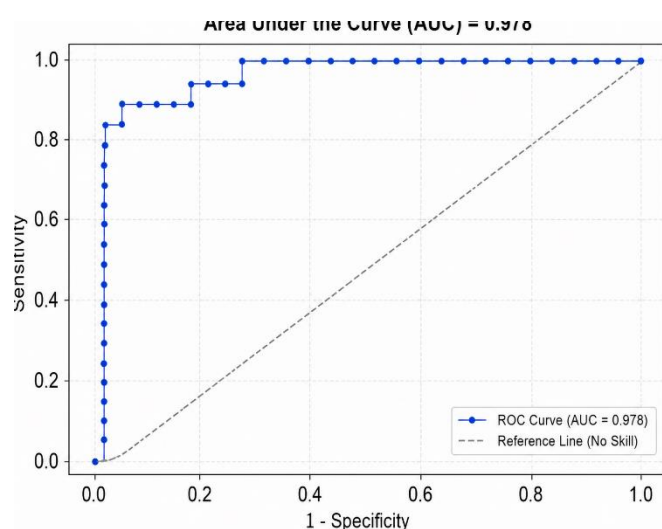


Figure 16. ROC curve of the logistic regression model for predicting cognitive impairment in rats with coronavirus infection.

The model is statistically significant (Wald Chi-square $p=0.002$), consistent with the initial data (Hosmer-Lemeshow test $p=0.470$), has satisfactory information content (Nagelkirk criterion $R^2=55\%$), sufficient sensitivity (62%) and high specificity (95%). Differences in indicators were statistically significant ($U=74.5$; $Z = -6.88$; $p < 0.0001$).

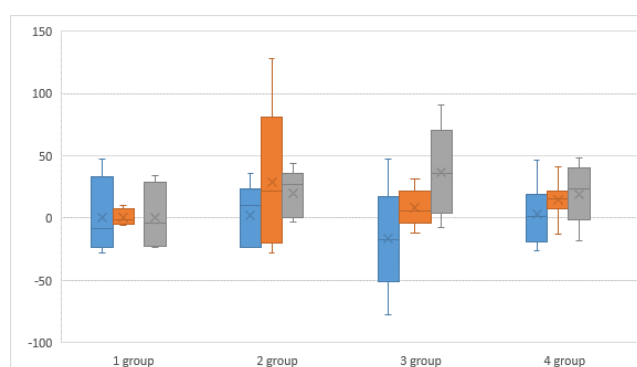


Figure 17. Study of the effect of the drugs used on the immunogenicity of SARS - CoV -2.

Analysis of the response dynamics in group 1 revealed a significant increase in antibody titers to the SARS - CoV -2 strain:

The values on day 7 significantly exceeded those on day 1 ($p = 0.0429$, Friedman test). By day 14, a 2.2-fold decrease in the average titer relative to peak values was observed, but this dynamic was statistically insignificant (Wilcoxon test, $p = 0.25$). In groups 2, 3, and 4, the titer dynamics between days 7 and 14 were characterized by the absence of statistically significant differences ($p = 0.75$).

Already on the 7th day after using Ganoderma Lucidum and Alkhadaya produced detectable antibody levels (mean titer 1:23), while in groups 2, 3, and 4, the levels did not reach the limit of detection (LOD). On day 7, a statistically significant increase in titers to 1:2987 ($p=0.0429$, Friedman test) was observed in group 1, while the response in groups 2, 3, and 4 remained below the LOD. By day 14, weak seroconversion was recorded in groups 2, 3, and 4, but the average titer in group 1 (1:1387) was 86 times higher.

When studying the immunogenicity of a combination drug based on Ganoderma Lucidum and Alkhadaya found that the inclusion of this drug in the coding of the SARS-CoV-2 S-protein does not cause antigen interference and does not reduce the effectiveness of influenza components compared to traditional drugs.

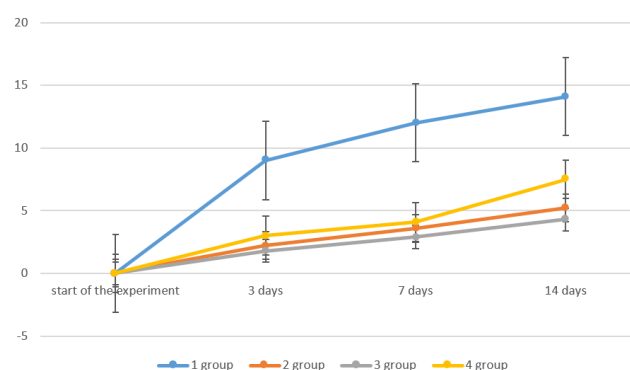


Figure 18. Comparative analysis of the immunogenicity of the SARS - CoV -2 S-protein in various drugs used.

Statistical comparison between the drugs used revealed statistically significant differences ($p < 0.05$), indicating the influence of the new combination drug based on Ganoderma. Lucidum and Alkhadaya on the immunogenicity of the coronavirus component.

Analysis of the geometric mean values of the area under the titration curve (AUC) revealed statistically significant differences between Group 1 and the other groups in ELISA with both antigenic determinants. A comparative assessment of the final titers of specific antibodies demonstrated a pronounced advantage of the new drug based on Ganoderma. Lucidum and Alkhadaya in comparison with other drugs: the level of antibodies to the spike protein of SARS - CoV -2 was 100 times higher ($p=0.0043$), and to the RBD domain – 1000 times

higher ($p < 0.0001$, according to the Mann-Whitney test).

On the 3rd day after infection, a quantitative analysis of the viral load in lung homogenates was performed using RT-PCR with normalization to the reference gene PDK1. In group 1, the average level of viral RNA was 3.5 lg copies/ml, which is significantly lower than the values in groups 2, 3, and 4 (6.65; 6.4 and 6.78 lg copies/ml, respectively).

Survival analysis showed that in Group 4, complete mortality occurred by day 14 (cumulative mortality of 100%). In Groups 2 and 3, mortality was 23%, while in Group 1, no animal deaths were recorded (survival rate of 100%).

Body weight monitoring revealed significant weight loss in animals in Group 4, beginning 2 days after infection. In Groups 2 and 3, weight loss continued for up to 3 days, followed by recovery. In Group 1, animals receiving Ganoderma No weight loss was recorded for Lucidum and Alahadya. Notably, on the first day, a short-term weight gain (3%) was observed in Group 2. However, from Days 2 to 10 of the experiment, minimal weight fluctuations and a stable clinical condition were predominantly characteristic of Group 1.

After infection with coronavirus, the survival rate and weight loss dynamics of mice were monitored. Three animals were also autopsied on day 3 post-infection to analyze the viral load in the lungs. Visually, foci of lung tissue necrosis were visible in the mice from group 4, while the lungs of animals treated with Ganoderma were intact. Lucidum and Alkhadya did not visually show any obvious foci of necrosis. Analysis of the viral load in the lungs on day 3 post-infection showed that treatment of mice with the combination drug resulted in significantly lower influenza virus loads in the lungs.

According to numerous studies, Omicron coronavirus strains do not exhibit 100% lethality in mouse models, which explains the design we chose to test the efficacy of the new drug. The viral load and cytopathic infectious dose (TCID₅₀) were determined in the obtained biological samples (lung homogenates). On day 7, TCID₅₀ analysis revealed no viable SARS-CoV-2 virus in the lungs of animals in groups 1 and 2. However, the lungs of animals in group 4 showed signs of tissue necrosis throughout the entire organ upon dissection. In addition to lung samples, blood was also collected from mice on days 4 and 7 for hematological analysis. Significant differences were detected when comparing the two parameters in the groups of mice that received Ganoderma. Lucidum and Alkhadya - relative number of eosinophils (RNE) and nucleated erythroid cells.

4. Conclusions

Considering the increased transmissibility of the virus and the fact that the reproductive number (R_0) is estimated to be quite high, as this is associated with the optimization of the virus for binding to ACE2 receptors in the upper respiratory tract, a new drug based on Ganoderma Lucidum and Alkhadya demonstrated superior viral killing compared to other drugs, which is associated with M2 receptors on macro-

phages, which it activates and facilitates better virus recognition and prevents its entry into liver cells. As the virus continues to evade the immune system, the new drug activates alarmins, particularly HMGB -1, which enables activation of the immune system via the classical and alternative complement pathways, resulting in complete viral eradication. Since the virus primarily replicates in the bronchi, rather than the lungs, colds and pharyngitis will be more noticeable.

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Author Contributions

Abilov Pulat Melisovich: Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing

Conflicts of Interest

The authors declare no conflicts of interest.

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