Features of the Treatment of Coronavirus Infection Caused by Covid-2019: A Randomized, Double-Blind, Placebo-Controlled Study

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Abstract. This article presents data on the treatment of coronavirus infection with a new unique drug based on G. Lucidum and Alkhadai according to a randomized, double-blind, placebo-controlled trial. There was a significant improvement in the course of the disease, prognosis and outcome after the use of the drug, in contrast to traditional methods of treating coronavirus infection caused by COVID-19.

Keywords: coronavirus; COVID-19; G. lucidum; Alkhadaya; disease prognosis; Exodus; WHO

Introduction. Coronavirus disease 2019 (COVID-19), a highly contagious viral disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has had a devastating impact on world demographics, causing more than 6 million deaths worldwide as of March people in 2022, the most serious global health crisis since the 1918 influenza pandemic [6, 8]. After the first cases of this predominantly respiratory viral disease were first reported in Wuhan, Hubei Province, China, at the end of December 2019, SARS-CoV-2 rapidly spread around the world in a short period of time, forcing the World Health Organization (WHO) to) declare it a global pandemic on March 11, 2020 [1, 5]. Since the announcement of a global pandemic, COVID-19 has devastated many countries around the world and overwhelmed many health systems [3,9]. The pandemic also resulted in the loss of livelihoods due to long shutdowns, which had a strong impact on the global economy [4, 7]. While significant advances in clinical research have led to a better understanding of SARS-CoV-2 and the treatment of COVID-19, limiting the continued spread of this virus and its variants is becoming an increasing challenge as SARS-CoV-2 continues to wreak havoc around the world. world, with many countries experiencing the second or third wave of outbreaks of this viral disease, mainly due to the emergence of mutant variants of the virus [2,10].

The aim of the study. To conduct a double-blind, randomized, placebo-controlled study of the effect of a new drug based on G. Lucidum and Alkhadai on the course and prognosis of coronavirus infection caused by COVID-19.

Materials and research methods. To achieve this goal, the results of treatment of 50 patients with coronavirus infection caused by COVID-19 were analyzed. All patients were divided into groups: group 1 - patients with coronavirus infection with a confirmed positive PCR test, treated with ivermectin at a dosage of 300 mg of body weight (n=15), group 2 - patients with coronavirus infection treated with baicalin at a dosage of 500 mg (n= 15), group 3 - patients with coronavirus infection treated with molnupiravir 25 mg/kg body weight (n=15), group 4 - patients with coronavirus infection treated with a new drug based on G. Lucidum and black cumin (n=15).

The experiments were carried out on 100 mature rats of both sexes weighing 220-250 g. The maintenance of animals, surgical interventions and withdrawal from the experiment were carried out on the basis of ethical principles declared by the European Convention for the Protection of Vertebrate Animals used for experimental and other purposes. The animals were kept in a vivarium with free access to food and water and a natural change of day and night. The experiments were carried out under conditions of spontaneous respiration and an ambient temperature

of 24-25°C. Virus isolation was carried out on viro cell culture from a virus-containing sample of clinical material (nasopharyngeal swab). The efficiency of replication of the SARS-Cov-2 virus in cell culture was assessed by the dynamics of the appearance of cytopathic action and the presence of viral RNA in the analysis of the culture fluid by polymerase chain reaction (PCR).

Rats were intranasally challenged with SARS-Cov-2 at 50% tissue culture median infectious dose (TCID 50) per 50 µl inoculum (live culture biologic) after intraperitoneal anesthesia using 2.5% sodium thiopental solution.

Statistical processing of the results was carried out using parametric and non-parametric research methods.

Research results.

After infecting rats with an experimental strain of SARS-Cov-2 coronavirus infection, PCR diagnostics were performed to confirm the presence of the virus.

When performing PCR diagnostics, 110 copies / ml were found in the blood of rats on days 5-6, which indicates high levels of the virus in the respiratory tract. The viral RNA detection rate was 95%.

During a physical examination of the respiratory tract using a probe, it was found that in almost 100% of rats, edema and hyperemia of the mucous membrane of the respiratory tract were detected. Also, palpation in 90% of rats revealed an increase and thickening of the lymph nodes. Hepatomegaly and splenomegaly were also found in 90% of rats. Also during the physical examination, a decrease in appetite was found in all animals (100%). The experimental animals were lethargic, practically did not react to the change of day and night. Examination of the conjunctiva of the eyes of laboratory animals revealed edema and hyperemia of the mucous membrane.

A biochemical analysis of blood revealed an increase in creatinine by 35%, which was 71.5±1.4 mmol/l in 80% of infected animals. Electrolytes (K+, Na+, Cl-) were increased by 40% (4.9±0.23, 198.0±2.6, 150.0±3.7 mmol/l). In 90% of the experimental animals, an increase in AST was observed by 45% and amounted to 68 U/l. In 92% of the experimental animals, there was an increase in ALT by 25% and amounted to 46 U/l. In 90% of the animals there was an increase in total bilirubin by 45% and amounted to 30 U/l. The content of D-dimer increased in all animals (100%) and amounted to 350 ng/l (N<250 ng/l).

The level of C-reactive protein in 90% of laboratory animals increased and ranged from 10 mg/l to 15 mg/l.

The results of panel testing before treatment are shown in Table 1.

Table 1. Results of testing a panel of PCR samples.

pattern	SARS-CoV-2	COVID-19
1 group (positive)	+	+
2 group (positive)	-	+
3 group (positive)	+	+
4 group (positive)	+	+

After electrophoresis, the results of 7 separate PCR reactions for each SARS-CoV-2 strain were converted into a binary matrix for subsequent in silico analysis. Positive amplification results were recorded as “1”, and negative ones as “0”, indicating the presence or absence of a specific DFR locus in a particular representative of SARS-CoV-2. The obtained DFR profiles of collection strains were supplemented with profiles of SARS-CoV-2 strains obtained as a result of in silico analysis of nucleotide sequences from the Broad Institute of MIT and Harvard database.

After treatment in group 1 (treatment with ivermectin), the results of PCR tests are shown in Table 2.

Table 2. Results of treatment with ivermetin (group 1) using PCR test.

Pool size	SARS-CoV-2	COVID-19
6 plasma samples	-	-
6 plasma samples	+	+
6 plasma samples	-	+
6 plasma samples	+	+

Table 3 shows the results of treatment with baicalin (group 2) using a PCR test.

Table 3. Results of treatment with baicalin (group 2) using a PCR test.

Pool size	SARS-CoV-2	COVID-19
6 plasma samples	+	+
6 plasma samples	-	+
6 plasma samples	+	-
6 plasma samples	-	+

Table 4 presents the results of treatment with monupiravir (group 3) using a PCR test.

Table 4. Results of treatment with molnupiravir (group 3) using PCR test.

Pool size	SARS-CoV-2	COVID-19
6 plasma samples	+	-
6 plasma samples	-	-
6 plasma samples	+	+
6 plasma samples	-	-

Table 5 presents the results of treatment of a new combination drug based on G. Lucidum and Alhadai.

Table 5. Results of treatment of a new combination preparation based on G. lucidum and black cumin.

Pool size	SARS-CoV-2	COVID-19
6 plasma samples	-	-
6 plasma samples	-	-
6 plasma samples	-	-
6 plasma samples	+	-

Thus, the treatment of coronavirus infection with a new combination drug based on G. Lucidum and black cumin is a pathogenetically substantiated method.

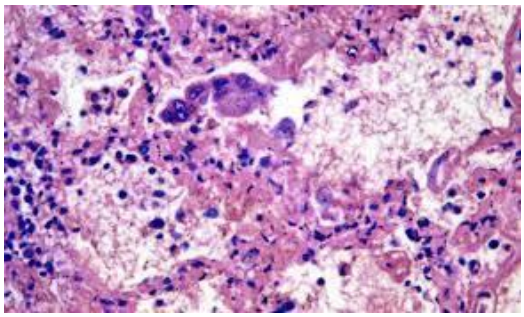
Table 6 presents data on the incidence of coronavirus infection.

Table 6. Epidemiological indicators of coronavirus infection COVID-19.

Groups	In the active phase of the disease	Total number of cases	Number of deaths
II	24	25	1
III	23	25	1
III	25	25	2

Histological examination revealed signs of inflammatory cell infiltrates around the bronchioles and blood vessels, an increase in the number of type II alveolocytes, and massive lesions were also observed in the epithelium of the mucous membrane of the nasal cavity and trachea (Fig. 1). Diffuse alveolar damage was also observed in combination with involvement in the pathological process of the vascular bed of the lungs and alveolar-hemorrhagic syndrome. Also, microangiopathy with thrombosis develops in the vessels of the lungs. Changes in the lungs macroscopically corresponded to the concept of "shock lung".

Rice. 1. Histological changes in the mucous membrane of the respiratory tract with a coronavirus infection caused by COVID-19. Diffuse alveolar damage (exudative phase); cytotoxic effect of the influenza virus on alveolar epithelial cells with the presence of punctate eosinophilic inclusions (possibly viral inclusions). Stained with hematoxylin and eosin, x 250.



After the detection of coronavirus infection COVID-19, 0.9% saline was administered to animals of group II by histological and biochemical methods, no positive changes were found.

In animals of group III, after the use of remdesavir, 60% of rats showed positive dynamics of treatment. Thus, in 50% of laboratory animals, a physical examination of the respiratory tract using a probe revealed a decrease in the degree of edema and not pronounced hyperemia of the mucous membrane of the respiratory tract. Also, palpation revealed a decrease in the size of the lymph nodes in 60% of the rats. Also during the physical examination, normalization of appetite was found in all animals. Physical activity in experimental animals returned to normal. When examining the conjunctiva of the eyes of laboratory animals, 60% showed a decrease in the severity of edema and hyperemia of the mucous membrane. However, 50% of rats still had signs of hepatomegaly and splenomegaly, which was characterized by an increase in biochemical parameters (Table 7)

Table 7. Changes in biochemical parameters before and after the use of remdesavir.

	n	Before treatment	After treatment	p
Креатинин	23	71,5±1,4 mmol/l	67,8±1,2	<0,05
Electrolytes Na+	20	198,0±2,6 mmol/l	185,0±2,0 mmol/l	<0,05
K^+		4,9±0,23 mmol/l	4,1±0,20 mmol/l	≤0,03
Cl^-		198,0±2,6 mmol/l	175,0±2,1 mmol/l	≤0,05
AST	22	68 Ед/л	55 Ед/л	
ALT	22	46 Ед/л	55 Ед/л	
total bilirubin	20	30 Ед/л	22 Ед/л	
D-dimer	22	350 нг/л	300 нг/л	
C-reactive protein	23	10 мг/л	8 нг/л	

So, 95% CI (confidence interval) in groups III and IV is between 2.4-4.0, which indicates an accurate estimate at p≤0.05. OR (odds ratio) was 0.9523107 between the use of a new drug based on Ganoderma Lucidum and Alkhadai and the severity of the pathological process in the lungs, $\chi 2$ (Wilconson test) is 0.9328071, U (Mann-Wini test) is 0.9413508 at p≤ 0.05

Table 1. Some non-parametric indicators for the main and control groups of treatment

Groups	95% CI	OR	$\chi 2$ (Wilconson test	U (Mann-Wini test)
Basic group	2.4-4.0	0.9523107	0.9328071	0.9413508
Control group	2.7-4.2	0.8455109	0.7566132	0.8121005

In animals of group III, after the use of remdesavir, there was a partial improvement in biochemical parameters. Since ALT and AST remained high, hepatomegaly and splenomegaly were observed.

Histological examination in animals of group III showed no signs of inflammatory cell infiltrates around bronchioles and blood vessels, normalization of the number of type II alveolocytes, and a significant decrease in lesions in the epithelium of the mucous membrane of the nasal cavity and trachea (Fig. 1). There was also a decrease in the degree of diffuse alveolar damage without involvement in the pathological process of the vascular bed of the lungs.

In animals of group IV, after the use of a new drug based on Ganoderma Lucidum and Alkhadai, a positive dynamics of treatment was found. So, in 90% of laboratory animals, during a physical examination of the respiratory tract using a probe, the disappearance of edema and hyperemia of the mucous membrane of the respiratory tract were revealed. Also, palpation in 90% of rats revealed normalization of the state of the lymph nodes. Also during the physical examination, normalization of appetite was found in all animals. Physical activity in experimental animals returned to normal. When examining the conjunctiva of the eyes of laboratory animals, 95% showed a decrease in the severity of edema and hyperemia of the mucous membrane. Almost all experimental animals (97%) did not show hepatomegaly and splenomegaly.

Biochemical analysis of blood in animals of group IV revealed a decrease in creatinine by 33%, which was 63.4±1.4 mmol/l in 95% of infected animals. Electrolytes (K+, Na+, Cl-) were reduced by 38% (4.0±0.23, 143.0±2.6, 100.0±3.7 mmol/l). In 90% of the experimental animals, normalization of AST was observed, which was 46 U/l. In 92% of the experimental animals, normalization of ALT occurred, which amounted to 46 U/L. In 90% of the animals there was a decrease in total bilirubin by 45% and amounted to 4 U/l. The content of D-dimer increased in all animals (100%) and amounted to 250 ng/l.

The level of C-reactive protein in 90% of laboratory animals decreased and amounted to 3 mg/l.

Histological examination in animals of group IV also showed no signs of inflammatory cell infiltrates around the bronchioles and blood vessels, normalization of the number of type II alveolocytes, and also the disappearance of lesions in the epithelium of the mucous membrane of the nasal cavity and trachea. The disappearance of diffuse alveolar damage was also observed without involvement of the pulmonary vascular bed in the pathological process.

Findings:

Thus, the use of a new drug based on Ganoderma Lucidum and Alkhadai in the treatment of coronavirus infection caused by COVID-19 is justified, since due to the amino acid complex there is no excessive accumulation of angiotensin II, which leads to the normalization of biochemical and histological parameters.

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