«8D10110» — Dissertation on "Medicine" presented by Serikbayeva Saltanat Zhaksylykovna for the degree of Doctor of Philosophy (PhD) in the specialty.

**"Substantiation of Hirudotherapy for Cognitive Impairments in Patients with Post-COVID-19 Infection"**

**ABSTRACT**

**Relevance of the Research Topic:**

One of the leading pathogenetic factors in nervous system damage in post-COVID syndrome is hemostasis destabilization, including microcirculation disorders accompanied by increased aggregation and adhesion of erythrocytes and platelets. For the first time (2010–2016), we established the neuroprotective properties of hirudotherapy in the treatment of patients with ischemic stroke of varying severity. Considering the above, we have set the objectives of this study.

**Research Objective:**  
Justification for the use of personalized hirudotherapy enriched with Actovegin in patients with the neurological variant of post-COVID syndrome.

1. **Research Tasks:**
2. To determine the comparative effectiveness of personalized standard and Actovegin-enriched hirudotherapy on cognitive manifestations of post-COVID syndrome, depression, and insomnia, depending on patients' sensitivity to xenobiotics.
3. To assess the state of spontaneous platelet aggregation and lipid peroxidation in saliva in patients with neurological variants of post-COVID syndrome when using personalized standard and Actovegin-enriched hirudotherapy.
4. To evaluate the impact of personalized standard and Actovegin-enriched hirudotherapy on the endogenous intoxication index in patients with post-COVID syndrome, as indicated by the level of medium-weight molecules in saliva.
5. To establish the comparative pharmacoeconomic efficiency of personalized standard and Actovegin-enriched hirudotherapy courses used for neurological variants of post-COVID syndrome.

**Scientific Novelty of the Study:**

1. For the first time, neurological and cognitive changes in post-COVID syndrome were studied depending on individual sensitivity to xenobiotics when using standard and enriched hirudotherapy.
2. The state of lipid peroxidation in blood platelets and oral fluid in patients with post-COVID syndrome was simultaneously examined in relation to xenobiotic sensitivity under the influence of standard and enriched hirudotherapy.
3. The detoxification efficacy of enriched and standard hirudotherapy in patients with post-COVID syndrome was established for the first time, depending on the dose, types of medicinal leeches, and individual sensitivity to xenobiotics.
4. Pharmacoeconomic characteristics of patients with post-COVID syndrome were determined based on their sensitivity to xenobiotics under the influence of standard and enriched hirudotherapy, with optimal efficiency observed after the 10th and 5th procedures, respectively.

### ****Theoretical Significance of the Study:****

The theoretical concepts and practical results of this dissertation are used in the educational process and scientific research at the departments of pharmacology, pharmacotherapy, and clinical pharmacology of the South Kazakhstan Medical Academy. Additionally, they have been integrated into the practical activities of City Hospital No. 2 in Shymkent.

### ****Practical Significance of the Study:****

The scientific and theoretical significance of the study lies in the use of comprehensive neurological methods to determine the degree of nervous system deviations in patients with post-COVID syndrome, depending on their sensitivity to xenobiotics. A pathogenetic analysis of hirudotherapy as a treatment method for patients with post-COVID syndrome was conducted, highlighting the peculiarities of their sensitivity to xenobiotics. Using reliable digital data, pharmacoeconomic indicators of standard (traditional) and original (Actovegin-enriched medicinal leech) hirudotherapy were determined, and the effectiveness of the applied treatment methods was evaluated. The obtained data are crucial for the training of hirudotherapists and are widely used in the educational process.

**Publications on the Topic of the Work:**

4 articles published in journals recommended by the Committee for Control in Education and Science of the Ministry of Education and Science of the Republic of Kazakhstan. 2 articles published in an international peer-reviewed scientific journal indexed in the Web of Science Core Collection, Science Citation Index Expanded, with a CiteScore percentile of at least 25 in the Scopus database. 11 abstracts presented at international conferences abroad.

### ****Positions to be Defended:****

1. Positive shifts in neurological and cognitive functions in patients with post-COVID syndrome during standard and enriched hirudotherapy depend on the individual sensitivity of the body to xenobiotics.
2. The value of the integral coefficient of lipid peroxidation/antioxidant system (LPO/AOS) in blood platelets and oral fluid in patients with post-COVID syndrome depends on individual sensitivity to xenobiotics. In the resistant group, the value is slightly elevated (66%), while in the highly sensitive group, it is significantly higher, with a difference of more than four times.
3. The detoxification efficacy of standard and enriched hirudotherapy in patients with post-COVID syndrome depends on individual sensitivity to xenobiotics. Optimal pharmacotherapeutic efficacy of standard and enriched hirudotherapy is observed after the 10th and 5th procedure, respectively. The optimal therapeutic dose of medicinal leeches for post-COVID syndrome depends on individual sensitivity to xenobiotics. The personalized dose of medicinal leeches for patients with post-COVID syndrome, particularly those who are sensitive and highly sensitive, exceeds the dose for resistant patients by 66.6% and more in a single application (116.6%).

**Materials and Methods of the Research:**

The study was conducted at the Department of Neurology, H.A. Yasavi International Kazakh-Turkish University, Department of Pharmacology and Clinical Pharmacology at the South Kazakhstan Medical Academy, in the Neurology and Ischemic Stroke Department of the Regional Clinical Hospital, and at the "Hirudomed" Medical Center during 2021-2023.

According to the objectives of the study, 30 healthy patients and patients with varying degrees of post-COVID syndrome were examined. The patients were aged between 25 and 65 years. They were divided into four groups based on the research tasks:

1. **Control Group:** 30 healthy individuals.
2. **Neuroprotective Group:** Patients received Actovegin 400 mg.
3. **Traditional Group:** Patients received certified standard medicinal leeches.
4. **Original Hirudotherapy Group:** Patients received Actovegin-enriched medicinal leeches.

The resistant, sensitive, and highly sensitive patients received medicinal leech therapy (MLT) at doses of 12.0 g, 20.0 g, and 26.0 g, respectively. To assess the state of cognitive activity of the nervous system, the following were conducted: MoCA (Montreal Cognitive Assessment), visual-constructive and executive skills (VCS), Shulte test, Beck Depression Inventory (BDI), and Pittsburgh Sleep Quality Index (PSQI) to evaluate sleep quality, as well as the Insomnia Severity Index (ISI).

### ****Research Results:****

When assessing the cognitive function of the nervous system in patients with post-COVID syndrome (PCS) using the MoCA scale, the values decreased by 31.7%, 47.3%, and 66.0% in resistant, sensitive, and highly sensitive patients, respectively.

The use of Actovegin-enriched certified medicinal leeches normalizes the cognitive functions of the central nervous system (CNS) in PCS patients earlier and is more cost-effective compared to standard hirudotherapy, as it reduces the number of procedures and the need for medicinal leeches.

In the case of standard hirudotherapy, the MoCA scale scores in resistant, sensitive, and highly sensitive patients increased by 36.5%, 27.9%, and 193.0%, respectively, after the 10th procedure. For the Actovegin-enriched medicinal leech group, the scores increased by 45%, 50%, and 99.0% after the 5th procedure, reaching the values seen in the control group. The Schulte test values increased by 43%, 100%, and 137% in resistant, sensitive, and highly sensitive patients, respectively.

When treating patients with PCS using standard medicinal leeches, Schulte test values decreased by 29%, 22.9%, and 22.9% after the 10th procedure. In the case of Actovegin-enriched medicinal leeches, the decrease after the 5th procedure was 30.1%, 37.3%, and 46.7%.

In resistant, sensitive, and highly sensitive patients, depression scores on the Beck scale increased by 1.86, 3.09, and 4.47 times, respectively.

In traditional (standard) hirudotherapy, depression symptoms decreased after five procedures in patients with resistant, sensitive, and highly sensitive PCS by 40.5%, 40.2%, and 40.2%, respectively. In contrast, with the use of Actovegin-enriched medicinal leeches, the reduction after the 5th procedure was 79.1%, 80.8%, and 88.2%, approaching the values seen in the control group. The values change depending on patients' sensitivity to xenobiotics, increasing by 96% in the resistant group compared to the control group, 244.4% in sensitive patients, and more than three times (375.9%) in highly sensitive patients with PCS.

In traditional (standard) hirudotherapy, the insomnia index (ISI) decreased after ten procedures in patients with resistant, sensitive, and highly sensitive post-COVID syndrome by 47.2%, 68.3%, and 68.3%, respectively. With the use of Actovegin-enriched medicinal leeches, the reduction after the 5th procedure was 37.1%, 86.7%, and 86.7%, approaching the values seen in the control group.

The numerical value of the visual-constructive and executive skills (VKE) scale in patients with post-COVID syndrome, depending on sensitivity to xenobiotics, decreased by 16.0% in resistant patients compared to the control group, 38.0% in sensitive patients, and 64.0% in highly sensitive patients.

When using hirudotherapy in resistant, sensitive, and highly sensitive patients with post-COVID syndrome at a single dose of 12.0 g, 20.0 g, and 26.0 g, the parameters of the VKE scale increased after ten procedures by 54.8%, 32.2%, and 161%, respectively. With the use of Actovegin-enriched medicinal leeches, the growth after the 5th procedure was 54.9%, 61.3%, and 178%, reaching values similar to the control group.

In post-COVID syndrome, platelet aggregation ability was found to be activated. In the resistant group, the index increased by 55.5%, in sensitive patients by more than 101.5%, and in highly sensitive patients by more than two times (201.5%).

Depending on sensitivity to xenobiotics, the coefficient of spontaneous platelet aggregation (SpAIC) after a single application of standard hirudotherapy decreased by 13.5%, 22.4%, and 29.1% in resistant, sensitive, and highly sensitive patients, respectively, and returned to normal by days 4, 8, and 9 of the study.

Under the influence of Actovegin-enriched hirudotherapy, the coefficient of spontaneous platelet aggregation (SpAIC) decreased compared to the standard group by 10.0% in the resistant group, by 15% in sensitive patients, and by 20% in highly sensitive patients. It also normalized one day earlier than in the traditional groups.

In post-COVID syndrome, the activation of lipid peroxidation processes in platelets was observed due to the depression of antioxidant system activity, as evidenced by the increase in the integral coefficient of PLO/OSC in the resistant group by 55.0%, and by 187.0% and 194.0% in sensitive and highly sensitive patients, respectively.

Normalization of the values of the integral coefficient PLO-OSC in platelets under the influence of standard hirudotherapy occurred after the tenth procedure, whereas with Actovegin-enriched hirudotherapy, normalization occurred after the fifth procedure.

In post-COVID syndrome, the activation of lipid peroxidation processes in saliva was observed due to depression of the antioxidant system and increased accumulation of PLO products, which was reflected in an increase in the integral coefficient of PLO/OSC in the resistant group by 66.0%, and by 177.0% and 365.0% in sensitive and highly sensitive patients, respectively.

Normalization or return of the values of the integral coefficient PLO-OSC in saliva to normal under the influence of standard hirudotherapy occurred after the tenth procedure, while under the influence of Actovegin-enriched hirudotherapy, this occurred after the fifth procedure.

In post-COVID syndrome, a fixed state of endotoxemia was established. The endotoxemia index in the resistant group increased by 53.0%, in sensitive patients by 70.0%, and in highly sensitive patients by 90.0%.

Treatment with traditional and Actovegin-enriched hirudotherapy demonstrated a detoxification effect. The total index of endogenous intoxication in saliva decreased in resistant patients after 5 and 4 procedures, in sensitive patients after 6 and 5 procedures, and in highly sensitive patients by 40.3% and 51.8%. The detoxification of saliva under Actovegin-enriched hirudotherapy occurred one day earlier than with traditional treatment and showed high activity.

The pharmacoeconomic indicators for patients with post-COVID syndrome (PCS) depending on their sensitivity to xenobiotics under standard and enriched therapy show significant changes, including the integral "cost-effectiveness" indicator.

When applying Actovegin-enriched hirudotherapy in resistant, sensitive, and highly sensitive patients with PCS, the "cost-effectiveness" value decreases compared to Actovegin and traditional hirudotherapy by: 48.5% and 73.3% for resistant patients, 48.5% and 54.6% for sensitive patients, 78.4% and 79.5% for highly sensitive patients.

This demonstrates that Actovegin-enriched hirudotherapy offers a more cost-effective treatment for PCS patients, especially in those with varying levels of sensitivity to xenobiotics.

### ****Conclusions:****

1. **Improvement in cognitive manifestations** of post-COVID syndrome as measured by the MoCA scale was observed with actovegin-enriched hirudotherapy in resistant, sensitive, and highly sensitive patients, showing improvements of 45.8%, 84.8%, and 193.1%, respectively. Actovegin-enriched hirudotherapy resulted in a decrease in comparison to standard hirudotherapy by 20.0%, 62.0%, and 127.4%.
2. In **actovegin-enriched hirudotherapy**, the insomnia severity index (ISI) decreased by 49.1%, 70.4%, and 78.6% in resistant, sensitive, and highly sensitive patients, respectively. In comparison, the reduction in standard hirudotherapy was 37.6%, 33.1%, and 33.4%, respectively.
3. **Platelet aggregation index (PAI)** decreased with actovegin-enriched hirudotherapy by 10.0%, 15%, and 20% in resistant, sensitive, and highly sensitive patients compared to the traditional groups. Normalization of lipid peroxidation (LPO) and antioxidant enzymes (AOE) in platelets occurred 2, 3, and 5 procedures earlier under actovegin-enriched hirudotherapy compared to the standard group.
4. **Lipid peroxidation-Antioxidant enzyme (LPO-AOE)** index values in saliva of post-COVID syndrome patients increased by 66.0%, 177.0%, and 365.0% in resistant, sensitive, and highly sensitive patients, respectively. After ten procedures of standard hirudotherapy, LPO-AOE index values in saliva decreased by 30.0%, 42.8%, and 72.3%. Under actovegin-enriched hirudotherapy, normalization occurred two procedures earlier than with the traditional method. The detoxification effect of actovegin-enriched hirudotherapy reduced the endotoxicity index by 32.7%, 41.1%, and 51.6% in resistant, sensitive, and highly sensitive patients, respectively, while the standard hirudotherapy showed an average efficiency of 13%.
5. **Cost-effectiveness** ratios for resistant patients in the actovegin, standard, and actovegin-enriched hirudotherapy groups were 286.8, 147.7, and 130.1 tenge, respectively. Actovegin-enriched hirudotherapy was 12.0% more cost-effective compared to standard hirudotherapy, with a savings of 139.1 tenge compared to the actovegin group and 17.6 tenge compared to the traditional group. In patients with sensitive and highly sensitive forms of post-COVID syndrome, the savings in the actovegin-enriched hirudotherapy group were 130.1 and 17.6 tenge compared to the actovegin group, and 462.0 and 7.3 tenge compared to the traditional group, respectively.