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Журнал «Фармация и фармакология» включен в перечень рецензируемых научных изданий, входящих в международные реферативные базы данных и системы цитирования, и в соответствии с пунктом 5 правил формирования перечня рецензируемых научных изданий, в которых должны быть опубликованы основные научные результаты диссертаций на соискание ученой степени кандидата наук, на соискание ученой степени доктора наук (Перечень ВАК), Scopus, Web of Science (ESCI), РИНЦ, eLibrary, ВИНИТИ, РГБ, Киберленинка, Соционет, EMBASE, Chemical Abstracts (CAS), Directory of Open Access Journals (DOAJ), EBSCO Discovery Service, RNMJ, University of CAMBRIDGE, Ulrich'sWeb, Google Scholar, Biefeld Academic Search Engine (BASE), Directory of Open Access Scholarly Resources (ROAD), Research Bible, Open Archives Initiative, Academic Keys, JournalTOCs, WorldCat, OpenAIRE, University of Oxford, The British Library, Universitait Gent, Université de Montréal, University of Saskatchewan.

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Pharmacy Compounding Regulation in the United Kingdom

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Pharmaceutical practice of advanced regulatory healthcare systems places high demands on the quality and safety of medicines, especially on compounding drugs by pharmacies. The United Kingdom is one of the countries with a developed system of legal regulation of drug treatment, which is based on the principles of effective management and is aimed not only at following formal procedures, but also at ensuring the ultimate goals, namely patient safety, quality of manufactured drugs and effectiveness of pharmaceutical processes.

The aim. To identify the key elements of the British system of regulation of drugs compounding by pharmacies, to assess their applicability in other legal systems and to form promising directions for improving Russian legislation.

Materials and methods. The study based on analysis of the UK regulatory framework governing circulation of medicines, as well as documents from government agencies and regulatory agencies. Comparative legal, content analysis, a systematic approach, analytical and empirical methods were used.

Results. Pharmaceutical activity is regulated by the General Pharmaceutical Council, which develops standards and guidelines that are binding. The production of medicines is carried out within the framework of a licensing system and is subject to the principles of good practices. The British system is based on a model of effective regulation, where the emphasis is on achieving targeted results rather than strictly following procedures. There is a separate license for the production of special drugs designed for a specific patient.

Conclusion. Legal and regulatory systems for manufacturing of medicines in the UK demonstrates high degree of harmonization with international standards, including GMP and PIC/S recommendations. It can serve as a model for improving legislation in other countries, including the Russian Federation, in terms of developing and implementing a unified system of regulatory support for manufacturing of medicines, including introduction of adapted GMP requirements into of pharmaceutical medicine manufacturing practice — good practices for the preparation of medicinal products.

Keywords: drugs compounding; compounding pharmacies; personalized medicine; Great Britain; good pharmacy practice; extemporaneously compounded medicines

Abbreviations: IPP — intra-pharmacy preparation; RMP — registered medicinal product; EAEU — Eurasian Economic Union; DF — dosage form; SOP — Standard operating procedure; ECM — extemporaneously compounded medicines; SMP — special medicinal product; GMP — Good manufacturing practices; BPh — British Pharmacopoeia; GPhM — General Pharmacopoeia Monograph; PhM — Pharmacopoeia Monograph.

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Нормативное правовое регулирование изготовления лекарственных препаратов аптечными организациями в Великобритании

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Фармацевтическая практика передовых регуляторных систем здравоохранения предъявляет высокие требования к качеству и безопасности лекарственных препаратов для медицинского применения (ЛП), особенно при их изготовлении аптечными организациями (АО). Великобритания представляет собой одну из стран с развитой системой правового регулирования обращения лекарственных средств (ЛС), которая основана на принципах результативного управления и направлена не только на соблюдение формальных процедур, но и на обеспечение конечных целей, а именно — безопасность для пациента, качество изготовленных ЛП и эффективность процессов осуществления фармацевтической деятельности.

Цель. Выявить ключевые элементы британской системы регулирования аптечного изготовления лекарственных препаратов, оценить их применимость в других правовых системах и сформировать перспективные направления совершенствования российского законодательства.

Материалы и методы. Исследование проведено на основе анализа нормативной правовой базы Великобритании, регулирующей обращение ЛС, а также документов государственных органов и профильных агентств. Применялись сравнительно-правовой, контент-анализ, системный подход, аналитический и эмпирический методы.

Результаты. Фармацевтическая деятельность регулируется Генеральным фармацевтическим советом, который разрабатывает стандарты и руководства, обязательные для исполнения. Изготовление ЛП осуществляется в рамках системы лицензирования и подчиняется принципам надлежащих практик. Британская система основана на модели результативного регулирования, где акцент делается на достижении целевых результатов, а не на формальном соблюдении процедур. Существует отдельная лицензия на производство специальных ЛП, предназначенных для конкретного пациента.

Заключение. Система правового регулирования изготовления ЛП в Великобритании демонстрирует высокую степень гармонизации с международными стандартами, включая GMP и рекомендации PIC/S. Она может служить моделью для совершенствования законодательства в других странах, в том числе в Российской Федерации в аспекте разработки и внедрения единой системы нормативного обеспечения деятельности по изготовлению ЛП, включающей установление «адаптированных» требований GMP в практику аптечного изготовления ЛП — надлежащей практики изготовления ЛП.

Ключевые слова: изготовление лекарственных препаратов; производственные аптеки; персонифицированная медицина; Великобритания; надлежащая аптечная практика; экстемпоральные лекарственные препараты

Список сокращений: АО — аптечная организация; ВАЗ — внутриаптечная заготовка; ГЛФ — зарегистрированный лекарственный препарат; ЕАЭС — Евразийский экономический союз; ЛП — лекарственный препарат; ЛС — лекарственное средство; ЛФ — лекарственная форма; СОП — Стандартная операционная процедура; МО — Медицинская организация; ЭЛП — экстемпоральный лекарственный препарат; СЛП — специальный лекарственный препарат; GMP — Надлежащая производственная практика; БФ — Британская фармакопея; ОФС — общая фармакопейная статья; ФС —фармакопейная статья.

INTRODUCTION

Pharmaceutical practice in developed regulatory systems in the healthcare sector places high demands on ensuring the quality and safety of medicines, especially in cases of prescribing and compounding of medicines for medical by pharmacies. According to some studies, in addition to numerous physical, chemical, instrumental, and microbiological tests, which are most common in the quality control of extemporaneous drugs, it also seems advisable to implement additional control measures to improve the quality level of safety guarantees [1–3].

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Today, there is an special interest in the activity of drugs compounding at various levels of the professional community. As part of the current tasks to improve the main elements of the regulatory legal regulation of drugs compounding, it is fundamentally important to ensure the transition from the current rules of the compounding and dispensing of drugs to the harmonized good compounding practice [4], opening up new prospects for the development of pharmaceutical infrastructure. Its provisions should meet the principles of applicability to any type of drugs that belongs to the segment of personalized medicine, high-tech healthcare, and health-saving technologies, as well as take into account the current features and processes of compounding pharmacies operating in Russia. At the same time, modern pharmaceutical practices require pharmacies to develop competencies in the field of development, testing, mastering, and implementation of advanced and most important technologies for the compounding of drugs[1] [5, 6].

The United Kingdom of Great Britain and Northern Ireland (hereinafter referred to as "Great Britain," "United Kingdom," "England," "Britain") is one of the countries with a developed regulatory system, where the regulatory legal framework combines the principles of precedent, parliamentarism, and constitutional customs (traditionally oriented legislation), including modern approaches to the provision of pharmaceutical care [7, 8]. The legal regulation of the drugs compounding in Great Britain is based on the principles of effective management, which implies an emphasis not only on compliance with formal procedures, but also on achieving the ultimate goals — patient safety, the quality of extemporaneously compounded medicines (hereinafter referred to as "ECMs"), and the efficiency of processes.

THE AIM. To identify the key elements of the British system for regulating the pharmacy compounding of drugs, to assess their applicability in other legal systems, and to form promising directions for improving Russian legislation. The latter is an urgent task in connection with the active discussion of the future of compounding pharmacies in the Russian Federation, including issues related to the implementation of good compounding and dispensing practice[2] of drugs [1, 9], the development and implementation of new provisions of the State Pharmacopoeia of the Russian Federation XV edition [10, 11], which should take into account modern international approaches and opportunities of pharmaceutical practice [12].

MATERIALS AND METHODS

The object of the study was the regulatory legal documents of Great Britain regulating the sphere of circulation of medicines, as well as documents of state bodies and specialized agencies of Britain (https://www.legislation.gov.uk/ — official archive and publishing house of the Government of Great Britain; https://www.parliament.uk/ — official website of the Parliament of Great Britain).

The United Kingdom is a state consisting of England, Wales, Scotland, and Northern Ireland. Naturally, each of these countries has common legislative norms on various issues, including features and (or) exceptions that will not be mentioned in this manuscript and are accepted as irrelevant.

This work is a continuation of a series of scientific research by the authors devoted to the issues of regulatory legal regulation of the drugs compounding in world healthcare systems, where the main legislative imperatives of the named activity were consistently studied (including issues of direct circulation of compounded drugs): at the supranational level — in the European Union (hereinafter referred to as "EU") [13], at the national level — in the USA [14], Germany [15–17], Latvia [18], the Netherlands [19], BRICS countries [12] and the CIS [13], as well as the Russian Federation [20–22].

Starting to study the regulatory legal regulation of the drugs compounding in the United Kingdom, it is necessary to describe the logic of the formation of English legislation, where the following are distinguished[3, 4]:

1. Constitutional documents (un-codified constitution):
   - Act of Settlement, 1701;
   - Bill of Rights, 1689.
2. Primary legislation (acts of parliament):
   - Employment Rights Act, 1996;
   - Equality Act, 2010.
3. Secondary legislation issued on the basis of primary legislation as part of (documents of the UK Government and its ministries):
   - Orders: The Companies (Miscellaneous Reporting) Order, 2018;

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- Regulations: The Working Time Regulations, 1998;
- Rules: The Immigration Rules, 2023.

4. Guidance and Standards:
   - Fundamental Standards of the Care Quality Commission.

The main research method was comparative legal analysis, which allowed us to identify the features of regulation of compounding of drugs by pharmacies in England and to correlate them with similar approaches in other countries. Content analysis of documents ensured the systematization of legal norms concerning the requirements of licensing, quality control, and distribution of responsibility in compounding of drugs by pharmacies. A systematic approach made it possible to consider the institution of drugs compounding in the context of the entire procedure for the circulation of medicines, to assess the role of professional self-regulatory organizations, and to identify approaches in public administration, including those based on international standards. The analytical method was applied to interpret the requirements for personnel, premises, equipment, and processes of drugs compounding. The empirical method consisted of studying practical examples of the implementation of legal norms. The comprehensive application of these methods allowed us to comprehensively consider the subject of the study and formulate reasonable conclusions.

In this work, an analysis of a wide range of relevant sources was carried out and information was obtained from regulatory acts regulating the activities of compounding pharmacies in Great Britain, which was implemented by the bibliometric method.

Information from various sources was used as materials. In the part of the analysis of regulatory legal documents were used: the electronic fund of regulatory technical and regulatory legal information of the Codex Consortium, the legal reference system of Great Britain Legislation.gov.uk. For analyze of relevant sources of information and data from search engines were used: PubMed, the eLIBRARY.RU, Russian National Library, National Electronic Library, and Google Academy. The search was carried out using the next key queries in English: “medicine”, “extemporaneous”, “compounded”, “drug formulations”, “pharmacy”, “compounding”, “drug”. The selection of literature was carried out for the period from 1968 to 2023. The choice of the period is due to historical events and the beginning of the active development of the activity of drugs compounding in the Soviet Union. The search of literature was in Russian and English.

RESULTS AND DISCUSSION

The main regulatory legal documents that regulate the circulation of medicines in Great Britain are the Medicines Act of October 25, 1968 (hereinafter referred to as the “English Law”)[5] and the Human Medicines Regulations of July 19, 2012 (hereinafter referred to as the “English Rules”)[6], where the word “preparation” is used in relation to the compounding of medicines, similar to that in the European Union (EU).

Key regulators in the market of medicines circulation in Great Britain

The structure of the Government of Great Britain includes[7, 8]:

1. The Department[9] of Health and Social Care [23], responsible for developing public policy in the field of healthcare. The subordinate organization is the Medicines and Healthcare products Regulatory Agency (hereinafter referred to as “MHRA”), which issues licenses to compounding of medicines, wholesale companies, pharmacovigilance, inspection of production sites for compliance with the rules of Good Manufacturing Practice (hereinafter referred to as “GMP”), Good Distribution Practice (hereinafter referred to as “GDP”)[10], and other functions.
2. The Department for Business and Trade[11], responsible, among other things, for the development of the pharmaceutical industry and international trade.

The professional activity of pharmaceutical and medical workers is regulated by independent regulatory bodies in the form of the General Pharmaceutical Council (hereinafter referred to as the “Pharmaceutical Council”)[12] and the General Medical Council[13], which were created by the “Pharmacy Order” No. 231 of February 10, 2010 (hereinafter referred to as the “Pharmacy Order”)[14] and the Medical Act[15] of July 26,

[5] Medicines Act 1968. Available from: https://clck.ru/3MEQvU
[6] The Human Medicines Regulations 2012. Available from: https://clck.ru/3MEfon
[7] National Health Service Act 2006. Available from: https://clck.ru/3MEfqG
[8] Health and Social Care Act 2012. Available from: https://clck.ru/3MEfrB
[9] In the United Kingdom, the words “department” and “ministry” are synonymous due to the historical development of public administration. Russian
[10] Medicines: good manufacturing practice and good distribution practice. Available from: https://clck. ru/3MkHF8
[11] Department for Business & Trade. Available from: https://clck.ru/3MEfsw
[12] General Pharmaceutical Council. Available from: https://www.pharmacyregulation.org/
[13] General Medical Council. Available from: https://clck.ru/3MkHSS
[14] The Pharmacy Order 2010. Available from: https://clck.ru/3MEftn
[15] Medical Act 1983. Available from: https://clck.ru/3MEfuw

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1983, respectively. According to these regulatory legal documents, these independent bodies are accountable to the Parliament of the United Kingdom, but act autonomously.

The National Health Service of Great Britain (hereinafter referred to as "NHS")[16] operates on the territory of the United Kingdom. A formed state healthcare system, created in 1948 and based on the principles of free access to medical services, the financing of which is carried out from taxes paid. The NHS covers the entire territory of Britain and consists of four autonomous but interconnected systems — in England, Scotland, Wales, and Northern Ireland. The system provides access to a wide range of services — from primary healthcare to highly specialized treatment in hospitals [24, 25].

Licensing of pharmaceutical activity

According to the English Rules, there are three main types of activity that are subject to separate licensing procedures:

- Retail Pharmacy Licence;
- Wholesale Dealer Licence;
- Manufacturing Licence.

In the United Kingdom, separate certificates of compliance with GMP or GDP requirements are not issued — the very presence of a wholesale or production license implies that the licensee complies with these rules.

However, the legislation of Great Britain on the circulation of medicines does not contain specific definitions of these types of activity.

Thus, section 8 of the English Rules defines retail pharmacy business, which includes the retail sale of drugs that are not subject to "free sale".

Section 5 of the English Rules presents a classification of drugs:

1. Prescription Only Medicine (hereinafter referred to as "POM").
2. Pharmacy Medicine (hereinafter referred to as "PM") dispensed without a prescription, but only in pharmacies.
3. Available for "free sale" (General Sale List; for example, in supermarkets, hereinafter referred to as "GSL").

The regulation of wholesale trade in medicines is covered in section 18 of the English Rules, where it includes the supply of drugs to medical organizations (hereinafter referred to as "MOs"), as well as other subjects of medicines circulation for the purposes of subsequent resale. However, manufacturers of medicines, when directly selling their products to pharmacies and MOs, do not have to obtain a wholesale license and can carry out supplies under a production license, which is due to the presence in GMP of instructions on compliance with GDP rules (Fig. 1).

The regulation of the activity of pharmacies is carried out according to standards and guidelines that are developed and published by the Pharmaceutical Council. The resulting licensing scheme in the field of medicines circulation is presented in Figure 2.

In accordance with sections 22, 167, as well as paragraphs 14, 22, 37 of Appendix No. 4 of the English Rules, a special license for the manufacturing (Manufacturing Specials Licence) is distinguished [26]. This type of license is intended for the production of "special" medicines (Special Medicines; hereinafter referred to as "SMs") [27], which are produced according to an individual technical specification provided by a doctor, dentist, or pharmaceutical worker and are intended for use by a specific patient, for the effectiveness of treatment of which the above-mentioned specialist bears direct responsibility.

This type of activity is separate and does not require the presence of a "standard" license for the production of drugs, while the compounding of SMs must comply with GMP requirements. There is also a separate MHRA guide for manufacturers of SMs[17], which gives instructions on the interpretation of individual provisions of GMP in relation to the specifics of the products being compounded. This approach is identical in the organization of work of 503B type pharmacies in the USA [28–30], where, as is known, such organizations must comply with GMP requirements, but a separate FDA guide introduces exceptions to these rules. That is, formally, compliance with GMP[18, 19] is required, but not for the entire scope of requirements [12].

SMs are supplied to MOs, where they were prescribed to the patient, at the same time, the possibility and the right to move the named products between Great Britain and Northern Ireland is established. In addition, MHRA has developed a special document on the distribution of SMPs for such cases[20] (Fig. 3).

[16] NHS website for England. Available from: https://www.nhs.uk/

[17] Guidance for 'specials' manufacturers. Available from: https://clck.ru/3MZb58

[18] Current Good Manufacturing Practice – Guidance for Human Drug Compounding Outsourcing Facilities Under Section 503B of the FD&C Act Guidance for Industry. Available from: https://clck.ru/3MkGfv

[19] Questions and Answers on Current Good Manufacturing Practice Requirements. Control of Components and Drug Product Containers and Closures. Available from: https://clck.ru/3MkGiE

[20] Medicines and Healthcare Products Regulatory Agency. The supply of unlicensed medicinal products («specials»); MHRA Guidance Note 14; 2014.

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Key features of the legislation on the compounding of medicines

In accordance with part 4 of the English Law, a legal basis was established at the legislative level for regulating retail pharmaceutical activity, including the provision of services for dispensing and drugs compounding.

However, most of the original provisions of this law were repealed or replaced after the entry into force of the English Rules — a regulatory legal document that, at the time of the study, plays a central role both in practical application and in the administrative enforcement of pharmaceutical legislation. Despite this, the English Law remains the legal basis on which modern rules are developed and operate. Many key principles noted in this document are relevant and formed the basis of the modern system of regulation of medicines circulation in the territory of the United Kingdom.

According to section 10 of the English Law, it is not required to obtain a production license for the following actions in pharmacies and MOs:

- compounding of drugs;
- intra-pharmacy packaging of registered medicines (hereinafter referred to as “RMs”).

Such types of activity are carried out exclusively by a pharmacist[21] and (or) under his supervision and can be carried out by pharmacies under a pharmaceutical license, as well as by MOs under a medical license.

In addition, section 10 of the English Law provides an exhaustive list of grounds for the drugs compounding and intra-pharmacy packaging of RMs:

- compounding and intra-pharmacy packaging of MPs are carried out according to a doctor’s prescription for a specific patient;
- compounding and intra-pharmacy packaging of medicines are carried out in the form of intra-pharmacy preparation (hereinafter referred to as “IPP”) for subsequent dispensing according to a doctor’s prescription;
- compounding of drugs at the request of an “individual” [15, 16] for medicines subject to over-the-counter dispensing (PM, GLS);
- drugs compounded by pharmacy are not subject to supply to MOs and can be dispensed to the patient only according to a doctor’s prescription and (or) on the basis of a request from an “individual”, i.e. supply to MOs can only be carried out for SMs, if there is a special license for the compounding of drugs.

In accordance with sections 69, 70, and 71 of the English Law, retail pharmaceutical activity can be carried out either by an individual or by a legal entity (body corporate) [31], and for each of these cases, its own requirements are established for the appointment of persons responsible for pharmaceutical activity.

If pharmaceutical activity is carried out by an individual (analogous to an individual entrepreneur in Russia), then for each address of pharmaceutical activity, a Responsible Pharmacist must be appointed, who is obliged to monitor compliance with pharmaceutical law on a daily basis. The name of the responsible pharmacist and his registration number must be placed in an accessible (visible) place of each pharmacy. He bears personal legal responsibility for compliance with standards when dispensing drug at his address of activity.

If pharmaceutical activity is carried out by a legal entity, then according to sections 69 and 70 of the English Law, a mandatory condition is the presence of a Superintendent Pharmacist [32], who is responsible for all pharmaceutical activity of the company. This specialist must be a pharmacist and be responsible for conducting business in all pharmacies owned by the company. At the same time, for each address of pharmaceutical activity, a responsible pharmacist must be appointed, who can be either the superintendent himself or another pharmacist acting under his guidance. Thus, the superintendent provides overall control and compliance with standards throughout the network, while responsible pharmacists bear operational responsibility for the pharmaceutical services provided and the work performed by specific pharmacies.

It is also worth noting here that English legislation uses the term “Assembling a Medicinal Product” [33], which also includes intra-pharmacy packaging of RMs, as well as: “relabeling of medicines” and “packaging of medicines” with other components (for example, syringes, dispensers). At the same time, the direct process of “assembly” of drug consists in the fact that there should be no qualitative and quantitative change in the drug or its dosage form (hereinafter referred to as “DF”).

According to part 1 of the English Rules, EMs and packaged RMs are not subject to state registration in the territory of Great Britain. In the context of the cycle of works by the authors of this study, the latter has a significant role in view of the presence of legal conflicts in the procurement of EMs and packaged RMs, since in Russian legislation [13, 34]:

[21] In the UK, there are pharmacists (higher pharmaceutical education — 4 years of study) and pharmaceutical technicians (secondary pharmaceutical education — 2 years of study).

– the definition of RM is not established;
– a number of bylaws of the contract system in the field of procurement establish barriers and restrictions in the procurement of RMs, which may be the basis for canceling ongoing procurements in cases where their subject is packaged RMs.

In turn, the English Rules directly indicate that a violation of the primary packaging automatically makes the medicine unregistered and the packaged RMs cease to comply with the registration certificate. A similar legal structure operates in Germany [15, 16].

Also, a feature that we consider necessary to note is the expanded right of pharmaceutical workers, provided for in section 12 of the English Rules, allowing the dispensing of prescription medicines without a doctor's prescription in the following cases:

– the doctor has confirmed (orally and (or) in writing) the patient's need for the drug, and in this case, the prescription must be provided to the pharmacy within 72 hours from the moment of dispensing the drug;
– during pharmaceutical counseling, the pharmacist came to the unambiguous conclusion that in this situation the patient cannot get a prescription for the drug, and any delay in taking the drug will lead to health risks and (or) the requested drug was prescribed to the patient earlier.

Compounding of medicines: A model of effective regulation

The analysis of the current regulatory legal documents devoted to pharmaceutical activity in the United Kingdom showed that the model of outcome-based regulation in modern pharmaceutical practice in Great Britain, in which the main emphasis is placed on achieving certain target results, and not on "strict" adherence to prescribed procedures [35–37]. Thus, there are no detailed documents in the British regulatory system that are comparable, for example, with the rules for the compounding and dispensing of drugs[22]. In other words, the regulatory acts establish a minimum level of general requirements, and not a descriptive part, in particular, a specific technology for the drug compounding.

The professional activity of pharmaceutical workers is regulated by the Pharmaceutical Council with powers in the following areas:

– creation and maintenance of a register of pharmaceutical workers, pharmacies, and their premises;
– establishment of Standards, Guidance, requirements for the implementation of professional activity of pharmaceutical workers;
– formation of educational programs for pharmaceutical workers, establishment of relevant requirements for their accreditation and continuing education;
– conducting inspections and investigations.

Mandatory and structural elements in the composition of the Pharmaceutical Council are:

– regulatory committee (combines functions similar to those for the Department of Medicines and Medical Devices Regulation and the Department of Medical Education and Personnel Policy in Healthcare of the Ministry of Health of Russia);
– inspectorate (analogous to Roszdravnadzor);
– appeals committee (appeal against orders issued by the inspectorate, there is no analogue in Russia).

The latter is an important part of the dialogue between control and supervisory bodies in any sphere of economic activity. For example, the pre-trial (out-of-court) procedure for appealing decisions and actions (inaction) of Roszdravnadzor (territorial body), as well as its officials, is regulated by paragraphs 110–132 of the order of Roszdravnadzor dated July 10, 2020 No. 5974[23], which states that complaints can be sent to the head of Roszdravnadzor or the Ministry of Health of Russia. In such an iteration, the interested person appeals the decisions of the inspectorate to it, and the higher organization (the Ministry of Health of Russia) does not have a structural unit that would perform such functions.

The Pharmaceutical Council has the right to determine independently other elements of its organizational structure, and is also obliged to consult with the professional community (existing business, expert and educational organizations, and others) before introducing any rules regulating the professional activity of pharmaceutical workers.

The Pharmaceutical Council is the main body that carries out the function of developing and implementing regulatory legal regulation of the professional activity of pharmaceutical workers. At

[22] Order of the Ministry of Health of the Russian Federation dated May 22, 2023 No. 249n "On Approval of the Rules for the compounding and Release of Medicines for Medical Use by pharmacy Organizations Licensed for pharmaceutical activities". Available from: https:// clck.ru/3MEfx2. Russian

[23] Roszdravnadzor Order No. 5974 dated July 10, 2020 "On Approval of the Administrative Regulations of the Federal Service for Healthcare Supervision on the Implementation of State Quality Control and Safety of Medical Activities. Available from: https://clck.ru/3MEfxu. Russian

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the time of this study, the Pharmaceutical Council has developed and implemented more than 20 different standards and guidelines that are mandatory for pharmaceutical workers[24] to comply with. The Standards of the Pharmaceutical Council consist of nine key principles:

1. The central role of the patient (pharmacists must put the interests of patients first, ensuring access to safe and effective pharmacotherapy).
2. Teamwork (pharmacists must cooperate with medical workers to ensure comprehensive care for patients).
3. Omnichannel communication model (doctor–patient–pharmacist).
4. Professional behavior (pharmacists must demonstrate high standards of professional behavior and ethics).
5. Professional knowledge and skills (pharmacists must maintain and develop their professional knowledge and skills).
6. Professional decisions (pharmacists must make informed decisions based on evidence and knowledge).
7. Respect and confidentiality (pharmacists must respect the rights of patients and ensure the confidentiality of their data).
8. Responsibility (pharmacists must demonstrate leadership qualities and take responsibility for their actions).
9. Risk management (pharmacists must identify and minimize risks associated with pharmaceutical practice).

The described principles are largely harmonized with the concept of providing pharmaceutical care adopted in the EU [13]. In our previous works, we have repeatedly described the European concept of providing pharmaceutical care in the form of a set of pharmaceutical services, as well as the gradual "simplification" of the concept of pharmaceutical activity, pharmacy infrastructure, pharmacy technologies, and personnel qualifications in the Russian Federation [13, 21, 38]. British law does not specify the full list of existing pharmacy services, however, the Standards of the Pharmaceutical Council for pharmacists[25], they include all services provided by pharmacies, including activities related to the retail sale of drugs, their dispensing, storage, transportation, compounding, clinical services of pharmacies (vaccination, screening, etc.), and pharmaceutical counseling. In 2022, amendments were made to the English Law, which introduced:

- definition of "Relevant Pharmacy Service" — a pharmaceutical service that is provided in MOs, nursing homes, prisons, and other social institutions;
- position of Chief Pharmacist [39, 40] — the authorized pharmacist for the provision of pharmaceutical services in the above-mentioned institutions, in the absence of a license for the retail sale of drugs.

Based on the content of the explanatory note[26], the described changes were introduced to strengthen the protection of medical workers from criminal liability in terms of providing pharmaceutical services without the presence of a pharmacy in the MO. At the same time, in Russia, against the background of the problem of the availability of medicines and pharmaceutical care, these issues have only developed due to the shortage of pharmacies and pharmaceutical workers in rural settlements in which there are no pharmacies. In particular, the solution is implemented through the retail sale of drugs through outpatient clinics, feldsher and feldsher-midwife stations, centers (departments) of general medical (family) practice (Article 55 of Federal Law-61). On the other hand, at the time of the study, a draft law was published, according to which it is proposed to expand the functionality of the Russian Post Office for the retail sale of over-the-counter drugs in remote and sparsely populated areas without obtaining a pharmaceutical license and complying with special storage conditions[27], leaving open questions about ensuring the quality of medicines, storage requirements, compliance with dispensing rules, and possible criminal liability in terms of providing pharmaceutical services, including pharmaceutical counseling.

For the purposes of this study, it is necessary to highlight the following general features from the main standards and guidelines[28, 29] of the Pharmaceutical Council:

[24] Standards and guidance for pharmacy professionals. Available from: https://clck.ru/3MDajU
[25] Standards and guidance for pharmacy professionals. Available from: https://clck.ru/3MDajU
[26] The Pharmacy (Preparation and Dispensing Errors – Hospital and Other Pharmacy Services) Order 2022. Available from: https://clck.ru/3MEVGG
[27] On amendments to certain legislative acts of the Russian Federation. Available from: https://clck.ru/3MgppC. Russian
[28] Standards for pharmacy professionals. Available from: https://clck.ru/3MDaUE
[29] Standards for registered pharmacies. Available from: https://clck.ru/3MEah7

1. Requirements for personnel:
– pharmaceutical workers are obliged to ensure the safety and quality of the services provided. If situations arise that go beyond their competence, they should seek help from other specialists;
– delegation of tasks should be carried out exclusively to qualified persons who have undergone appropriate training, with mandatory provision of the proper level of control over the performance of assigned functions;
– all employees involved in the process of compounding and dispensing of drug must have the necessary professional knowledge, skills, and experience.
2. Requirements for premises:
– must be safe, clean, properly maintained, and suitable for the pharmaceutical services provided and comply with established sanitary standards (including confidentiality requirements for clinical services).
3. Requirements for equipment. All equipment used must:
– come from trusted suppliers;
– correspond to its purpose;
– be protected from unauthorized access by third parties;
– be properly operated and maintained.
4. Documentation and registration:
– all records related to the provision of pharmaceutical services must be kept and maintained up to date, all personal information about patients must be processed in such a way as to ensure its confidentiality.
5. Obligations of the pharmacy owner:
– Pharmacy owners must ensure that all personnel are familiar with the standards and guidelines of the Pharmaceutical Council, understand the importance of their compliance, and are aware of all responsibility for their non-compliance.

Basic principles of drugs compounding in pharmacy

For the activity of pharmacies in the compounding of drugs in England, a separate guide to the compounding of drugs (hereinafter referred to as the "British Guide")[30] is provided, according to which:

– responsibility for compliance is borne by the owners of pharmacies, who are also obliged to ensure the implementation of other regulatory requirements related to the legislation on the circulation of medicines;
– deviations from the guiding principles described in the guide are permissible in cases where the achievement of established standards can be implemented in other ways and provided that the final results;
– compounding of IPPs is allowed provided that such EMs are intended for subsequent sale through pharmacies of one pharmacy (as a legal entity);
– patient can expect that the compounded drug will correspond in quality and safety to the same drug in the case of its production under a production license.

From the point of view of good practices, the British Guide distinguishes five principles that must be observed in production pharmacies:

Principle No. 1. Ensuring the safety of EMs for patients through an effective risk management system.

Each production pharmacy must develop and approve regulatory provisions that allow minimizing the risks to patients associated with the drug compounding. This principle covers the following key aspects.

1.1. Risk assessment

According to the British Guide, risk assessment means a systematic analysis of factors that can cause harm to patients or other persons, as well as the formation of necessary measures to neutralize such factors.

The risk assessment methodology must demonstrate its effectiveness for each address of pharmaceutical activity and, in particular, include provisions and procedures on:

– pharmaceutical examination of the prescription (checking, incompatibilities, doses, etc.);
– verification of the method of drug compounding;
– identification of specific risks associated with the raw materials;
– ensuring the necessary level of training and qualification of personnel;
– compliance of premises, equipment, and sanitary regime with the types of compounding nomenclature;
– preventing the risk of contamination;
– circumstances under which a re-assessment of risks will be required.

[30] Guidance for registered pharmacies preparing unlicensed medicines. Available from: https://clck.ru/3MEf3X

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1.2. Internal audit

For the purposes of ensuring compliance with safety and quality standards of EMs, it is necessary to conduct a regular audit of the processes of drugs compounding. At the same time, the frequency of the internal audit is determined by the parmacy independently, where the time intervals must be justified and reflect the specifics of the activity of each pharmacy at the address of pharmaceutical activity. The audit must include at least:

- a comprehensive inspection of premises (temperature, humidity, lighting, sanitary standards);assessment of equipment and its operating conditions;
- analysis of the processes of drug compounding and their quality control;
- assessment of the level of training and qualification of personnel;
- verification of record keeping, including in relation to the methods used for drug compounding, ensuring traceability of raw materials, proper labeling of EMs, and data storage.

The results of the audit should be used to adjust existing procedures, eliminate identified shortcomings, and improve the overall level of quality of pharmaceutical services provided.

1.3. Review of risk assessment

If there are changes in the working conditions of the pharmacy that affect the process of drug compounding, it is necessary to review the risk assessment and related procedures. Such changes may include:

- replacement of pharmaceutical workers;
- introduction of new personnel into the processes of drug compounding;
- modernization or replacement of equipment;
- change of suppliers of raw materials and packaging materials;
- registration of incidents or complaints filed by the public and the medical community;
- change of environmental conditions or technical characteristics of premises.

The review of the risk assessment must be documented and serve as the basis for conducting a new risk assessment if required.

1.4. Recall procedures

The pharmacy must have developed and validated procedures for recalling, withdrawing from circulation, and subsequent destruction of compounded drug in case of detection of factors that pose a risk to patients. Such procedures must indicate the person who is responsible for the implementation of these procedures, as well as establish appropriate procedures, including the sequence of actions and indication of supervisory authorities and (or) organizations that must be notified of the procedure for recalling, withdrawing from circulation, and subsequent destruction of EMs. The instructions must provide additional mechanisms that allow each employee of the pharmacyto report a suspicion that the drug may be substandard or unsafe.

1.5. Responsibility

For each address of pharmaceutical activity, records must be kept that allow determining which of the pharmacists is responsible for the compounding of a specific medicine, as well as which pharmaceutical technicians and other employees of the PO participated in this process (if applicable).

1.6. Record keeping

All stages of drug compounding must be documented. The duration of record keeping must be justified and determined by the pharmacy independently. At the same time, the period of their storage must be sufficient to conduct an investigation in case of incidents or complaints. The records must include information at least in relation to:

a) The process of drug compounding, including:

- description of the key stages of drug compounding;
- detailed calculations with double checking;
- date of compilation of the worksheet (standard operating procedure for drug compounding);
- surname and name of the pharmacist who preparation the drug, full name of the pharmacist who approved the release of the finished product;
- surname and name of the pharmaceutical technician who participated in the process of drug compounding (if applicable);

b) raw materials, including:

- source of receipt (compounding of drug or wholesale distributor);
- certificate of conformity (if applicable)31;
- certificate of analysis (if applicable)32;
- batch number;
- expiration date (if available);
- surname and name of the pharmacist who checked the raw materials;
- description of the packaging.

[31] A certificate of conformity confirming that the raw materials meet the established requirements or specifications, but do not contain test results.

[32] A certificate containing the results of the test results of the feedstock together with an assessment of its compliance with the declared specification.

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c) compounded drug, including:
- date of compounding;
- batch number;
- expiration date;
- date of dispensing to the patient.

d) patient, including:
- surname and name;
- patient's address;
- contact details (phone, email, etc.);
- sample of the labeling that was applied to the drug;
- surname and name of the person who created the label layout.

e) prescription (in case of dispensing by prescription), including:
- surname and name of the doctor (contact details);
- patient's age;
- other prescription data.

f) incidents and complaints, including:
- reports of suspected adverse reactions;
- complaints and comments.

Principle No. 2. High professional competence and continuous education.

All employees of the pharmacy who participate in the drugs compounding must have the necessary qualifications, undergo appropriate training, and constantly improve their knowledge, skills, and abilities.

2.1. Training

Admission to the implementation of activities for the drugs compounding is granted only after mastering the corresponding level of the educational program. If an employee is in the process of training, then a mentor must be assigned to him, who will exercise proper control.

To work with EMs, the personnel of the pharmacy require knowledge and skills that go beyond the retail sale of RMs.

If an employee does not have sufficient qualifications, the owners of the pharmacymust organize additional training. Special attention should be paid to regular knowledge and skills checks, especially in situations where work is carried out with specific raw materials and (or) methods of drug compounding, including with raw materials and methods that are used in relatively rare cases.

Employees working with highly hazardous and (or) active raw materials (cytostatics, hormones, biological raw materials) [41] or in conditions requiring compliance with special sanitary measures (compounding of sterile DFs) must undergo special training corresponding to the nature of the work performed.

2.2. Training records

The owners of the pharmacy are obliged to keep records of the training of personnel and store information for as long as it is considered reasonable and justified. Such records must be available upon request from authorized bodies and organizations.

Principle No. 3. Compliance of the production environment with the nature of the work performed.

Premises intended for the drugs compounding must comply with the required level of cleanliness classes. It is required to implement all measures to prevent contamination and the risks of its occurrence.

3.1. Measures to minimize contamination

Premises intended for the drug compounding must be designed to correspond to the nature of the work performed. If there is a need, it is necessary to have separate work areas for the drug compounding of different classes. The working environment of the premises must correspond to the established cleanliness class.

Specific measures should be taken to prevent or minimize the risk of cross and microbiological contamination during the drug compounding.

The described factors must be taken into account at the stage of conducting the initial risk assessment.

3.2. Records on compliance with the sanitary regime

It is necessary to keep records on compliance with the sanitary regime, including standard operating procedures for cleaning premises and equipment, as well as assessments of microbiological purity. Records should be kept for as long as it is considered reasonable and justified.

Principle No. 4. The procedure for providing pharmaceutical services must ensure the effectiveness and safety of EMs.

4.1. Raw materials

The quality of the finished product directly depends on the quality of the raw materials. The pharmacy must ensure that it comes from trusted suppliers.

4.2. Quality Assurance

The quality control system must consist of at least the following elements:
- use of worksheets;
- confirmation of the quantity and identity of ingredients;
- availability of qualified and trained personnel;
- properly maintained equipment.

The pharmacy must guarantee the proper quality of EMs. When compounding a series of drugs, including in the form of IPPs, the quality control system must be more extensive (for example, conducting full chemical control) than in the case of a single manufacture of EMs.

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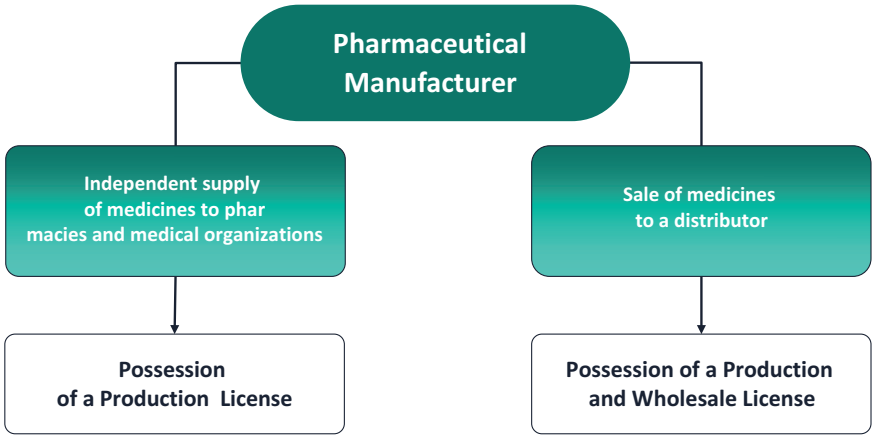

Figure 1 – Requirements for the availability of licenses for manufacturers of medicines

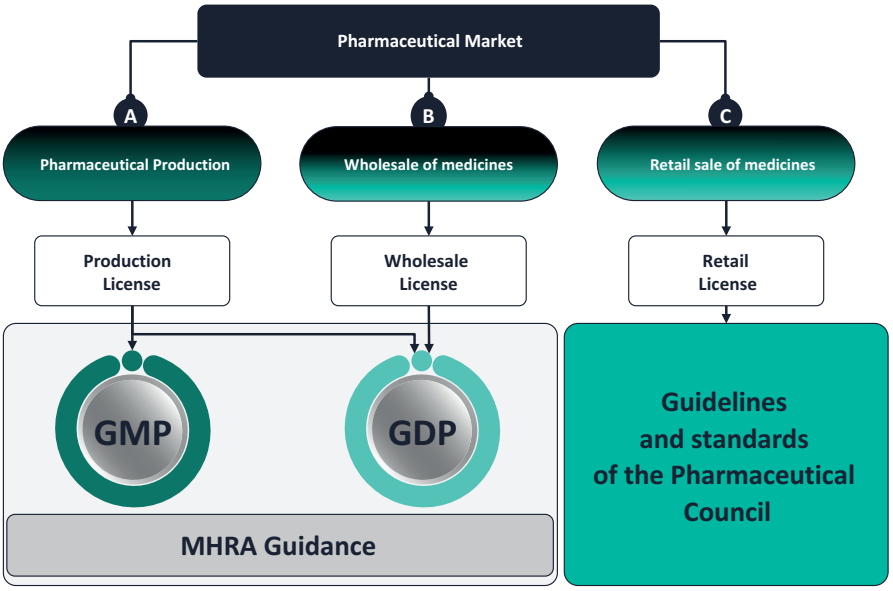

Figure 2 – Licensing requirements in the United Kingdom.

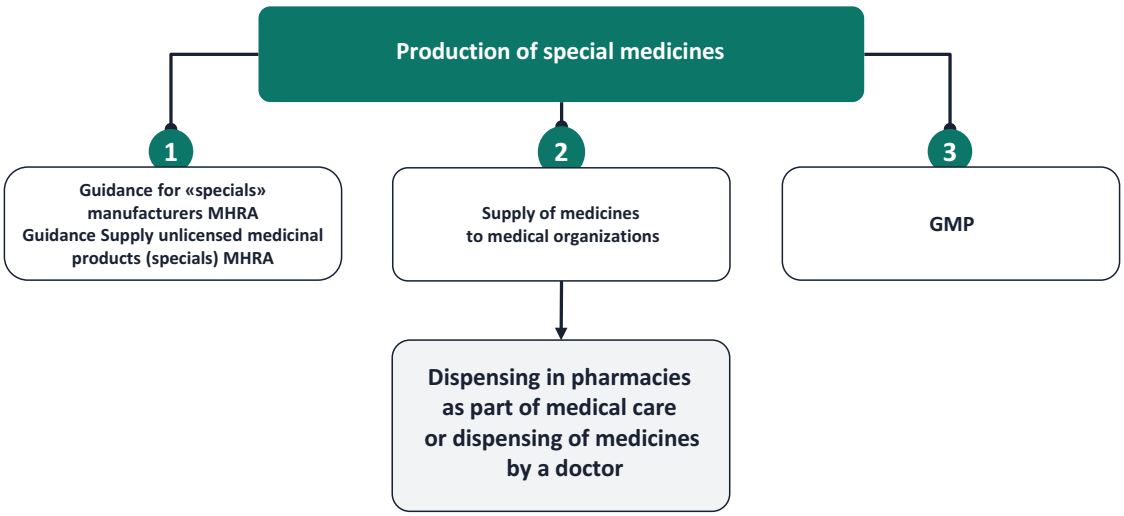

Figure 3 – Licensing requirements for special medicines.

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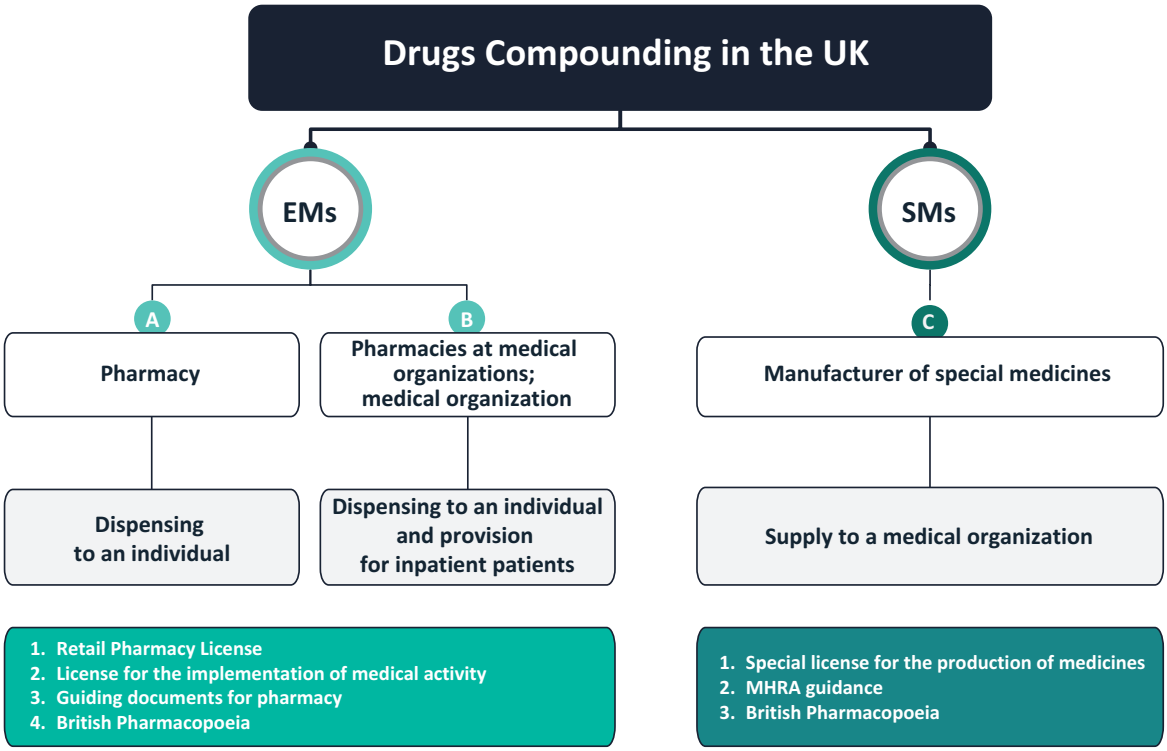

Figure 4 – Schematic diagram of organizational approaches to compounding of drugs in the UK.
Note: ELP — extemporaneous medicines; SLP — special medicines.

4.3. Information for patients

Each production pharmacy must have a patient information system, according to which the responsible pharmacist (or other competent pharmaceutical worker) informs him that he will be dispensed an unregistered drug, where, in certain cases, there is no clinical trial data and information on the safety and effectiveness of such medicine.

Principle No. 5. Compliance of equipment and premises with the nature of the work performed.

5.1. Specialized equipment and premises

The pharmacy must have equipment and premises specially designed for the purposes for which they will be used by employees. The equipment must be of high quality and accuracy (if necessary) to guarantee the compounding of high-quality and safe EMs.

5.2. Equipment maintenance logs

For each type of specialized equipment, it is necessary to keep maintenance logs, including data on: conducted verifications and calibrations; planned and unscheduled maintenance; identified malfunctions and their elimination. Records should be kept for as long as it is considered reasonable and justified.

The British Guide recommends that pharmacies additionally use the following in their activities for the drugs compounding:

- The MHRA Orange Guide to Rules and Guidance for Pharmaceutical Manufacturers and Distributors;
- PIC/S Guide to Good compounding Practice for Medicinal Products;
- Resolution on the requirements for the quality and safety of the compounding of medicines in pharmacy for the special needs of patients.

Extemporaneous compounding in the British Pharmacopoeia

The British Pharmacopoeia (hereinafter — BP)[33] contains 94 pharmacopoeial monographs on individual formulations of ELP in different dosage forms, as well as a number of general pharmacopoeial monographs (hereinafter — GPM) devoted to various aspects of drug compounding:

1. GPM “Pharmaceutical preparations” — from the European Pharmacopoeia and introducing the classification of ELP, including those made in the form of AIS [13];
2. GPM “Unlicensed Medicines”.

In contrast to other global regulatory systems that have been previously studied by the authors of this study, this GPM is devoted to both EMs and SMs (hereinafter jointly referred to as NMs), which repeatedly emphasizes the continuity of good practices. The document defines key aspects of

[33] The authors have access to the full version of the British Pharmacopoeia 2022 edition.

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quality assurance in the compound (production) of NMs, including compliance with aseptic conditions, microbiological control, shelf life, labeling and stability of drugs, and also indicates the features of dissolution and uniformity of dosage tests for oral suspensions. Particular attention is paid to the need for compliance of the active and excipient substances used with pharmacopoeial standards, as well as ensuring safety for patients through strict control over compounding processes. In particular, the GPM contains the following features:

- it is emphasized that the provisions of this article do not apply to actions for intra-pharmacy packaging of RMs;
- NMs must comply with pharmacopoeial requirements during testing, however, the use of alternative quality control methods is allowed if it is impossible to regularly apply the entire scope of full chemical control and (or) specific methods of analysis;
- each series of sterile compounded / produced NMs must be tested for sterility, if the shelf life of the NMs is less than 90 days, the analysis is carried out retrospectively and is part of the control in the compounded / produced process;
- in the case of individually compounded / produced NMs, the batch size is equal to one package, under these circumstances, one package of NMs compounded (produced) during one work shift is subjected to a sterility test (continuous period of work with the participation of the same employees34, under the same environmental conditions, using the same equipment and materials). If no series of NMs has been compounded (produced) during one work shift, then control samples (English Dummy samples) must be provided;
- sterility tests may not be carried out in the case of validation of the compounding (production) process of NMs.

3. The appendix of the fifth volume of the BP contains an additional chapter devoted to NMs, also entitled “Unlicensed Medicines” (NMs Guide). It emphasizes that this type of drug therapy plays an important role in modern medical practice, while such drugs require special attention from doctors, pharmaceutical workers and their compounding / produced.

The document states that, as a rule, owners of a special license for the production of medicines also have a retail pharmaceutical license and, in fact, carry out two types of activities on the basis of a joint-stock company. The MHRA guidance for manufacturers of SMs refers to the same fact. The described practice is not unique to the UK. As noted above, a similar model is implemented in the USA — with 503A [42–44] and 503B pharmacies, and in the EU [45–47] — the mentioned model echoes the European resolution [48, 49][35], according to which GMP rules[36] are recommended to be used as a reference when compounding the “high-risk drugs” group, and the PIC/S Guide[37] to Good compounding Practice for Medicinal Products — when compounding “low-risk drugs”.

In addition, additional chapters cover issues related to:

- the use of NMs without preservatives, especially in pediatric and neonatal practice;
- bioequivalence of oral liquids, especially in the case of drugs with a narrow therapeutic index, since data on the bioavailability of NMs are usually absent, there is a risk of mismatch between clinically significant indicators of the concentration of the active substance in the blood and the prescribed dosage of the drug, and therefore it is recommended in some cases to carry out the necessary clinical monitoring;
- stability of NMs, which is assessed taking into account an extensive list of factors listed in the BP that affect the shelf life of compounding (produced) products. Some PMs devoted to specific NMs formulations provide recommended shelf lives. However, the final value of the shelf life is established by the persons responsible for the compounding or production of drugs.

Within the NMs Guide, a special subsection is dedicated to standards for aseptic compounding (production) of drugs. The following key features of the document should be highlighted:

1. It is emphasized that a special license for the production of medicines appeared in the British regulatory system as a logical development of the activity of dilution (reconstitution) of medicines, which is directly related to the need to minimize the risk of microbiological contamination, ensure accurate dosing and the possibility of full documentary support of the entire

[34] The qualifications of employees depend on the type of license.

[35] Committee of Ministers, Council of Europe. Resolution CM/Res (2016) 1 on Quality and Safety Assurance Requirements for Medicinal Products Prepared in Pharmacies for the Special Needs of Patients, 2016.

[36] Scheme P.I.C. Guide to good manufacturing practice for medicinal products annexes, 2009.

[37] Scheme P.I.C. PIC/S guide to good practices for the preparation of medicinal products in healthcare establishments. PE 010i; 2014.

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compounding process (production of medicines).

2. In aseptic premises (English Aseptic Units; aseptic unit) a wide range of drugs are manufactured / produced (parenteral nutrition solutions, cytostatics, radiopharmaceuticals, etc. It is separately emphasized that the implementation of activities for dilution (reconstitution) of drugs is pharmacoeconomically beneficial.
3. The subsection emphasizes the need to implement a comprehensive quality assurance system covering all stages of the drug's life cycle – from the purchase of raw materials to the storage and supply of finished products. Such a system should include standard operating procedures, regular monitoring of the environment and finished products, process validation, training and certification of personnel, internal and external audits, etc.
4. Any manipulations with cytostatic drugs should be carried out in biological safety cabinets of type II or in a pharmaceutical isolator, including operations of dilution (reconstitution) of toxic drugs.

In the subsection of this work devoted to the Pharmaceutical Council, it was noted above that it is obliged to consult with the professional community (existing business, expert and educational organizations, etc.) before introducing any rules regulating the professional activities of pharmaceutical workers. The regulatory documents of the Pharmaceutical Council, as well as the BP, directly refer to the guidelines of the Royal Pharmaceutical Society (RPS)[38], which has been continuously operating since 1841. Thus, the aforementioned subsection of the BP, devoted to standards for aseptic compounding (production) of drugs, recommends organizing activities for the compounding of drugs (without a special license for the production of drugs) in accordance with the RPS Guide[39] "Ensuring the quality of services for aseptic preparation of medicines", which in its content is similar to chapter 797 of the US Pharmacopoeia (USP)[40]. The UK Guidance, as well as the NHS Standard for ensuring aseptic preparation of drugs in hospital pharmacies[41], refers to it. There are other NGOs in Britain that issue similar documents that are taken into account in the professional activities of joint-stock companies and medical organizations, but RPS is undoubtedly the leading one.

In general, the entire set of standards, guidelines and other regulatory legal documents studied in the course of this work, devoted to the features and directly the processes of drug compounding, is based on the basic principles of good practices, which are highly comparable with chapters 795, 797 and 800 USP, as well as with the PIC/S Guide to Good compounding Practice for Medicinal Products, which was discussed in detail in the corresponding monograph [13] and individual scientific publications [15, 16].

The final scheme of organizational approaches to drug compounding in the United Kingdom is presented in Figure 4.

Study Limitations

Only the full version of the BP 2022 edition was available to the authors of the study, while the online version of the British Pharmacopoeia (in the 2026 edition), containing current updates and additions, is not available for free access and requires a special subscription.

CONCLUSION

Despite the fact that there is a special license for the production of medicines, under this type of activity, it is undoubtedly worth understanding precisely the pharmacy compounding of drugs, which is confirmed both by the current practice of Great Britain and by the analysis of the regulatory legal framework. This mechanism is distinguished by analogy with the approach to the activities of 503B pharmacies in the United States. The authors of the work failed to find separate guidelines specifically developed for compounding of drugs by medical organizations without a pharmaceutical license, but the analysis allows us to conclude that the same standards, methods and norms are used in such activities as are applied to joint-stock companies. Thus, we can talk about the formation of a unified system of regulatory support for drug compounding activities, including elements of regulation of both the European Union and the United States, as well as the introduction of "adapted" GMP requirements into the practice of pharmacy drug compounding. Such a regulatory structure indicates cross-border unification of approaches and harmonization of requirements for the quality and safety of EMs.

The described unification of regulatory requirements emphasizes the increasing role of pharmacy drug compounding in the modern healthcare system, which is directly related to the expansion of the

[38] So L. About the royal pharmaceutical society; 2014.
[39] Quality Assurance of Aseptic Preparation Services: Standards. Available from: https://clck.ru/3Mc5pY
[40] USP-NF/PF online. Available from: https://clck.ru/3MkGLp
[41] Assurance of aseptic preparation of medicines. Available from: https://clck.ru/3Mc68g

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functions of joint-stock companies in providing patients with personalized drugs. All the approaches considered in this study and in the previous works of the authors demonstrate a steady trend towards the fact that the activities of joint-stock companies are becoming a key link in providing patients with personalized drugs of all types of therapy, which seems to be a logical and appropriate direction for the development of pharmaceutical activities, which contributes to a more flexible and responsible provision of special medical needs of patients.

Such an expansion of the functionality of joint-stock companies is impossible without the corresponding development of the concept of pharmaceutical care as a systemic element of healthcare, which is confirmed by the practice of countries with a developed regulatory system. As the results of the study showed, pharmaceutical workers are able to make a significant contribution to the accessibility and quality of medical and social care to the population through the development of the concept of providing pharmaceutical care, in the development of which it is necessary to consider the issues of citizens' access to the necessary drugs in a comprehensive manner. The British healthcare regulatory system has separate legislative documents that define the foundations in the field of organization of pharmaceutical affairs, the functioning of joint-stock companies, the provision of pharmaceutical care by them and the provision of pharmaceutical services in a wide range of competencies.

In these circumstances, the lag of the Russian regulatory legal framework in the regulation of pharmacy drug compounding looks especially noticeable, which confirms the need for systemic changes. This study has once again demonstrated that the current state of legal regulation of drug compounding activities in Russia needs to be improved in terms of the circulation of Ems and packaged RMs, which is not sufficiently regulated by current legislation and should be harmonized with international norms.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

Devi D. Mamedov — analysis of normative and legal information, search and collection of literary sources, writing and editing the text of the manuscript; Dmitry S. Yurochkin — analysis of normative and legal information, search and collection of literary sources, writing and editing the text of the manuscript; Svetlana N. Egorova — analysis of normative and legal information, search and collection of literary sources, writing and editing the text of the manuscript; Zakhar M. Golant — analysis of normative and legal information, search and collection of literary sources, writing and editing the text of the manuscript; Igor A. Narkevich — analysis of regulatory and legal information, search and collection of literary sources, writing and editing of the manuscript text. All authors confirm that their authorship meets the international ICMJE criteria (all authors have made significant contributions to the development of the concept, research and preparation of the article, read and approved the final version before publication). All authors made an equivalent and equal contribution to the preparation of the publication. All authors confirm that their authorship meets the international ICMJE criteria (all authors made a significant contribution to the development of the concept and preparation of the article, read and approved the final version before publication).

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REVIEW

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Prospects for the use of an original medicine based on hexapeptide succinate for organ protection in various diseases

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The aim. To analyze and summarize the available results of experimental and clinical scientific studies of synthetic analogs of leu-enkephalin; to evaluate the expansion of the spectrum of application of the original medicine based on hexapeptide succinate in patients with various diseases of different etiologies.

Materials and methods. An analysis of scientific literature data in the PubMed, eLibrary.ru and CyberLeninka databases was carried out. The search depth was 50 years (from 1976 to 2024), the list of references includes 60 scientific papers.

Results. It was revealed that receptors for endogenous opioid peptides are widespread in living organisms. Modern neuropharmacology recognizes 4 main types of opioid receptors: μ (MOR) — mu, δ (DOR) — delta, κ (KOR) — kappa and NOP — nociceptive receptor, which bind to endogenous opioids such as enkephalins, endorphins, endomorphins, dynorphins and nociceptin. Due to their antioxidant, immunomodulatory, and anti-inflammatory properties, analogs of opioid peptides are widely used in Russian medicine. A number of experimental and clinical studies are presented, to prove that agonists of delta-opioid receptors can "soften" organ damage in a variety of diseases accompanied by oxidative stress and endothelial dysfunction. The dose-dependent regulatory effect is realized with intravenous, intramuscular and intranasal administration. The therapeutic efficacy of leu-enkephalin analogs based on tyrosyl-D-alanyl-glycyl-phenylalanyl-leucyl-arginine is analyzed, and the prospects for using the new original Russian medicine Ambervin® Pulmo with the inclusion of succinic acid salts are also discussed.

Conclusion. The information on the functional properties of leucine-enkephalin analogs presented in the article indicates broad prospects for the use of medicines based on tyrosyl-D-alanyl-glycyl-phenylalanyl-leucyl-arginine with the inclusion of succinic acid salt in the complex therapy of many diseases for the purpose of organ protection.

Keywords: delta opioid receptor; leucine-enkephalin analogs and derivatives; tyrosyl-D-alanyl-glycyl-phenylalanyl-leucyl-arginine diacetate (dalargin); succinate; acute respiratory distress syndrome; cytokine storm; COVID-19; organ protection; ischemia-reperfusion

Abbreviations: WHO — World Health Organization; ARVI — acute respiratory viral infection; ACE 2 — angiotensin-converting enzyme 2; ARDS — acute respiratory distress syndrome; OP — opioid peptides; IL — interleukin; TNF — tumor necrosis factor; CHD — coronary heart disease; LPO — lipid peroxidation.

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Перспективы применения оригинального препарата на основе сукцината гексапептида с целью органопротекции при различных заболеваниях

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Цель. Проанализировать и обобщить имеющиеся результаты экспериментальных и клинических научных исследований синтетических аналогов лей-энкефалина и оценить возможности расширения спектра применения оригинального препарата на основе сукцината гексапептида в перспективе у пациентов с заболеваниями различной этиологии.

Материалы и методы. Проведён анализ данных научной литературы в базах PubMed, eLibrary.ru и CyberLeninka. Глубина поиска составила 50 лет (с 1976 по 2024 гг.), список литературы включает 60 научных работ.

Результаты. Установлено, что благодаря антиоксидантным, иммуномодулирующим, противовоспалительным свойствам, аналоги опиоидных пептидов широко применяются в России и за рубежом. Приведён ряд экспериментальных и клинических исследований, который доказывает, что агонисты δ-опиоидных рецепторов могут «смягчать» повреждения органов при самых разных заболеваниях, сопровождающихся оксидативным стрессом и эндотелиальной дисфункцией. Установлено, что их дозозависимый регуляторный эффект реализуется при внутривенном, внутримышечном и интраназальном введении. Проанализирована терапевтическая эффективность аналогов лей-энкефалина на основе тирозил-D-аланил-глицил-фенилаланил-лейцил-аргинина и отражены перспективы использования в клинической практике нового оригинального отечественного лекарственного препарата Амбервин® Пульмо с включением солей янтарной кислоты.

Заключение. Информация о функциональных свойствах аналогов лейцин-энкефалина, представленная в обзоре научных публикаций, свидетельствует о широких перспективах применения лекарственных препаратов на основе тирозил-D-аланил-глицил-фенилаланил-лейцил-аргинина с включением соли янтарной кислоты в комплексной терапии различных заболеваний с целью органопротекции.

Ключевые слова: дельта-опиоидный рецептор; аналоги лейцин-энкефалина и производные; тирозил-D-аланил-глицил-фенилаланил-лейцил-аргинина диацетат (даларгин); сукцинат; острый респираторный дистресс-синдром; цитокиновый шторм; COVID-19; органопротекция; синдром ишемии-реперфузии

Список сокращений: ВОЗ — Всемирная организация здравоохранения; ОРВИ — острая респираторная вирусная инфекция; АПФ 2 — ангиотензинпревращающий фермент 2; ОРДС — острый респираторный дистресс-синдром; ОП — опиоидные пептиды; ИЛ — интерлейкин; ФНО — фактор некроза опухоли; ИБС — ишемическая болезнь сердца; ПОЛ — перекисное окисление липидов.

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INTRODUCTION

The COVID-19 pandemic was officially declared over by the World Health Organization (WHO) in May 2023, but periodic, including seasonal, increases in the incidence of this infection continue to pose a threat to the health of all mankind[1]. Cases of the disease that require hospitalization and lead to death are still registered. A key problem is the rapid evolution of the virus: emerging variants of SARS-CoV-2 strains are increasingly resistant to immune protection formed after previous infections or vaccination.

The virus binds to the angiotensin-converting enzyme 2 (ACE2) receptor on the cell surface and penetrates inside, using cellular mechanisms to replicate its genome and synthesize viral particles. This process initiates an inflammatory response with activation of immune cells and release of pro-inflammatory cytokines, which leads to the development of inflammation in the affected tissues [1, 2].

The variety of clinical manifestations in the acute period of the disease is due to the fact that ACE2 is the main entry receptor for SARS-CoV-2, which is expressed on the membranes of cells of various organs and tissues. Cells of the mucous membrane of the oral and nasal cavities, lungs, heart, digestive tract, liver, kidneys, spleen, brain, as well as the walls of blood vessels can be affected, which explains the significant spectrum of potential organ damage during SARS-CoV-2 infection [1, 2].

Recent studies have shown that some symptoms can persist for a long time after an infection. This condition, which can develop both in patients with a mild course of COVID-19 and in those who have had the disease in a severe form, affecting various organs and systems, including the respiratory, cardiovascular, nervous, gastrointestinal and musculoskeletal systems, was called “Long COVID” and was recognized by the scientific community. The most common symptoms of Long COVID are fatigue, shortness of breath, heart disorders, cognitive disorders, including problems with memory and concentration, sleep disturbance, symptoms of post-traumatic stress disorder, myalgia and headaches [1].

[1] After the end of the international public health emergency, WHO/Europe launches a plan to move to a new stage of action in connection with COVID-19. Available from: https://www.who.int/europe/ru/news/item/12-06- 2023-with-the-international-public-health-emergency-ending--who-europe-launches-its-transition-plan-for-covid-19. Russian

It is known that the deterioration of patients against the background of a new coronavirus infection SARS-CoV-2 with an increase in the content of cytokines, which can lead to complications such as pneumonia and acute respiratory distress syndrome (ARDS) [2, 3]. In turn, the outcome of these conditions can be multiple organ failure and death.

Oxidative stress and endothelial dysfunction underlie many severe diseases of various etiologies and lead to damage to organs and tissues at the cellular level. In this regard, the search for effective anti-inflammatory drugs with organoprotective properties has become an especially urgent task during the COVID-19 pandemic and continues to be one of the most sought-after requests of the medical community. Safety of a wide range of doses, high efficiency and a minimum number of adverse reactions are the main requirements for medicines. It is known that analogues of opioid peptides (OPs), which meet these parameters, are used in clinical practice in Russia [4].

Currently, sufficient evidence has already been accumulated that agonists of delta-opioid receptors can mitigate damage to various organs. Synthetic analogues of leu-enkephalin have found application in schemes of pathogenetic therapy for many diseases: gastric and duodenal ulcers, pancreatitis, pneumonia of various origins, COVID-19, ARDS, metabolic and toxic and chemical liver damage, multiple organ failure syndrome, septic shock, ischemic stroke, myocardial infarction. Despite the heterogeneity of the etiology of diseases in which synthetic analogues of leu-enkephalins were used and the unclear mechanism of their action, they demonstrated pronounced organoprotective properties, which was shown in numerous experimental and clinical studies. The development of drugs based on regulatory peptides today is one of the promising areas and allows expanding the range of their use in various diseases.

THE AIM. To analyze and summarize the available results of experimental and clinical studies of synthetic analogues of leu-enkephalin and to evaluate the expansion of the spectrum of application of the original drug based on hexapeptide succinate in the future in patients with various diseases, regardless of etiology.

MATERIALS AND METHODS

PubMed, CyberLeninka, eLibrary.ru search databases were used. The following keywords were used for the search: opioid receptor; leu-enkephalin,

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leucine / analogs and derivatives; tyrosyl-D-alanyl-glycyl-phenylalanyl-leucyl-arginine / dalargin; succinate; acute respiratory distress syndrome; cytokine storm; COVID-19; organ protection; ischemia-reperfusion injury.

A simple search for “leu-enkephalin” in PubMed (as of July 11, 2024) showed about 30,000 results. The peak of publication activity on the keywords “opioid receptor” or “opioid peptide” is noted in the 2000s and 2010s. For example, in 1986, about 700 articles were found for these requests, while in 2016 — 2500, and in 2021 — 2800. The number of articles on “opioid peptide” is stable at hundreds per year. In 2024 (data until July 11, 2024), > 300 articles have already been published on this request.

The search depth was 50 years (from 1976 to 2024). Most studies were conducted on experimental models *in vitro* or models *in vivo.*

RESULTS AND DISCUSSION

Preclinical studies

In vitro studies

Opioids are natural, synthetic or semi-synthetic chemical compounds that interact with opioid receptors, causing analgesia and other physiological effects [5]. Their clinical use began after the isolation of morphine from opium poppy (*Papaver somniferum* L.) in 1806. Among the alkaloids of opium, morphine (10–15 %), codeine (1–3 %), noscapine (4–8 %), papaverine (1–3 %) and thebaine (1–2 %) are of pharmacological significance [6]. These exogenous ligands bind to the same receptors as endogenous OPs, including enkephalins, endorphins, endomorphins, dynorphins and nociception / orphanin.

The hypothesis about the existence of opioid receptors was first proposed in the 1950s, but experimental evidence of their diversity was obtained only in the 1970s when studying the mechanisms of action of analgesics. Thus, the first two types of opioid receptors were discovered, which were named after the drugs used in these studies to distinguish them: mu (μ) for morphine and kappa (κ) for ketocyclazocine [7]. The third type of opioid receptors was found in the vas deferens of mice: its pharmacological profile differed significantly from previously identified (μ and κ) and was named delta (δ) to indicate this difference [8]. Together μ, κ and δ, opioid receptors (MOR, KOR, DOR, respectively) are considered classical opioid peptide receptors based on their structural homology and sensitivity to the non-selective opioid receptor antagonist — naloxone [9].

Later, a fourth type of receptor was identified, structurally homologous to classical opioid receptors, but not sensitive to traditional opioid ligands [10]. After the discovery of its endogenous ligands (nociceptin [11] and orphanin [12]) the receptor known as NOR (nociceptin receptor). Despite structural similarity, NOR is often excluded from the classical group due to its insensitivity to naloxone.

The four main families of endogenous OPs demonstrate preferential activity against the corresponding receptor systems: β-endorphins (MOR), dynorphins (KOR), enkephalins (DOR) and nociception / orphanin (NOR) [13]. However, both endogenous and exogenous opioids rarely exhibit absolute selectivity and more often activate different types of receptors [14].

Opioid receptors are widely represented in the CNS and peripheral nervous system [15]. In the periphery, they are expressed in the lungs, heart, kidneys, small intestine and pancreas, modulating organ functions, inflammatory processes and homeostasis [16]. Additional localization includes neuroendocrine cells (adrenal glands, pituitary gland), immune cells (leukocytes) and ectodermal cells, where opioid receptors are involved in the regulation of nociception and inflammation [5]. Signal transduction is carried out through interaction with endogenous ligands (β-endorphins, dynorphins, enkephalins, nociceptins), ensuring the maintenance of homeostasis under physiological conditions.

The importance of OPs in the regulation of physiological systems was consistently revealed in the second half of the XX to early XXI century. Initially, their action was associated exclusively with the CNS through μ-, δ- and κ-receptors [14, 17], but later their peripheral effects were revealed, including modulation of the immune response (for example, met-enkephalin in rheumatoid arthritis) [18, 19]. OPs are formed from three main precursors (proopiomelanocortin, proenkephalin, prodynorphin) by proteolytic processing with subsequent modifications (acetylation, phosphorylation), which determine their activity [20].

Peripheral effects (decreased peristalsis, modulation of inflammation) are mediated by all receptors: MOR — respiratory depression, constipation; DOR — cardioprotection, antidepressant effect; KOR — sedation, mood modulation, diuresis; NOR — mediates

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bipolar pain effects: hyperalgesia (low doses) and analgesia (high doses) [21]. In addition, two subtypes of δ-receptors are known that bind to enkephalins (the precursor of which is proenkephalin). They play an important role in analgesia and reducing gastric motility [20, 22].

The opioid system is considered one of the most complex neurotransmitter systems of the body, playing the most important role in main biological processes. The use of exogenous opioids for analgesia has limitations due to their undesirable side effects, including sedation, respiratory depression and constipation. However, experiments have shown that drugs that bind to δ-opioid receptors (DOR) do not have side effects on the respiratory system and gastrointestinal tract. Activation of DOR enhances signals that promote survival and reduces oxidative damage to neurons [23], and activation of DOR reduces ion imbalance caused by hypoxia [24]. It has been shown that the degree of respiratory depression depends on the stimulated receptor: MOR causes a more significant decrease in respiratory rate than DOR and KOR [25]. Thus, the development of drugs specific to DOR may be clinically useful.

Recent studies show that enkephalins act as modulators of cardiac function and play a vital role in aging, ischemic preconditioning, heart failure and hypertension [26]. The biological activity of peptides is largely determined by their post-translational modifications, such as acetylation, phosphorylation, methylation and glycosylation [27, 28]. Of particular interest are OPs — endorphins and enkephalins are actively involved in the regulation of immune processes. Their synthesis is carried out by various cells of the immune system, including macrophages, lymphocytes, monocytes and mast cells [29].

The production of these peptides is under cytokine control: in particular, the level of enkephalin increases under the influence of interleukin-4 (IL-4), IL-10 and transforming growth factor beta (TGF-β) [30, 31].

Different classes of peptides demonstrate multidirectional effects on antibody formation in inflammation models, while α-endorphin and leu-enkephalin suppress this process, β-endorphin enhances it by 2–3 times [32].

The effect of OPs on immune reactions is characterized by dose dependence: physiological concentrations stimulate immunity; high concentrations suppress for it [33]. The study by S.V. Gein et al. (2020) demonstrated their modulating effect not only on the humoral, but also on the cellular component of immunity, including the functional activity of NK cells, proliferation of T-effectors and chemotaxis of leukocytes: β-endorphin increases the activity of NK cells through MOR receptors, and enkephalins inhibit the proliferation of T-lymphocytes [34].

An important aspect is the participation of OPs in regenerative processes. It has been established that leukocytes enhance the synthesis of endorphins in the focus of inflammation, which in turn stimulate the phagocytic activity of neutrophils [35]. In addition, increased secretion of enkephalins by immunocompetent cells also enhances the bactericidal activity of blood serum [36].

In vivo studies

According to the study by C.W. Tang et al., experimental administration of leu-enkephalin at a dose of 5 mg/kg increased the survival rate of laboratory animals. It has been shown that this effect is associated with a decrease in serum levels of not only "early" pro-inflammatory cytokines (TNFα, IL-1), but also the "late" mediator of inflammation is HMGB1. It is important to note that the effectiveness of OPs persisted both at the initial stage of development of the septic shock model and in the later phase [37].

The anti-inflammatory properties of the synthetic analogue of leu-enkephalin were confirmed in the works of Russian scientists (2020): on a model of acute respiratory distress syndrome in mice, its use significantly reduced mortality [38, 39].

In a series of experiments on rats, it was found that tyrosyl-D-alanyl-glycyl-phenylalanyl-leucyl-arginine (dalargin) improves collagen metabolism in liver and kidney tissues under conditions of repeated stress, and also enhances metabolic activity of the kidneys in experimental diabetes mellitus [40].

The stress-protective activity of the drug was demonstrated in several experimental and clinical studies. For example, a 28-day course of intranasal administration of hexapeptide (0.2 mg/kg) against the background of ulcerogenic stress led to normalization of the distribution index of medium-mass molecules in the blood serum of rats [41]. Normalization of the area of hepatocyte nucleoli and a decrease in the intensity of lipid peroxidation (LPO) were noted in the liver and blood serum of rats that underwent antenatal hypoxia and received intraperitoneal administration

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of an analogue of leu-enkephalin (100 μg/kg), which indicates pronounced antioxidant protection [42, 43]. In addition, in the work of N.A. Bebyakova et al. it was shown that against the background of acute stress, the administration of the peptide to rats caused a significant increase in the level of NO compared to the control (p < 0.001), which confirms its stress-limiting properties and interaction with endothelial factors [44].

Experimental data also indicate the participation of OPs in the neuroendocrine regulation of processes related to food intake and digestion. It turned out that enkephalins play an important role in the control of cognitive functions, such as learning and memory, as well as in the regulation of appetite, thirst and sexual behavior. According to the results of a comparative study by T.A. Czyzyk et al. (2012), conducted on mice receiving a high-calorie diet, it was found that the introduction of endogenous opioids contributes to an increase in energy metabolism and reduces the intensity of weight gain. In this regard, agonists of δ-opioid receptors can be characterized not only as agents capable of correcting eating behavior, but also eliminating metabolic disorders [45]. Of course, the data obtained during tests on animal models still need to be studied for use in clinical practice.

Clinical studies

The world's first peptide drug based on an analogue of leu-enkephalin — tyrosyl-D-alanyl-glycyl-phenylalanyl-leucyl-arginine (Tyr-D-Ala-Gly-Phe-Leu-Arg) — was developed in the USSR in 1983 in the laboratory of Professor M.I. Titov (All-Union Cardiology Research Center of the USSR Academy of Medical Sciences) [46]. Since its creation, it has found successful application in gastroenterology to stimulate tissue regeneration in gastric and duodenal ulcers, acute and chronic pancreatitis, as well as pancreonecrosis [46, 47]. In addition, the drug has been shown to correct disorders of lipoperoxide homeostasis in patients with erosive and ulcerative gastropathy [35].

In a study of Crohn's disease, the addition of tyrosyl-D-alanyl-glycyl-phenylalanyl-leucyl-arginine to standard 5-ASA therapy led to a significant improvement in histological and proliferative activity, as well as a decrease in the intensity of free radical oxidation compared to monotherapy [48].

The ability of the leu-enkephalin analogue to protect the body from the effects of stress factors has been shown in many studies. Its organoprotective properties are manifested by a decrease in the release of cortisol and catecholamines, the intensity of the LPO process, and a decrease in the formation of free radicals. All these processes reduce the harmful effects of free radicals on the lipids of cell membranes, contributing to effective protection of cells from damage caused by oxidative stress [46].

The effect of hexapeptide on carbohydrate metabolism was evaluated in a clinical study involving 123 patients (mean age 56.7 ± 5.1 years) with stable coronary heart disease (CHD) and metabolic syndrome. Of the 123 patients, 63 received standard CHD therapy (control group), and 60 patients in the main group were additionally prescribed tyrosyl-D-alanyl-glycyl-phenylalanyl-leucyl-arginine intranasally at a dose of 2 mg/day in courses of 10 days for 3 months.

As a result, patients receiving peptide therapy showed a significant decrease in initially elevated indicators: insulin level by 21.7 % (p < 0.001), C-peptide by 8.9% (p=0.017) and HOMA-IR index by 38.2 % (p < 0.001). No significant changes in carbohydrate metabolism were noted in the control group, while in the main group the positive effect persisted throughout the entire observation period.

Thus, intermittent intranasal administration of tyrosyl-D-alanyl-glycyl-phenylalanyl-leucyl-arginine in combination with standard therapy contributed to a decrease in hyperinsulinemia and insulin resistance in patients with CHD and metabolic syndrome, which confirmed its ability to correct carbohydrate metabolism disorders [49].

The ability of the synthetic analogue of leu-enkephalin to stabilize metabolic homeostasis by reducing oxidative stress and inhibiting pathological processes has also been demonstrated in liver diseases associated with toxic effects and metabolic disorders [50].

In cases accompanied by endothelial dysfunction (multiple organ failure syndrome, septic shock, ischemic stroke, myocardial infarction, ARDS), the peptide at a dose of 50–200 μg/kg (with intravenous administration) has been shown to inhibit the degradation of intercellular contacts of endothelial cells and prevent their apoptosis [51].

At the same time, the breakdown products of the drug can circulate in the blood for a long time, despite the fact that the half-life is on average 15 min. At the same time, endonasal administration of

peptide solutions entails direct penetration into the cerebrospinal fluid, bypassing the blood-brain barrier. Therefore, intranasal administration of tyrosyl-D-alanyl-glycyl-phenylalanyl-leucyl-arginine diacetate has been proven and is used to enhance the effect [52].

Therapy with hexapeptide diacetate has demonstrated significant efficacy in patients with COVID-19 complicated by moderate to severe ARDS. Against the background of treatment, a significant reduction in the frequency and duration of non-invasive ventilation (NIV) was noted. On the 6th day of therapy, the need for NIV was recorded 4.9 times less often (p = 0.020), and on the 7th day — 6.7 times less often (p = 0.013) compared with the control group.

In the main group receiving the leu-enkephalin analogue, positive dynamics according to CT studies were observed 3.2 times more often (p = 0.040) than in the control group. The data obtained indicate that the use of the drug affects the key pathogenetic mechanisms of ARDS development: it helps to reduce the area of lung tissue damage and increases the oxygenation index. These effects, most pronounced on the 4–7th day of therapy, probably caused positive clinical dynamics in patients [53].

Thus, the results of the pilot study demonstrated the high clinical efficacy of tyrosyl-D-alanyl-glycyl-phenylalanyl-leucyl-arginine diacetate for pathogenetic therapy of COVID-19.

As a result of the studies, pulmono-, cardio-, hepato-, gastro-, pancreato-, cerebroprotective, as well as antinociceptive effects have been proven. Further study of the role of neuropeptides and the opioid system proved to be promising for the development of new drugs based on synthetic OPs.

Studies of the leu-enkephalin analogue Ambervin® Pulmo during the COVID-19 pandemic

The long-term experience of successful use of tyrosyl-D-alanyl-glycyl-phenylalanyl-leucyl-arginine in various fields of medicine, supported by positive results of preclinical and clinical studies, became the basis for studying its organoprotective properties in the context of the COVID-19 pandemic. In this context, main attention was paid to the pulmonoprotective effect of this synthetic analogue of leu-enkephalin [54].

In 2021, the pharmaceutical company PROMOMED RUS developed the original medicine Ambervin® Pulmo, which for the first time implemented a rational combination of two biologically active compounds with proven efficacy — succinic acid salt and synthetic hexapeptide (tyrosyl-D-alanyl-glycyl-phenylalanyl-leucyl-arginine). The use of succinic acid (SA) in clinical practice dates back to 1972, when the Pharmcommittee of the Ministry of Health of the USSR issued a permit for its medical use. The ability of succinate to modulate energy metabolism due to participation in tissue respiration and oxidative phosphorylation processes, as well as to maintain ATP synthesis under hypoxia, underlies the wide therapeutic use of SA [55–58].

In the course of studies to assess the specific pharmacological activity of the Ambervin® Pulmo on the ARDS model with intravenous and combined intramuscular and inhalation administration to C57Bl mice, the pulmonoprotective effect was confirmed [59]. The results of determining the level of cytokines in the lung tissue showed a significant increase in the level of IL in the control group compared with other control groups, which indicates the development of a “cytokine storm” in animals of this group. The use of the Ambervin® Pulmo contributed to a significant (more than 2 times) decrease in the level of pro-inflammatory cytokines IL-1 and IL-6 in the lung tissue.

The results of histological analysis showed positive changes in the lung tissue with the introduction of the Ambervin® Pulmo, expressed in a moderate degree of interstitial edema, the absence of edematous fluid in the alveoli, bronchi and bronchioles.

Preclinical studies of tyrosyl-D-alanyl-glycyl-phenylalanyl-leucyl-arginine succinate demonstrated that intramuscular administration against the background of ARDS development reduced the area of spread of inflammatory infiltrate. Moreover, hexapeptide succinate almost 2 times more often increased the survival rate of animals compared with hexapeptide diacetate: the survival rate increased by 70 %, and in the comparison group — by 35 %.

Thus, as a result of experimental studies, it was shown that a pronounced anti-inflammatory effect with the introduction of tyrosyl-D-alanyl-glycyl-phenylalanyl-leucyl-arginine succinate is formed due to the suppression of the synthesis of one of the main pro-inflammatory mediators of the cytokine storm in the lungs — IL-6 and other pro-inflammatory cytokines (in particular IL-1β), and a simultaneous increase in the formation of the anti-inflammatory cytokine IL-10 [59]. It is possible that the effectiveness of tyrosyl-D-alanyl-glycyl-phenylalanyl-leucyl-arginine succinate

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is explained by the inclusion in the structure of the medicine, on the one hand, of a fragment of SA, which enhances its antioxidant properties, and on the other hand, of a δ-opioid receptor agonist hexapeptide, which suppresses the synthesis of pro-inflammatory cytokines in the lungs and inhibits the entry into the systemic circulation of one of the main mediators of the cytokine storm — IL-6.

A clinical study to assess the efficacy, safety and tolerability of intramuscular and inhalation use of hexapeptide succinate in complex therapy compared with standard therapy in patients with moderate COVID-19 was conducted on the basis of 10 research centers in Russia in 2022 and confirmed the results obtained at the preclinical stage [59].

Patients (*n* = 312) older than 18 years, hospitalized with moderate COVID-19, were randomized into 3 groups: the first received standard therapy in accordance with the current version of the Interim Guidelines of the Ministry of Health of Russia "Prevention, diagnosis and treatment of new coronavirus infection (COVID-19)" (hereinafter is Interim Guidelines) for 10 days; the second — hexapeptide succinate intramuscularly at 1 mg/day; the third — the same medicine by inhalation at 10 mg/day for 10 days. Therapy with hexapeptide succinate, both with intramuscular and inhalation administration, contributed to a reduction in the duration of the disease and the complete disappearance of symptoms of the disease in more than 80% of patients. The effectiveness of the medicine was also confirmed in comorbid patients. The use of the Ambervin® Pulmo was associated with the restoration of lung function, normalization of oxygenation, disappearance of shortness of breath, a decrease in the level of C-reactive protein below 10 mg/L and a reduction in the duration of symptoms compared with standard therapy.

There was also a decrease in indicators of inflammation (ESR, IL-6, d-dimer) and metabolic parameters (lactate, triglycerides). At the same time, there were no statistically significant differences in the frequency and nature of adverse events between the therapy groups [59].

Thus, the data obtained indicate that the use of tyrosyl-D-alanyl-glycyl-phenylalanyl-leucyl-arginine succinate in the complex therapy of COVID-19 in inhalation or intramuscular form in patients leads to a reduction in the duration and a decrease in the severity of symptoms of COVID-19. In addition to efficacy, the safety of hexapeptide succinate was shown, which confirms the feasibility of using the drug in schemes of pathogenetic therapy of COVID-19. In results the medicine was included in the Interim Guidelines [59].

Later, in a clinical study conducted by O.A. Radaeva et al. also studied the cytokine-mediated effects of Ambervin® Pulmo in the acute period of COVID-19 and in the convalescence period. Three groups of patients with COVID-19 were formed. In group 1 (*n* = 14), in addition to standard COVID-19 therapy, patients were injected intramuscularly with the medicine according to the scheme of 1 mg 1 per day for 10 days; group 2 (*n* = 13), in addition to standard COVID-19 therapy, received the Ambervin® Pulmo by inhalation at 10 mg 1 per day for 10 days; group 3 (*n* = 17) received standard therapy in accordance with the Interim Guidelines [60].

The reduction in the number of days of hospitalization in the groups receiving hexapeptide succinate and the increase in the level of IFN-β against the background of suppression of HMGB1 growth (a cytokine mediator associated with an unfavorable course of COVID-19) in the blood serum of patients with COVID-19 reflect positive dynamics, more pronounced with intramuscular administration of this analogue of leu-enkephalin [60].

It is known that a decrease in HMGB1 minimizes the risk of developing cardiovascular post-covid complications, while against the background of an increase in HMGB1, the synthesis of endothelial adhesion molecules increases and the barrier function of endothelial cells is impaired [39, 60]. A decrease in the level of HMGB1 is one of the most significant advantages of the drug based on hexapeptide succinate, which allows expanding the possibility of using this drug in the therapy of other diseases.

CONCLUSION

According to WHO experts, SARS-CoV-2 will continue to evolve, which will lead to the emergence of new variants of the virus with unpredictable properties. Despite previous immune experience (previous illness or vaccination), the risk of re-infection remains, including co-infection with various strains. In this regard, the search for new effective and safe pharmacological agents with anti-inflammatory and organoprotective activity remains relevant.

The organoprotective effect of hexapeptide

succinate established in preclinical and clinical studies, as well as its ability to correct disorders of antiviral immunity, allow us to consider OPs as promising compounds for the treatment of respiratory infections of the upper and lower respiratory tract of various etiologies (ARVI, pneumonia), post-infectious conditions (including post-covid syndrome) and other diseases of various etiologies. The prospects for their study are additionally confirmed by the involvement of OPs in the pathogenesis of a wide range of conditions — from mental (schizophrenia, addiction) and metabolic (obesity) disorders to somatic pathology, which opens up opportunities for the development of new therapeutic strategies in various fields of medicine. Thus, the creation of drugs based on regulatory peptides with a wide range of pharmacological activity is one of the most promising areas of pharmacology.

The pharmacological profile of hexapeptide succinate, including its clinical efficacy and safety, is confirmed by experimental and clinical studies. The combination of succinate with OP modulates the immune response, affecting the activity of macrophages and suppressing the production of pro-inflammatory cytokines. This manifestation enhances the anti-inflammatory activity of the compound and opens up prospects for its use in diseases such as rheumatoid arthritis and inflammatory bowel diseases.

In addition, succinate in the composition of the drug improves energy metabolism in neurons and provides protection against oxidative stress, which underlies the observed neuroprotective effects, including reducing anxiety and improving emotional state. Further controlled clinical trials are needed to confirm efficacy in neurodegenerative diseases, depression, chronic neuropathic pain, and cognitive impairment.

The synthetic analogue of leu-enkephalin-leucyl-arginine succinate also demonstrates a vasoprotective effect, which is realized through a decrease in the permeability of the vascular wall, normalization of microcirculation and stimulation of reparative processes in damaged tissues. The cardio- and pulmonoprotective properties of the compound allow us to consider it as a promising component of complex therapy of cardiovascular diseases and pneumonia of various etiologies, especially community-acquired.

The analysis revealed a number of limitations associated with the insufficient study of the molecular mechanisms of action of leu-enkephalin analogues, in particular, their selectivity for subtypes of δ-opioid receptors. In addition, there are no data on long-term follow-up of patients, which requires further research with a well-designed design and long-term monitoring of efficacy and safety.

Despite these limitations, the presented data demonstrate a favorable safety profile, proven efficacy and a wider therapeutic potential of hexapeptide succinate compared to the precursor drug. Promising directions for further research include a detailed study of its pharmacodynamics, expansion of indications for use and optimization of dosing regimens for the treatment of various diseases, primarily respiratory, especially community-acquired pneumonia.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

Khadizhat G. Omarova — writing the article, collecting and analysing information,
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REVIEW

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Compounding of Orphan Drugs as an indicator of systemic obstacles in pharmacy practice: the experience of the Russian Federation

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The aim. To identify existing obstacles hindering the introduction of extemporaneous orphan drugs into circulation in the Russian Federation, in order to develop recommendations for improving legislative regulation and organizing the activities of pharmacies with the right to manufacture drugs.

Materials and methods. The study was performed using a comprehensive approach, including logical, comparative, structural-functional, and conceptual analysis. Information retrieval was conducted in international scientific indexes, search engines (PubMed, Google Scholar), and legal reference systems (ConsultantPlus, GARANT, Kontur.Normativ). Regulatory legal acts of the Russian Federation and foreign countries, modern scientific publications, as well as the practical experience of Russian pharmacies manufacturing drugs for the treatment of orphan diseases were considered.

Results. The main obstacles in the development of pharmacy compounding of orphan drugs were identified: low availability of active pharmaceutical ingredients, limitation of the mechanism for establishing the shelf life of extemporaneous dosage forms, prohibition of the manufacture of registered drugs, heterogeneity of pharmacies in terms of equipment and competencies, lack of stable demand, restriction of dispensing compounded drugs for outpatient treatment, and uncertainty of requirements for the therapeutic effectiveness of extemporaneusly compounded drugs. Based on the analysis, proposals were formulated to improve the regulation of mechanisms for admitting substances to the market, using flexible approaches to establishing shelf life, differentiating requirements for pharmacies depending on their capabilities, and legally establishing the rights to manufacture certain categories of drugs, simplifying the dispensing of extemporaneous dosage forms, and involving federal and regional institutions in the formation of stable demand for pharmacy drugs.

Conclusion. Overcoming the identified obstacles is of strategic importance not only for the pharmacotherapy of orphan diseases, but also for the development of pharmacy compounding in general. The implementation of the proposed solutions will create a sustainable system capable of providing patients with vital medicines regardless of market conditions and the political situation. Global practice confirms that key competition in the field of orphan drugs unfolds precisely at the level of regulatory systems, and in this perspective, the development of manufacturing pharmacies can become a tool for the Russian Federation to protect the interests of patients, increase access to therapy, and strengthen drug sovereignty.

Keywords: drug supply; orphan medicines; emergency formulation; pharmacy compounding

Abbreviations: CC RF — Civil Code of the Russian Federation; SRMs — State Register of Medicines; GPhM — General Pharmacopoeial Monograph; FL — Federal Law; FDA — Food and Drug Administration.

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Изготовление орфанных лекарственных препаратов как индикатор системных барьеров в аптечной практике: опыт Российской Федерации

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Цель. Определить существующие барьеры, препятствующие введению в оборот экстемпоральных орфанных лекарственных препаратов на территории Российской Федерации, для выработки рекомендаций по совершенствованию законодательного регулирования и организации деятельности аптечных организаций с правом изготовления лекарственных препаратов.

Материалы и методы. Исследование выполнено с использованием комплексного подхода, включающего логический, сравнительный, структурно-функциональный и концептуальный анализ. Информационный поиск проводился в международных научных индексах, поисковых (Scopus, Web of Science, PubMed, Google Scholar) и справочно-правовых системах (КонсультантПлюс, ГАРАНТ, Контур.Норматив). Рассматривались нормативные правовые акты Российской Федерации и зарубежных стран, современные научные публикации, а также практический опыт российских аптечных организаций, изготавливающих лекарственные препараты для лечения орфанных заболеваний.

Результаты. Выявлены основные барьеры развития аптечного изготовления орфанных препаратов: низкая доступность активных фармацевтических субстанций, ограничение механизма установления сроков годности экстемпоральных лекарственных форм, запрет на изготовление зарегистрированных препаратов, неоднородность аптечных организаций по уровню оснащённости и компетенций, отсутствие устойчивого спроса, ограничение отпуска изготовленных лекарственных препаратов для амбулаторного лечения, а также неопределённость требований к терапевтической эффективности экстемпоральных аналогов промышленных лекарственных препаратов. На основе анализа сформулированы предложения по совершенствованию регулирования механизмов допуска субстанций на рынок, использования гибких подходов к установлению сроков годности, дифференциации требований к аптечным организациям в зависимости от их возможностей и законодательному закреплению прав на изготовление определённых категорий препаратов, упрощению отпуска экстемпоральных лекарственных форм, а также вовлечению федеральных и региональных институтов в формирование устойчивого спроса на аптечные лекарственные препараты.

Заключение. Преодоление выявленных барьеров имеет стратегическое значение не только для фармакотерапии орфанных заболеваний, но и для развития аптечного изготовления в целом. Внедрение предложенных решений позволит выстроить устойчивую систему, способную обеспечивать пациентов жизненно необходимыми лекарственными препаратами независимо от конъюнктуры рынка и политической ситуации. Мировая практика подтверждает, что ключевая конкуренция в сфере орфанных препаратов разворачивается именно на уровне регуляторных систем, и в этой перспективе развитие производственных аптек может стать для Российской Федерации инструментом защиты интересов пациентов, повышения доступности терапии и укрепления лекарственного суверенитета.

Ключевые слова: лекарственное обеспечение; орфанные лекарственные препараты; экстемпоральная рецептура; аптечное изготовление

Список сокращений: ГК РФ — Гражданский кодекс Российской Федерации; ГРЛС — Государственный реестр лекарственных средств; ОФС — общая фармакопейная статья; ФЗ — Федеральный закон; FDA — Управление по контролю качества пищевых продуктов и лекарственных средств, США.

INTRODUCTION

In recent years, the Russian Federation has seen a consistent development of regulatory legal regulation regarding the organization of extemporaneous compounding of medicines [1]. State policy in the field of healthcare increasingly views pharmacies with the right to compound medicines (hereinafter referred to as compounding pharmacies) not only as a resource for implementing personalized medicine tasks, but also as an effective tool for ensuring the accessibility of

pharmacotherapy [2–4]. Strengthening the regulatory legal framework, gradually adapting international principles of good pharmacy practice, separation of a special type of economic activity[1], implementation of requirements for the organization of a quality system[2], development and approval of new pharmacopoeial monographs[3], form the basis for the transformation of compounding pharmacies [5]. From a rudimentary and fragmented structure, they are turning into a full-fledged patient-oriented link in the drug supply system, capable of responding quickly to healthcare needs both to expand pharmacotherapy options and to rationally use resources [6–9].

At the same time, despite the significant potential, the still limited range and low profitability of compounded medicines determine the need to search for new directions for using extemporaneous drug formulations [10–13].

Given the incoming investments, the introduction of new high-tech approaches in pharmacy practice, and integration with the industrial sector of the pharmacy, the most promising area is the compounding of medicines with high potential in the therapy of orphan diseases[4].

Pharmacotherapy for patients with orphan diseases is a global problem, primarily due to the complexity of developing medicines, including difficulties in conducting full-fledged clinical trials, as well as a limited market [14]. In the Russian Federation, orphan diseases are diseases with a prevalence of no more than 10 cases per 100 000 population[5]. The situation differs in the European Union[6] (no more than 5 cases per 10 000 people) and the USA[7] (any disease or condition affecting less than 200 000 people in the USA), but even in these conditions, the market remains small [15]. As a result, healthcare systems are forced to develop and implement various incentive programs, which in most cases provide pharmaceutical companies with simplified market access, exclusive status, and also allow them to maintain a high price, often not determined by market, especially in the case of long-known medicines that were subsequently registered for the treatment of orphan diseases [16–19].

In the Russian Federation, there is a steady increase in the number of patients with orphan diseases receiving pharmacotherapy under state programs [20]. Thus, the Federal Program “High-Cost Nosologies” is gradually expanding, and the list of drugs purchased by the Circle of Good Foundation for Supporting Children with Severe Life-Threatening and Chronic Diseases, including Rare (Orphan) Diseases (hereinafter referred to as the Circle of Good[8]), is increasing. The taken measures allow covering a large list of patients, but also cause a rapid increase in the total costs of orphan drugs. Thus, the volume of federal funding for orphan programs has increased many times over the past years, and the total number of patients receiving therapy has increased many times over. As a result, all key sources demonstrate a single trend of increasing burden on drug provision for patients with rare diseases [21].

Simultaneously with the growth in demand, the limited resources of the healthcare system become obvious. Federal spending on orphan drugs today amounts to tens of billions of rubles annually, and regional budgets are often unable to fully cover the need for expensive treatment outside of centralized programs[9]. In practice, patients who do not fall under federal benefits (for example, the adult group of orphan patients) often face interruptions in the provision of

[1] Приказ Росстандарта от 09 апреля 2025 года № 268-ст «Об утверждении Изменения 80/2025 ОКВЭД 2 к Общероссийскому классификатору видов экономической деятельности». – [Электронный ресурс]. – Режим доступа: https://www.consultant.ru/document/cons_doc_LAW_503144/

[2] Order of the Ministry of Health of the Russian Federation dated May 22, 2023 No. 249n “On Approval of the Rules for the Compounding and Release of Medicines for Medical Use by Pharmacies Licensed for pharmaceutical activities”. Available from: https://www.consultant.ru/document/cons_doc_LAW_448335/

[3] Order of the Ministry of Health of the Russian Federation dated March 13, 2024 No. 120 “On Approval of General Pharmacopoeial Monographs and Pharmacopoeial Monographs”, Order of the Ministry of Health of the Russian Federation dated April 11, 2025 No. 188 “On Approval of General Pharmacopoeial Monographs and Pharmacopoeial Monographs”, Order of the Ministry of Health of the Russian Federation dated August 4, 2025 No. 460 “On Approval of Pharmacopoeial Monographs”. Available from: https://pharmacopoeia.regmed.ru/ approval-orders/

[4] Badyina E. A production pharmacy will be built in Sirius for 1 billion rubles – what is known about it? Pharmedprom. Available from: https://pharmmedprom. ru/Monographs/chem-privlech-molodezh-na-predpriyatie-farmkompanii-podelilis-sekretami/. Russian

[5] Federal Law No. 323-FZ of November 21, 2011 “On the Basics of Public Health Protection in the Russian Federation”. Available from: https://www.consultant.ru/document/cons_doc_LAW_121895/bbfb814a127237db75b90e15 4333ef3f085f4e7f/

[6] Orphan medicinal products. European Commission. – [Электронный ресурс]. – Режим доступа: https://health.ec.europa.eu/medicinalproducts/orphan-medicinal-products_en

[7] 21 U.S.C. § 360bb (Orphan Drug Act). – [Электронный ресурс]. – Режим доступа: https://www.law.cornell.edu/uscode/text/21/360bb

[8] Circle of Goodness Foundation for the Support of Children with Severe life-threatening and Chronic Diseases. public 2024 annual report. Available from: https://xn--80abfdb8athfre5ah.xn--p1ai/ wp-content/uploads/2025/05/15-may-AnnualReport-2024-RGB-2.pdf

[9] Kryukov V., Surinskaya Ya., Lekovskaya V. The lack of regional funds for the treatment of orphan diseases will be compensated by the federal budget. Vedomosti. Available from: https://www.vedomosti.ru/society/Monographs/2025/03/11/1097178-nehvatku-regionalnih-sredstv-na-lechenie-orfannih-boleznei-vospolnyat-fedbyudzhetom. Russian

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medicines and are forced to "seek" drugs through additional support mechanisms. Experts note that with the increase in the number of patients who have reached the age of 19 and are "graduating" from the care of the Circle of Good Foundation, the obligations of the regions to finance their further treatment are increasing — a burden that regional budgets can hardly bear[10]. Thus, the existing funding model is working at the limit of its capabilities, which emphasizes the need to introduce new approaches to drug provision for orphan patients.

In the context of limited resources, flexible, technology-oriented solutions, such as extemporaneous compounding of medicines, are of particular relevance [22–24]. At the same time, despite the introduction of modern technologies in pharmacy practice and the improvement of instrumental methods for quality control of extemporaneous drug formulations [25, 26], the first attempts to compound orphan drugs in the Russian Federation faced a number of diverse obstacles, limiting not only the development of the compounding pharmacy system, but also its existence.

THE AIM of this review is to identify existing barriers that prevent the introduction of extemporaneous orphan drugs into circulation in the Russian Federation, in order to develop recommendations for improving legislative regulation and organizing the activities of compounding pharmacies.

MATERIALS AND METHODS

The information search was conducted (2005–2025) throughout the entire period from December 2022 to July 2025 in international scientific indexes and search engines (Scopus, Web of Science, PubMed, Google Scholar), as well as using national reference legal systems (ConsultantPlus, GARANT system, "Kontur.Normativ") and international resources (official portal of the legislation of the European Union, official portal of the legislation of Germany, library of law of Cornell University, official website of the Ministry of Health of Canada, official websites of the European Medicines Agency, the Food and Drug Administration of the United States and the World Health Organization). In the context of limited access to a number of foreign databases after 2022, previously formed collections and open sources listed above were used.

The search was carried out using keywords and phrases in Russian and English, including: «орфанные лекарственные препараты», «редкие заболевания», «аптечное изготовление», «экстемпоральное изготовление», as well as their English equivalents — orphan drugs, rare diseases, pharmacy compounding, extemporaneously compounding drugs, regulatory framework for compounding. The selection of sources was carried out in several stages: removal of duplicates, exclusion of irrelevant publications based on titles and abstracts, subsequent full-text analysis. Articles devoted to orphan drugs, therapy of rare diseases and issues of pharmacy compounding were included. Priority was given to modern peer-reviewed publications and official regulatory documents. As a result, an array of sources was formed, including 50 scientific articles, 29 regulatory legal acts and 13 other relevant materials. The process of selecting sources is presented in a block diagram (Fig. 1), prepared in accordance with the recommendations of PRISMA.

The processing and analysis of the collected data included systematization of information and comparison of the identified information with international practice. The applicability of foreign experience to the Russian regulatory organizational environment was assessed. During the analysis, key problems were identified that hinder the development of pharmacy compounding of orphan drugs in the Russian Federation.

In addition to the analysis of literature and documents, the practical experience of pharmacy compounding in Russia was studied as part of the empirical part of the study. Applied aspects of the organization of extemporaneously compounding drugs were analyzed, including issues of importing the necessary pharmaceutical substances, building logistics supply chains, as well as internal preparation of compounding pharmacies for compounding and ensuring the quality of finished dosage forms.

Based on the results obtained, proposals were developed to improve the regulatory framework and practice of pharmacy compounding of orphan drugs. These recommendations and approaches are formulated taking into account the specifics of the legislation of the Russian Federation.

RESULTS AND DISCUSSION

Availability of substances

The problem of the lack of substances that can be used for the purposes of pharmacy compounding

[10] Nevinnaya I. Experts discussed how to provide treatment to "graduates" of the Circle of Goodness Foundation. Rossiyskaya Gazeta. Available from: https://rg.ru/2024/08/09/ eksperty-obsudili-kak-obespechit-lecheniem-vypusknikov-fonda-krug-dobra.html. Russian

is fundamental [27, 28]. Thus, in accordance with Art. 56 of Federal Law No. 61-FZ[11] of April 12, 2010, as well as Order No. 249n of the Ministry of Health of the Russian Federation dated May 22, 2023 "On approval of the rules for compounding and dispensing medicines for medical use by pharmacies licensed for pharmaceutical activities"[12] (hereinafter — Order No. 249n) it is established that in the compounding of drugs by pharmacies, and (or) pharmaceutical substances included in the State Register of Medicines for medical use (hereinafter — SRMs), the Unified Register of Registered Medicines of the Eurasian Economic Union are used.

Art. 34 of Federal Law No. 61-FZ establishes the procedure for including a pharmaceutical substance produced for sale in the SRMs, which provides for a complex and expensive procedure (Fig. 2), including the certification of the substance manufacturer for compliance with international standards, as well as a wide range of laboratory tests. Thus, few compounding pharmacies are able to initiate the inclusion of a pharmaceutical substance of interest to them in the SRMs.

It is advisable to provide for alternative mechanisms for the admission of substances, including those existing in international practice. For example, in Canada, in accordance with Health Canada POL-0051[13], a medicinal product in a compounding pharmacy must be compounded from a registered drug or active pharmaceutical substance already used in a registered medicinal product, or an active pharmaceutical substance for which there is a pharmacopoeial monograph in a pharmacopoeia recognized by Canada[14] (US Pharmacopoeia, European Pharmacopoeia, British Pharmacopoeia, etc.).

In the Russian Federation, information about the substance used in industrial production is also entered into the SRMs as part of the state registration of the corresponding medicinal product. Thus, it can be established that compounding pharmacies have the right to use substances both included in the SRMs.

Another solution implemented in Australia, as well as in a number of European Union countries, including Germany[15] and the Netherlands[16], is to allow compounding pharmacies to use all substances whose quality complies with the Pharmacopoeia, and the manufacturer guarantees compliance with good manufacturing practice standards, which is confirmed by a certificate of conformity, and not an on-site inspection at the manufacturer's site at the expense of the compounding pharmacy [29].

In the case of active pharmaceutical substances used in the therapy of orphan diseases, the situation is also complicated by the fact that most of the necessary and relevant substances are patented [30].

In A.V. Alekhin et al. [2] showed that the violation of patent rights occurs precisely at the moment of transfer of the substance from the supplier to the compounding pharmacy. Thus, in a situation where it is impossible for a compounding pharmacy to legally obtain a substance, the rule of law (clause 5 of Article 1359 of the Civil Code of the Russian Federation[17], containing an exception from patent law in the case of a one-time compounding in pharmacies according to doctors' prescriptions of medicines, including orphan drugs, which include patented substances), — does not actually work.

At the same time, this provision of the Civil Code of the Russian Federation corresponds to international practice, is conceptually formulated and enshrined in the legislation of more than 30 states — members of the World Intellectual Property Organization[18], and is key to ensuring the patient's right to receive the necessary pharmacotherapy in the absence of registered industrial analogues or the presence of medical indications for prescribing an extemporaneously compounded drug.

[11] Federal Law No. 61-FZ of April 12, 2010 "On the Circulation of Medicines". Available from: https://www.consultant.ru/document/cons_doc_ LAW_99350/. Russian

12 Order No. 249n of the Ministry of Health of the Russian Federation dated May 7, 2023 "On Approval of the Rules for the Manufacture and Release of Medicines for Medical Use by Pharmacy Organizations Licensed for Pharmaceutical Activities". Available from: https://normativ.kontur.ru/document?moduleId=1&documentId=449637. Russian

[13] Policy on Manufacturing and Compounding Drug Products in Canada. Available from: https://www.canada. ca/en/health-canada/services/drugs-health-products/compliance-enforcement/good-manufacturing-practices/guidance-documents/ policy-manufacturing-compounding-drug-products.html

[14] Food and Drugs Act (R.S.C., 1985, c. F-27). Available from: https://laws-lois.justice.gc.ca/eng/ acts/f-27/page-11.html

[15] Verordnung über den Betrieb von Apotheken (Apothekenbetriebsordnung – ApBetrO): Verordnung vom 09.02.1987 (BGBl. I S. 547); neugefasst durch Bek. v. 26.09.1995 (BGBl. I S. 1195); zuletzt geändert durch Art. 8z4 G. v. 12.12.2023 (BGBl. 2023 I Nr. 359). Available from: https://www.gesetze-iminternet.de/apobetro_1987/

[16] Inspectie Gezondheidszorg en Jeugd (IGJ). Eigen bereidingen apotheek. Available from: https://www.igj.nl/zorgsectoren/geneesmiddelen/beschikbaarheid-vangeneesmiddelen/eigen-bereidingen-apotheek

[17] The Civil Code of the Russian Federation. Available from: https://www.consultant.ru/document/cons_doc_LAW_64629/11c74717682795e6ef099015bbcba71081a5b918/

[18] Committee on the Law of Patents. Draft Reference Document on the Exception Regarding Extemporaneous Preparation of Medicines: SCP/36/3. Available from: https://www. wipo.int/edocs/mdocs/scp/en/scp_36/scp_36_3.pdf

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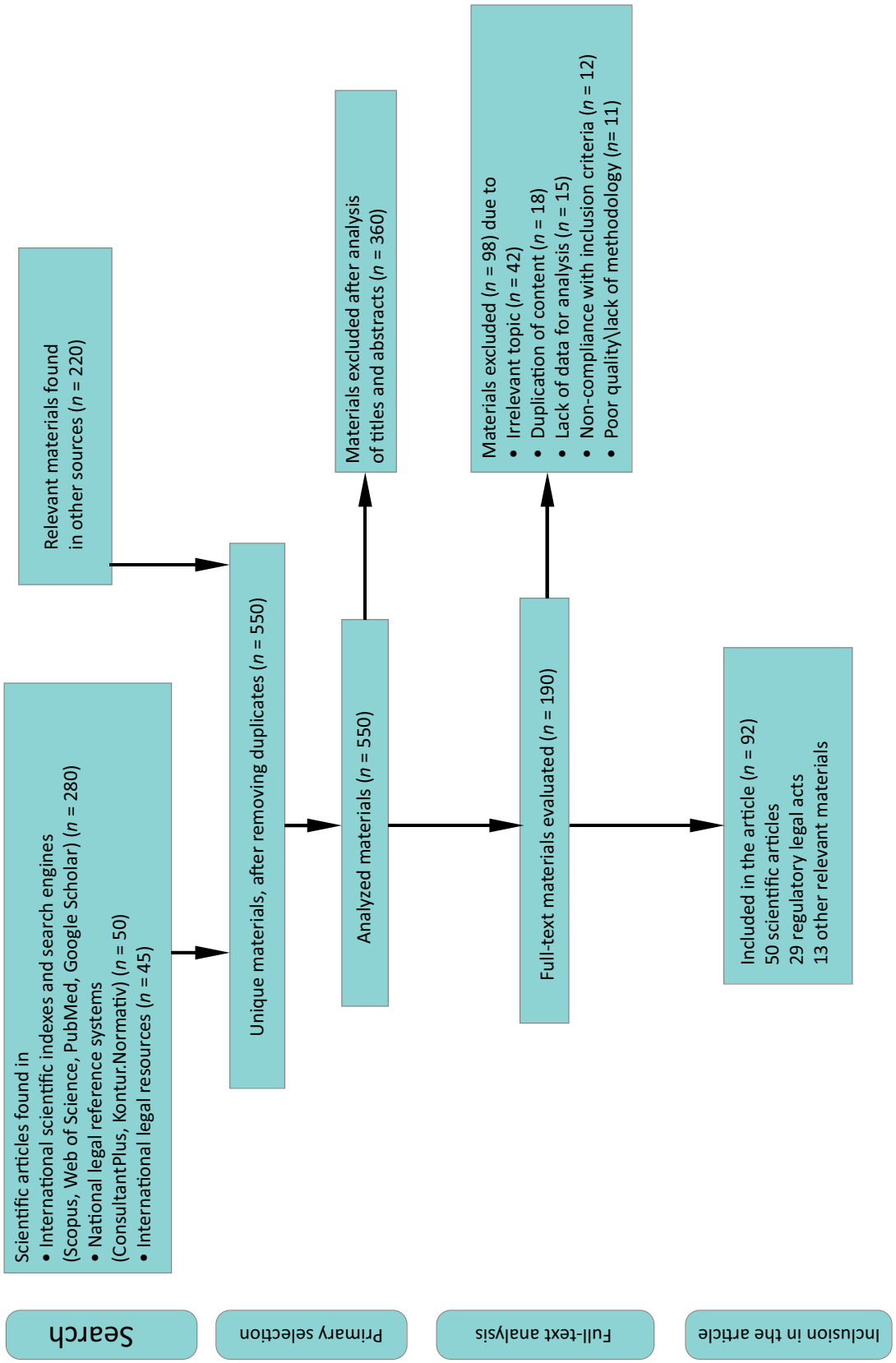

Figure 1 – Block diagram of source selection.

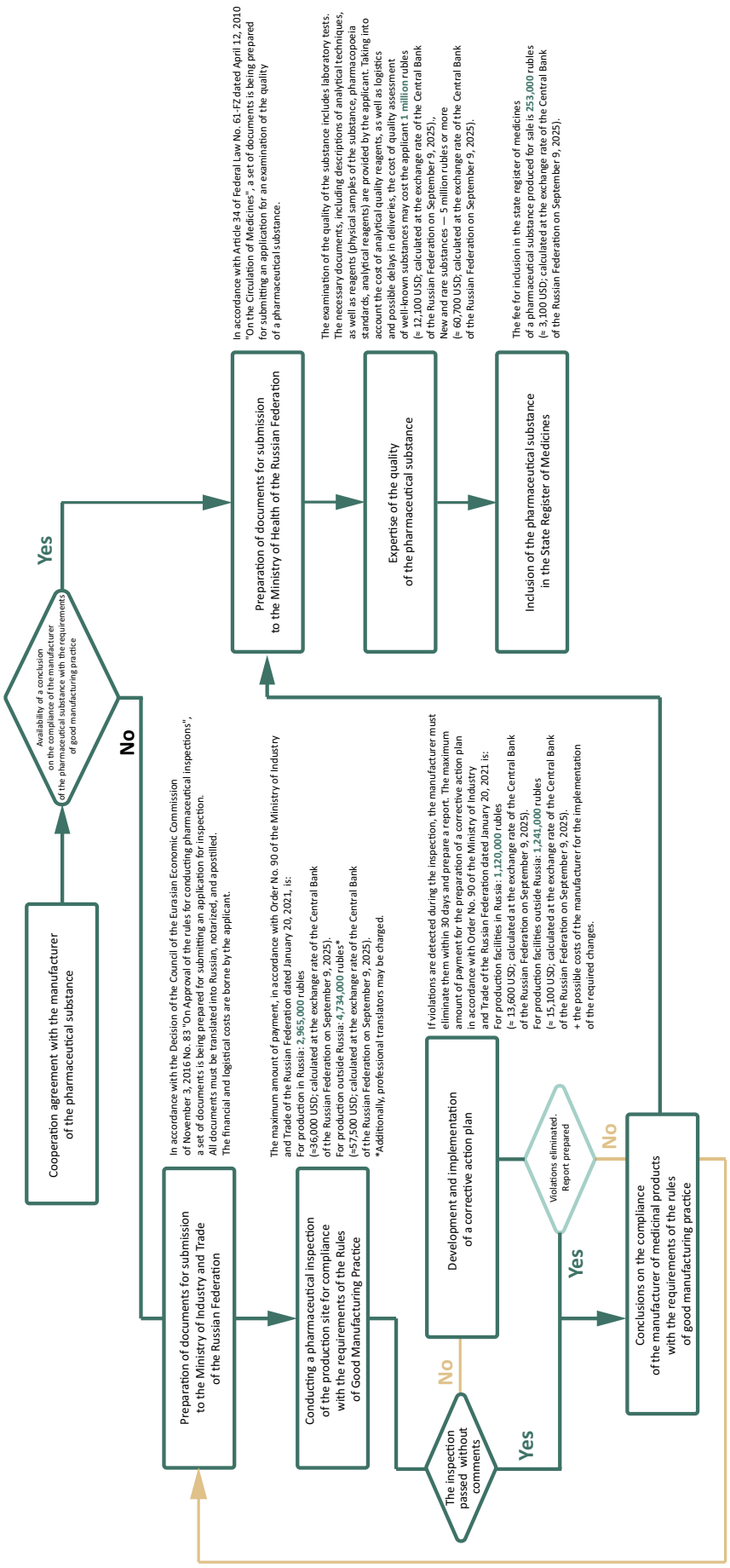

Figure 2 – Procedure for including a pharmaceutical substance in the State Register of Medicines of the Russian Federation.

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In these conditions, the statement that compounding pharmacies can solve the problem of periodically occurring shortages, often put forward in support of the development of the compounding pharmacy system, is untenable[19]. Even if the supplier has a substance, compounding pharmacies will not be able to solve the problem of a sudden shortage due to the effect of patent law, the inability to quickly include substances in the SRMs, as well as the prohibition on compounding registered medicines.

Therefore, the solution to this problem with the availability of substances should be comprehensive and not limited to expanding the substances available for pharmacy compounding. Thus, it seems appropriate to provide for an exception in Art. 56 of Federal Law No. 61-FZ, allowing the compounding of registered medicines in cases determined by the Government of the Russian Federation.

Such a legislative norm does not violate the balance between industrial production and pharmacy compounding, but gives the state a regulatory lever, both to combat shortages and to curb manufacturers' prices. International experience shows the high efficiency of such a solution.

For example, in the United States, a similar mechanism is implemented when a medicinal product is included in the official list of deficient drugs (Drug Shortages List), compounding pharmacies are allowed to compound the corresponding registered medicinal product[20]. At the same time, the use of substances that are not included in the list of compounds allowed for extemporaneous compounding (Bulks List), formed by the Food and Drug Administration (hereinafter — FDA) is allowed[21]. In the event of the end of the shortage, compounding must be stopped, and the dispensing of previously compounded drugs is possible for another 60 days after the exclusion of the medicinal product from the list of deficient drugs. Thus, during the COVID-19 pandemic, this norm made it possible to provide critically important drugs (including sedatives) to hospitalized patients[22]. Also, in March 2022, the FDA included semaglutide in the list of deficient drugs, which allowed American compounding pharmacies to legally prepare semaglutide injection solutions from the original substance, even despite the patent protection of the original drug. In early 2025, the FDA announced the resolution of the semaglutide shortage and notified compounding pharmacies of the termination of the authorization to compound and dispense the pharmacy analogue of semaglutide within 60 and 90 days for compounding pharmacies of type 503A and 503B, respectively[23].

Limitation of the shelf-life establishment mechanism

Another obstacle to the development of pharmacy compounding of orphan drugs is the issue of short-shelf lives of extemporaneous dosage forms. The standard shelf lives approved by Order No. 249n do not allow ensuring the proper "uninterrupted" receipt of medicines by patients. Moreover, they practically exclude the use of extemporaneous forms by маломобильными and lonely citizens. It is also impractical to deliver extemporaneous medicines to regions of the Russian Federation remote from the compounding pharmacy. To solve this problem, GPhM.1.8.0008 "Stability and shelf life of medicines compounded in pharmacies"[24] (hereinafter — GPhM.8.0008) was developed and approved, which provides for a mechanism for increasing shelf life based on stability testing of a medicinal product compounded in accordance with the approved intra-pharmacy standard operating procedure.

It should be noted that stability testing of a medicinal product in accordance with GPhM.1.8.0008 is an expensive procedure. Thus, studying the stability of an external dosage form for the purpose of establishing a shelf-life of 1 year in an accredited federal-level laboratory in 2025 will cost 250 thousand rubles

[19] Pichugina E. The production of drugs in pharmacies has become endangered. Moskovsky Komsomolets. Available from: https://www.mk.ru/economics/2023/06/16/proizvodstvo-preparatov-v-aptekakhokazalos-pod-ugrozoy-ischeznoveniya.html. Russian

[20] U.S. Food & Drug Administration. Compounding when Drugs are on FDA's Drug Shortages List. Available from: https://www.fda.gov/drugs/human-drug-compounding/compounding-when-drugs-are-fdas-drug-shortages-list

[21] Compounding under Section 503B of the FD&C Act. Available from: https://www.fda.gov/drugs/humandrug-compounding/bulk-drug-substances-used-compounding-undersection-503b-fdc-act

[22] U.S. Food & Drug Administration. Temporary Policy for Compounding of Certain Drugs for Hospitalized Patients by Outsourcing Facilities During the COVID-19 Public Health Emergency (Guidance for Industry). Available from: https://downloads. regulations.gov/FDA-2020-D-1136-0011/attachment_1.pdf

[23] U.S. Food & Drug Administration. FDA clarifies policies for compounders as national GLP-1 supply begins to stabilize. Available from: https://www.fda. gov/drugs/drug-safety-and-availability/fda-clarifies-policies-compounders-national-glp-1-supply-begins-stabilize

[24] Order of the Ministry of Health of the Russian Federation dated May 6, 2024 No. 233 "On Approval of the General Pharmacopoeial Article and Amendments to the Order of the Ministry of Health of the Russian Federation dated March 13, 2024 No. 120 "On Approval of General Pharmacopoeial Monographs and Pharmacopoeial Monographs"". Available from: https://base.garant.ru/409036856/

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(≈ 3 000 USD; calculated at the rate of the Central Bank of the Russian Federation (hereinafter — the Central Bank of the Russian Federation) on September 9, 2025). These tests involve long-term studies under controlled conditions, which requires specialized equipment, highly qualified personnel and significant resources, so this price is justified and corresponds to the market. However, conducting such a scientific study does not guarantee that the corresponding shelf life can be established.

At the same time, the most negative thing in this situation is that stability tests are carried out strictly for a specific formulation, compounded in accordance with the intra-pharmacy standard operating procedure. Any deviation from the original formulation, including a change in dosage, list of excipients or technology, can be considered as the compounding of a new medicinal product, which makes the results of stability tests and the established shelf life inapplicable. As a result, there is a need for new stability tests, which makes it impossible to respond flexibly to the individual needs of patients. Thus, the very essence of pharmacy compounding, namely ensuring the personalization of pharmacotherapy, contradicts the logic of determining the stability of a medicinal product in accordance with OFS.1.8.0008.

To overcome this barrier associated with stability testing and shelf-life restrictions, it is necessary to develop flexible regulatory mechanisms that would take into account the specifics of pharmacy compounding and its focus on individualizing pharmacotherapy. One of the promising approaches is the possibility of conducting stability tests not for one fixed formulation, but for a range of dosages and compositions. This approach will allow compounding pharmacies to obtain data applicable to a whole range of individualized medicines, and not just to one formulation, which will significantly expand the possibilities of personalization and reduce the cost of conducting commercial research.

The implementation of this solution can be achieved by introducing relevant provisions into OFS.1.8.0008. In particular, the pharmacopoeial monograph should provide for the possibility of indicating the permissible range of concentrations of the active substance and the main characteristics of the drug, within which stability tests will be recognized as valid. This approach will allow compounding pharmacies to conduct stability tests for a whole class of formulations, ensuring high quality and safety of extemporaneous medicines, as well as the flexibility of their use in clinical practice.

In addition, it is necessary to consider the possibility of determining a list of organizations authorized to conduct stability tests of extemporaneous dosage forms or provide for liability for the establishment by compounding pharmacies of shelf lives based on false results of stability tests.

Registration of a medicinal product — prohibition on compounding

The prohibition on compounding registered medicines by compounding pharmacies is an international norm. In the Russian Federation, it is enshrined in Art. 56 of Federal Law No. 61-FZ.

At the same time, this norm is considered exclusively as the inadmissibility of reproducing an already registered medicinal product by a compounding pharmacy. However, the situation is often the opposite, and it is the industrial manufacturer who registers a medicinal product already compounded by the pharmacy [31].

In a mini-review, K. Hendrickx and M. Dooms, for example, provide a list of well-known compounds traditionally used as food additives, but which have been granted the status of medicinal products for the treatment of orphan diseases [32]. At the same time, the authors argue that these compounds were discovered long ago and are “international property”, and the owners of registration certificates appropriated them and maintain a monopolistically high price, without making a significant innovative contribution, using open medical and scientific data. Moreover, it is indicated that many of these drugs were previously compounded in compounding pharmacies and cost many times less.

A classic example of such a situation was the case of the medicine Makena® (INN — hydroxyprogesterone caproate), intended for the prevention of premature birth. An analogue of this medicinal product was compounded in compounding pharmacies for many years according to prescriptions for women at risk and cost several tens of dollars per dose. However, after KV Pharmaceuticals received FDA approval for the medicine Makena® with the assignment of orphan status and exclusivity for 7 years, the pharmacy analogue turned out to be вне закона. The price of the original drug rose to 1500 dollars (≈ 125 thousand rubles; calculated at the rate of the Central Bank of the Russian Federation on September 9, 2025) per injection, which increased the cost of the course to 30 thousand dollars (≈ 2.5 million rubles; calculated at

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the rate of the Central Bank of the Russian Federation on September 9, 2025) [33]. Due to the wide public outcry, the FDA announced in March 2011 that it would not take action against compounding pharmacies that continue to compound an analogue of the medicinal product Makena®[25] according to a doctor's prescription. KV Pharmaceuticals' appeal to the court and accusation of the FDA of exceeding its authority were unsuccessful[26], and the precedent became an example of how a regulator can weaken barriers to protect patients and reduce economic costs [34].

The European Union is also discussing a reform of pharmaceutical legislation affecting orphan drugs[27]. The proposals of the European Commission and the European Parliament do not directly mention pharmacy compounding, but there is an idea of differentiated exclusivity for orphan drugs. In particular, it is planned to reduce the period of exclusivity to 4 years for drugs approved on the basis of already known bibliographic data. Thus, if a company registers an orphan drug based on existing published information without significant new research, it will receive not 10 years of protection, but significantly less. If this change is adopted, the period during which a pharmaceutical company can maintain a high monopoly price will decrease. Indirectly, this will legalize the earlier appearance of inexpensive alternatives.

In the Russian Federation, there is also a risk that investments and intellectual resources invested by compounding pharmacies in the development of medicines for the treatment of orphan diseases, the inclusion of relevant substances in the SRMs, the development of technology, the preparation of internal standard operating procedures, as well as the study of stability, may be used by large pharmaceutical companies.

In this case, it is advisable to provide by law a norm according to which the publication of a private pharmacopoeial monograph on an extemporaneous medicinal product secures the right of compounding pharmacies to compound it, even in the case of subsequent registration of an industrial analogue. This will avoid a situation where large pharmaceutical companies will use the developments of compounding pharmacies to register a drug, thereby effectively blocking the possibility of its compounding, as in the case of the medicinal product Makena®. Such legislative consolidation will protect the investments of compounding pharmacies in the development and testing of drugs, create a fairer competitive environment and ensure long-term availability of orphan drugs for patients.

Heterogeneity of compounding pharmacies

A key challenge for the system of pharmacy compounding of orphan drugs is the significant heterogeneity of compounding pharmacies in terms of equipment level, competencies and the ability to ensure quality [35]. In the Russian Federation, there are and are preparing to launch compounding pharmacies that have modern equipment, qualified personnel and are able to fulfill all the requirements for the compounding of medicines up to compliance with good manufacturing practice standards and the introduction[28] of advanced developments [36–39]. At the same time, a significant part of compounding pharmacies is limited in technical capabilities, experiences a shortage of personnel and does not have a well-established quality assurance system, which may affect the safety and effectiveness of the dosage forms they compound [40–42].

The complexity of the situation is aggravated by the fact that in the current legal field there is no differentiation of compounding pharmacies according to the level of their technological and organizational capabilities. As a result, the same regulatory requirements apply to compounding pharmacies at different levels of development. Such a uniform regulatory system, which does not take into account the real differences between compounding pharmacies, can create risks for the health of patients, as well as be used by interested parties to discredit pharmacy compounding in general. In particular, cases of unfair practice or insufficient control in compounding pharmacies with a low level of equipment can form a negative public opinion and be used by opponents of pharmacy compounding, including from large pharmaceutical manufacturers.

[25] U.S. Food & Drug Administration. Makena (hydroxyprogesterone caproate) – information page (история одобрения и последующее изъятие). Available from: https:// www.fda.gov/drugs/postmarket-drug-safety-information-patients-and-providers/makena-hydroxyprogesterone-caproate-injection-information

[26] K-V Pharmaceutical Company et al. v. FDA et al. Memorandum Opinion, 06.09.2012. United States District Court for the District of Columbia, No. 1:12-cv-01105 (ABJ). Available from: https://law.justia.com/cases/federal/district-courts/ district-of-columbia/dcdce/1%3A2012cv01105/155076/23/

[27] European Parliament. Parliament adopts its position on EU pharmaceutical reform. Available from: https://www.europarl.europa.eu/news/en/press-room/20240408IPR20308/parliament-adopts-its-positionon-eu-pharmaceutical-reform

[28] Sirius scientists have developed manufacturing technologies for three orphan disease drugs. Available from: https://sirius-ft.ru/tpost/uchenye-siriusa-razrabotali-tekhnologii-izgotovleniya-trekh-preparatov-ot-orfannykh-zabolevaniy. Russian

Thus, in order to ensure the safety of patients and increase confidence in pharmacy compounding, including orphan drugs, it is advisable to develop a system for classifying compounding pharmacies, taking into account both the level of their technical and personnel equipment, and the categories of medicines compounded. This approach will allow establishing reasonable and differentiated requirements for compounding pharmacies depending on their capabilities and goals, minimizing risks for patients and ensuring a fairer regulatory burden.

As an example, we can cite the experience of the United States of America, where a three-level system of regulation of pharmacy compounding has developed [43]. Ordinary retail pharmacies, although focused on dispensing finished medicines, retain the right to compound. With the exception of a number of states[29], it is limited to simple non-sterile forms that are compounded "under a prescription" from a doctor. More opportunities are provided to compounding pharmacies of category 503A[30, 31], which are authorized to compound a wide range of dosage forms, including sterile medicines (injectable drugs, eye drops, etc.). Their activities are regulated at the state level and must comply with the requirements of the pharmacopoeia. Such pharmacies have become a key link in providing patients with personalized medicines.

The next level is pharmacies of type 503B[32], which are an intermediate link between a pharmacy and a pharmaceutical production: they can produce medicines in series, without a doctor's prescription, mainly for medical organizations. At the same time, they are obliged to comply with good manufacturing practice standards and are under the direct control of the FDA.

This model demonstrates an institutionalized separation of functions: from limited individual compounding in retail pharmacies to full-fledged serial production for medical organizations [44].

Not identical, but a similar approach can be implemented in the Russian Federation within the framework of Decree of the Government of the Russian Federation No. 547 dated March 31, 2022 "On approval of the Regulations on licensing pharmaceutical activities"[33] and Order of the Ministry of Health of the Russian Federation No. 780n dated July 31, 2020 "On approval of the types of pharmacies"[34], which will allow more flexibly regulating the activities of compounding pharmacies. Order No. 249n may enshrine the requirements for different types of compounding pharmacies. Thus, for organizations with a minimum technological base that do not claim to compound innovative medicines, including those of protein, cellular or nucleotide nature, minimum requirements may be established. In the case of compounding pharmacies with modern equipment and the appropriate infrastructure, provide for stricter requirements for the organization of the quality assurance system and staffing, but allow the use of the existing high-tech potential, expanding the possibilities for compounding complex personalized medicines.

Thus, the development of a system for differentiating compounding pharmacies, taking into account their equipment and competencies, seems to be an important step for improving the safety and sustainability of pharmacy compounding, as well as for forming a more fair and effective regulatory system.

Low demand

The lack of a stable sales market and formed demand is also one of the most significant barriers to realizing the potential of pharmacy compounding of orphan drugs. In the context of the current regulation, compounding pharmacies, even with the necessary production capacities and quality assurance capabilities, face serious difficulties in promoting their medicines on the market. As a result, the economic feasibility of projects for compounding orphan drugs in pharmacy conditions is reduced, allowing manufacturers to set higher prices.

At the same time, unjustified price increases in a number of countries have begun to initiate changes in state policy regarding orphan drugs [45]. For example, in the Netherlands in 2018, the Amsterdam University

[29] Texas Administrative Code, Title 22, Part 15: Texas State Board of Pharmacy (§ 291.133 «Pharmacies Compounding Sterile Preparations»; § 291.106 «Pharmacies Compounding Sterile Preparations (Class A-S)»; § 291.3 «Required Notifications»). Available from: https://www.law.cornell.edu/regulations/texas/22- Tex-Admin-Code-SS-291-36

[30] Florida Administrative Code. 64B16-28.802 – Special Sterile Compounding Permits for Pharmacies and Outsourcing Facilities; Tallahassee, FL: Florida Board of Pharmacy. Available from: https://www.law.cornell.edu/regulations/florida/Fla-Admin-Code-Ann-R-64B16-28-802

[31] United States Code. Title 21. § 353a – Pharmacy compounding. Washington; DC: U.S. Government Publishing Office. Available from: https://www.govinfo.gov/link/ uscode/21/353a

[32] United States Code. Title 21. § 353b – Outsourcing facilities; Washington, DC: U.S. Government Publishing Office. Available from: https://www.govinfo.gov/link/ uscode/21/353b

[33] Decree of the Government of the Russian Federation dated March 31, 2022 No. 547 "On Approval of the Regulations on Licensing Pharmaceutical Activities". Available from: https://www.consultant.ru/document/cons_doc_LAW_413815/

[34] Order of the Ministry of Health of the Russian Federation dated July 31, 2020 No. 780n "On Approval of types of pharmacies". Available from: https://base.garant. ru/74647990/. Russian

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Medical Center reached a general agreement with the largest insurance companies on the compounding of capsules of chenodeoxycholic acid for all Dutch patients with cerebrotendinous xanthomatosis syndrome [14]. The reason for this decision was a sharp increase in the price of the original drug CDCA Leadiant, which until 2008 was sold under the name Chenofalk® at a price of 46 euros (≈ 4 400 rubles; calculated at the rate of the Central Bank of the Russian Federation on September 9, 2025) per package of 100 capsules. After the acquisition of rights by Leadiant, it rose in price to 885 euros (≈ 84 000 rubles; calculated at the rate of the Central Bank of the Russian Federation on September 9, 2025), and then, with the receipt of orphan status and the receipt of a registration certificate throughout the European Union, it consistently reached a price of 14 000 euros (≈ 1 335 000 rubles; calculated at the rate of the Central Bank of the Russian Federation on September 9, 2025) for the same package. At the same time, chenodeoxycholic acid is a compound known since the 1970s [46]. In these conditions, the initiative of the Amsterdam University Medical Center received approval from both the Dutch Health Inspectorate and the Ministry of Medical Care and was supported by the government, which put the interests of society first, despite initial problems with the quality of the substance used in compounding and complaints from Leadiant, which had exclusive rights[35]. Moreover, in 2021, Leadiant was found guilty of abuse and fined 19.5 million euros (≈ 1.72 billion rubles; calculated at the rate of the Central Bank of the Russian Federation on September 9, 2025)[36]. The court decision noted that the company did not conduct new research, but used orphan status for an unjustified multiple increase[37].

The situation is aggravated by the established social and cultural attitudes in the medical community and among patients, who are oriented mainly towards industrial and foreign medicines. This attitude is due to both a high level of trust in manufacturers and a lack of sufficient awareness of the capabilities of modern compounding pharmacies. Scientific and practical publications note that cases of negative experience with the use of pharmacy dosage forms in the past, associated with inadequate quality, have led to the formation of persistent caution among doctors and patients [47–49]. As a result, an additional limitation of the potential market for pharmacy medicines is formed.

In these conditions, it is extremely important to develop a set of measures that would stimulate the interest of the medical community and patients in pharmacy dosage forms, as well as provide reliable guarantees of their sale.

One of the promising areas is the active involvement of state institutions, including the “Circle of Good” Foundation, created to provide children with severe and rare diseases with high-cost medicines, in the mechanisms for purchasing extemporaneous medicines.

In addition, it seems appropriate to involve the constituent entities of the Russian Federation in the organization of regional programs for the purchase of orphan extemporaneous medicines, especially in cases where industrially produced drugs are absent or unavailable, for example, for the purpose of providing patients over 19 years of age who have ceased to be wards of the “Circle of Good” Foundation.

Restriction on the dispensing of extemporaneous medicines intended for outpatient treatment

In accordance with the current regulatory legal regulation, a medical organization for the purpose of ensuring the treatment process has the right to contact a pharmacy, including one that is not a structural unit of this medical organization, for the purpose of compounding and dispensing medicines on the basis of a requisition[38]. In a hospital setting, medicines received from a compounding pharmacy can be used as part of the provision of a medical service[39].

At the same time, orphan drugs are in most cases used on an outpatient basis, while they are of high cost and require centralized purchases with subsequent distribution through medical organizations.

In turn, the procedure for transferring a medicinal product compounded according to a requisition to

[35] Pharmaceutical Accountability Foundation. Amsterdam UMC resumes supply of compounded CDCA. 22.01.2020. Available from: https://www.pharmaceuticalaccountability. org/2020/01/22/amsterdam-umc-resumes-supply-of-compounded-cdca

[36] Autoriteit Consument & Markt. Autoriteit Consument & Markt imposes fine on drug manufacturer Leadiant for CDCA’s excessive price. Пресс-релиз от 19.07.2021. Гаага. Available from: https://www.acm.nl/en/publications/acm-imposes-fine-drug-manufacturer-leadiant-cdcas-excessive-price

[37] Autoriteit Consument & Markt (ACM). Summary of decision on abuse of dominant position by Leadiant. Available from: https://www.acm.nl/sites/default/files/documents/ summary-of-decision-on-abuse-of-dominant-position-by-leadiant.pdf

[38] Order of the Ministry of Health of the Russian Federation No. 100n dated March 7, 2025 “On Approval of the Rules for the release of medicines”. Available from: https://normativ.kontur. ru/document?moduleId=1&documentId=492852. Russian

[39] Federal Service for Healthcare Supervision. Medicines. Licensing of pharmaceutical activities: answers to frequently asked questions. “Is it mandatory for medical organizations to obtain a license for pharmaceutical activities?”. Available from: https://roszdravnadzor.gov.ru/drugs/licensingpharm/faq/c1324/107. Russian

a patient for outpatient use is not regulated by law, and such actions in the absence of a pharmaceutical activity license from a medical organization may be qualified by Roszdravnadzor as a violation, since they are considered as retail trade in medicinal products. Obtaining a pharmaceutical activity license by a medical organization and establishing a structural unit — a pharmacy — is also not a solution to this problem, since the current legislation does not provide for the possibility of transferring a medicinal product from a compounding pharmacy that compounds the medicinal product to another pharmacy.

The scheme of internal circulation of medicines, received according to a requisition from a compounding pharmacy, from a medical organization to its structural unit — a pharmacy with subsequent dispensing, implemented in practice, requires complex documentation and the writing of a repeated prescription. In addition, it is necessary to conduct a repeated control during dispensing.

The described scheme is formal in nature, but is associated with significant administrative and financial costs, which actually limits the possibility of providing outpatient patients with extemporaneous orphan drugs.

To eliminate the described barrier, it seems appropriate to enshrine the possibility for medical organizations to transfer medicines for outpatient treatment. It is also necessary to provide a mechanism for transferring an extemporaneous medicinal product from a compounding pharmacy to another pharmacy for subsequent dispensing.

Therapeutic effectiveness of extemporaneous analogue medicines

The argument about the lack of preclinical and clinical studies or evidence of therapeutic effectiveness is one of the key tools that supporters of the large pharmaceutical industry use to limit extemporaneous compounding. This statement is put forward as a universal objection against the possibility of using extemporaneous medicines instead of industrial analogues, which is already prohibited by Art. 56 of Federal Law No. 61-FZ.

In this regard, it is necessary to clearly differentiate the problem. The question of proving the therapeutic effectiveness of pharmacy medicines can arise only in two cases, and one of them is hypothetical and is possible only if compounding pharmacies are allowed to compound registered deficient medicines. Currently, only the compounding of complete non-personalized analogues of medicines not registered in the Russian Federation is a situation when the question of confirming therapeutic effectiveness from a scientific point of view is reasonable.

In turn, there is no mechanism in world practice for confirming the therapeutic effectiveness of pharmacy dosage forms. The US experience with semaglutide demonstrates this. Compounded analogues of Ozempic® and Wegovy® filled the needs of patients for more than three years. In addition, some compounding pharmacies used semaglutide in salt form, which makes the drug pharmaceutically non-equivalent. The FDA directly pointed out the inadmissibility of using semaglutide salts, but there were no other requirements that in any way related to therapeutic effectiveness[40].

Despite the fact that the benefit in this case outweighs the potential risks, since there is no regulatory mechanism “biowaiver for pharmacies” or a legal model where responsibility for the use of extemporaneous analogues of unregistered industrial medicines without their proven therapeutic effectiveness is distributed between the attending physician and the compounding pharmacy, this vulnerability will be used to restrain the pharmacy compounding system.

A possible way for solving this issue is to consolidate at the level of the State Pharmacopoeia a gradation of requirements for determining the equivalence of complete extemporaneous forms of analogues of unregistered industrially produced medicines, depending on the dosage form and their therapeutic effect.

Thus, for dosage forms with local action, it is sufficient to confirm pharmaceutical equivalence, that is, the identity of the qualitative and quantitative composition in one dosed unit of the medicinal product.

In the case of injectable medicines in which bioavailability is close to 100%, as well as non-modified immediate-release dosage forms, basic in vitro studies should be additionally carried out.

For complex dosage forms, including modified-release, transdermal systems, inhalations with a systemic effect, consolidate the mechanism of medical necessity according to the “Right to Try” principle,

[40] Food and Drug Administration. FDA’s Concerns with Unapproved GLP-1 Drugs Used for Weight Loss. Available from: https://www.fda.gov/drugs/postmarket-drug-safety-information-patients-and-providers/fdas-concerns-unapproved-glp-1- drugs-used-weight-loss

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when responsibility for the prescription is assigned to the attending physician, and the compounding pharmacy guarantees quality [50]. This approach would allow the legitimate compounding of unregistered medicines without requiring the impossible from compounding pharmacies — conducting full-scale clinical trials.

Study Limitations

The search for sources was conducted mainly in Russian and English, so publications in other languages may not have been taken into account. The analysis included only the most significant and relevant materials, which led to the risk of incomplete coverage of secondary studies and local regulatory legal acts.

CONCLUSION

The analysis of the identified barriers shows that the problem of providing patients with orphan drugs goes beyond pharmaceutical practice and affects the foundations of the state's drug safety. Pharmacy compounding in this context is not just an auxiliary mechanism, but a strategic tool capable of compensating for structural imbalances in the market.

World practice shows that the key competition in the field of orphan drugs occurs not between pharmaceutical companies, but at the level of regulatory systems of various countries and integration associations. At the same time, it is the developed national system of compounding pharmacies that becomes a weighty argument in the formation of pricing policy, ensuring sustainable access to therapy and protecting the interests of patients.

In conditions when global companies are increasingly using orphan status to maintain the high cost of drugs, compounding pharmacies are able to play the role of a "counterweight", providing patients with more effective, timely and affordable pharmacotherapy. Russia has a regulatory and organizational framework for the formation of such a system, but its further development requires the elimination of existing barriers and the institutionalization of pharmacy compounding as a full-fledged element of national drug policy.

Solutions aimed at overcoming the identified obstacles will be useful not only for the segment of orphan drugs, but also for pharmacy compounding in general, creating the basis for its sustainable development. Orphan drugs in this sense become an indicator of systemic problems, and overcoming them can become a point of growth for the entire industry.

Thus, the elimination of barriers will not only expand access to therapy for patients with rare diseases, but also turn pharmacy compounding into one of the key factors of pharmaceutical independence of Russia.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

M.A. Mandrik — idea, design, collection and critical analysis of scientific literature and regulatory legal documents, interpretation of the results, draft writing and editing, final approval of the article; A.V. Bykov — analysis of scientific literature and regulatory legal documents, discussion, editing and final approval of the article; V.S. Fisenko — search and analysis of literary sources, draft editing and final approval of the article; E.A. Maksimkina — analysis of scientific literature and regulatory legal documents, discussion, draft editing and final approval of the article. All authors confirm that their authorship meets the international ICMJE criteria (all authors made a significant contribution to the development of the concept, conduct of the study and preparation of the article, read and approved of the final version before the publication).

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Modern Aspects of Pharmacognostic Analysis of Wild Carrot Fruits (*Daucus carota* L.)

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The aim. Pharmacognostic study of wild carrot fruits: identification of the main microscopic features; chromatographic-mass spectrometric study of volatile compounds and determination of numerical commodity indicators of medicinal plant raw materials.

Materials and methods. Morphological and anatomical features of wild carrot root raw materials were studied by light microscopy; luminescence of tissues and working standard samples of substances were studied by luminescent microscopy; crystalline structures were studied by polarization microscopy; the profile of volatile compounds in raw materials was assessed using chromatographic-mass spectrometry. The preparation of microscopic slides, the determination of numerical commodity indicators of medicinal plant raw materials were carried out according to the requirements of the State Pharmacopoeia of the Russian Federation XV edition.

Results. Morphological and anatomical analysis established the characteristics of carrot fruits: oval shape of the fruit, tapering towards the apex, brown in color with seven veins; schizogenous containers of angular shape with non-cellular septa; endosperm parenchyma of isodiametric, oval cells; endocarp of parquet type from elongated, thick-walled, tightly closed cells; stellate druses in the tissues of the embryo with four symmetrical rays. Luminescence in the excitation range of 330–400 nm of the mesocarp — bright blue; epithelial cells — light yellow; resinous secretion — blue; chromatographic-mass spectrometric study of hexane and chloroform fractions of the extract from carrot fruits determined the profile of volatile compounds, with carotol being predominant (25.9% and 30.1%, respectively).

Conclusion. The identified microscopic features, the main biologically active substance, and numerical commodity indicators are of practical importance for inclusion in the draft pharmacopoeial monograph “Wild Carrot Fruits”.

Keywords: wild carrot fruits; light microscopy; luminescent microscopy; polarization microscopy; anatomical and morphological features; diagnostic features; chromato-mass spectrometry; carotol; numerical commodity indicators

Abbreviations: MPRMs — medical plant raw materials; BASs — biologically active substances; SPh RF — State Pharmacopoeia of the Russian Federation; BAAs — biologically active additives (food supplements); GPM — general pharmacopoeial monograph; RS — reference standard; PM — pharmacopoeial monograph; GC-MS — gas chromatography-mass spectrometry; UV light — ultraviolet light.

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Современные аспекты фармакогностического анализа плодов моркови дикой (*Daucus carota* L.)

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Цель. Фармакогностическое изучение плодов моркови дикой: выявление основных микроскопических признаков; хромато-масс-спектрометрическое исследование летучих соединений и определение числовых товароведческих показателей лекарственного растительного сырья.

Материалы и методы. Морфолого-анатомические признаки сырья плодов моркови дикой исследовали световой микроскопией; люминесценцию тканей и рабочих стандартных образцов субстанций изучали люминесцентной микроскопией; кристаллические структуры исследовали поляризационной микроскопией; профиль летучих соединений в сырье оценивали с помощью хромато-масс-спектрометрии. Изготовление микропрепаратов, определение числовых товароведческих показателей лекарственного растительного сырья осуществляли по требованиям Государственной Фармакопеи Российской Федерации XV издания.

Результаты. С помощью морфолого-анатомического анализа установили особенности плодов моркови: овальная форма плода, зауженная к верхушке, коричневого цвета с семью жилками; схизогенные вместилища угловатой формы с перегородками неклеточного строения; паренхима эндосперма из изодиаметрических, овальных клеток; эндокарпий паркетного типа из вытянутых, толстостенных, плотно сомкнутых клеток; звёздчатые друзы в тканях зародыша с четырьмя симметричными лучами. Люминесценция в диапазоне возбуждения 330–400 нм мезокарпия — ярко-голубая; клеток эпителия — светло-желтая; смолянистого секрета — голубая. Хромато-масс-спектрометрическое исследование гексановой и хлороформной фракции извлечения из плодов моркови определило профиль летучих соединений, преобладающим является каротол (25,9 и 30,1%, соответственно).

Заключение. Выявленные микроскопические признаки, основное биологически активное вещество и числовые товароведческие показатели имеют практическое значение для включения в проект фармакопейной статьи «Моркови дикой плоды».

Ключевые слова: плоды моркови дикой; световая микроскопия; люминесцентная микроскопия; поляризационная микроскопия; анатомо-морфологические признаки; диагностические признаки; хромато-масс-спектрометрия; каротол; числовые товароведческие показатели

Список сокращений: ЛРС — лекарственное растительное сырье; БАВ — биологически активные вещества; ГФ РФ — Государственная фармакопея Российской Федерации; БАД — биологически активные добавки к пище; ОФС — общая фармакопейная статья; СО — стандартный образец; ФС — фармакопейная статья; ГХ-МС — газовая хроматография с масс-спектрометрией; УФ-свет — ультрафиолетовый свет.

INTRODUCTION

The fruits of wild carrot (also known as common carrot) represent a medicinal plant raw material (MPRM) and a promising source of biologically active compounds (BACs) of phenolic and terpenoid nature. The interest in wild carrot fruits has been demonstrated by numerous domestic and international researchers. This raw material is known to exhibit antibacterial, antifungal [1, 2], and anti-inflammatory activities [3–5]. In particular, studies have reported its diuretic and choleretic effects in both wild and cultivated carrot fruit extracts [6–8]. An aqueous-alcohol extract of wild carrot fruits is an official substance used in the pharmaceutical preparations “Urolesan” (ART-PHARM LLC, Russia) and “Urokhol” (VIFITEH CJSC, Russia). Extractive substances from this MPRM are also included in the dietary supplement “Urolit” (VITAUKT-PROM LLC, Republic of Adygea,Russia). However,

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despite the phytotherapeutic significance of this plant species, no pharmacopoeial monograph for this raw material is included in the current edition of the State Pharmacopoeia of the Russian Federation (SPh RF).

Key stages of the standardization and subsequent quality control of MPRM include the establishment of identity through external (morphological) and microscopic (anatomical) features, detection of major BACs, and assessment of raw material quality by the determination of numerical quality indicators. Previously, researchers identified taxonomic and phylogenetic relationships between representatives of the genus *Daucus* L. [9], and studied the main anatomical and diagnostic features of pharmacopoeial MPRM [10]. Nevertheless, in the course of updating the regulatory documentation for medicinal products from wild carrot fruits, we are implementing the task of updating the pharmacopoeial monograph on this type of MPRM for the new edition of the SPh RF, taking into account better practices and updated requirements for microscopic examination. Modern chromatographic analysis methods make it possible to better characterize the component composition of the low-polar volatile fraction of secondary metabolites, thereby providing a more accurate identification and reliable assessment of the goodness of MPRM containing essential oils.

THE AIM of the study is a pharmacognostic study of wild carrot fruits: identification of the main morphological and anatomical diagnostic features, taking into account modern requirements for microscopy techniques; chromato-mass spectrometric study of the profile of volatile organic compounds and determination of numerical commodity indicators of the quality of medicinal plant raw materials.

MATERIALS AND METHODS

he objects of study

The object of study was industrial samples of wild carrot fruits provided by VITAUKT-PROM LLC (Republic of Adygea, Russian Federation). The raw materials were harvested in 2023 in the Krasnodar Territory (Ust-Labinsky district) and comply with the requirements of GOST 32592-2013 “Seeds of vegetable, melon crops, fodder root crops and fodder cabbage. Varietal and sowing qualities. General technical conditions” for the category of reproductive seeds.

Macroscopic, microscopic, microchemical and histochemical analyses

The external features of MPRM were examined visually and using a Motic DM 39 stereomicroscope (Motic Xiamen, China), based on the requirements of the general Pharmacopoeia monograph GPhM.1.5.1.0007 “Fruits”[1] and GPhM.1.5.1.0008 “Seeds”[2].

Anatomical (microscopic) signs of raw materials were examined using light microscopy in transmitted and reflected light on a Motic DM1802 light microscope (Motic Xiamen, China). Micro-preparations were prepared according to GPhM.1.5.3.0003 “Microscopic and microchemical analysis of medicinal plant raw materials and medicinal products of plant origin”[3] and GPhM.1.5.3.0003.15 “Technique of microscopic and microchemical examination of medicinal plant raw materials and medicinal herbal preparations”[4]. Reagents for microchemical and histochemical analysis were prepared in accordance with GPhM.1.3.0001 “Reagents. Indicators”[5].

The analysis of the luminescence of fruit tissues, as well as working standard samples of substances and plant raw materials, was carried out using an Altami LUM-2 luminescent microscope (Altami LLC, Russian Federation) with a special configuration and using blue and yellow light filters of 32 mm. The light source was a high–voltage mercury lamp (HBO 100W); luminescence excitation spectral range: blue excitation filter — 420–550 nm; UV-filter — 330–400 nm. The standard samples (SS) Quercetin and SS Coumarin provided by the Pharmacy Research Center of the Federal State Budgetary Educational Institution of Higher Medical Education of the Ministry of Health of the Russian Federation were used as working standard samples of phenolic nature. The luminescence of SS substances was evaluated directly in air–dry form by irradiating crystals of substances on a slide under an Altami LUM-2 microscope at various magnifications. The luminescence of crystals of SS substances in the form of a suspension in purified water on a slide covered with a cover glass was also evaluated.

[1] GPhM.1.5.1.0007 “Fruits”. State Pharmacopoeia of the Russian Federation. Available from: https://pharmacopoeia.regmed.ru/pharmacopoeia/
izdanie-15/1/1-5/1-5-2/plody/

[2] GPhM.1.5.1.0008 “Seeds”. State Pharmacopoeia of the Russian Federation. Available from: https://pharmacopoeia.regmed.ru/pharmacopoeia/ izdanie-15/1/1-5/1-5-2/semena/

[3] GPhM.1.5.3.0003 “Microscopic and microchemical analysis of medicinal plant raw materials and medicinal products of plant origin”. State Pharmacopoeia of the Russian Federation. Available from: https://pharmacopoeia.regmed.ru/pharmacopoeia/ izdanie-15/1/1-5/1-5-1/mikroskopicheskiy-i-mikrokhimicheskiy-analiz-lekarstvennogo-rastitelnogo-syrya-i-lekarstvennykh-sred/

[4] GPhM.1.5.3.0003.15 “Technique of microscopic and microchemical examination of medicinal plant raw materials and medicinal herbal preparations”. State Pharmacopoeia of the Russian Federation. Available from: https://pharmacopoeia. regmed.ru/pharmacopoeia/izdanie-13/1/1-5/1-5-3/1-5-3-3/ tekhnika-mikroskopicheskogo-i-mikro

[5] GPhM.1.3.0001 “Reagents. Indicators”. State Pharmacopoeia of the Russian Federation. Available from: https://pharmacopoeia.regmed.ru/ pharmacopoeia/izdanie-15/1/1-3/reaktivy-indikatory

If necessary, for contrasting, the method of polarization microscopy on a PLM 213 polarization microscope (LOMO JSC, Russia) was used in the study of crystalline structures. Cross-sections of fruits were prepared manually (for luminescence of dry raw materials), as well as on an HM 325 Thermo FS microtome (Thermo Fisher Scientific, USA).

Sample preparation for GC-MS analysis

To analyze the volatile organic compound (VOC) profile via gas chromatography–mass spectrometry (GC–MS), aqueous-ethanol extracts were prepared using 60% ethanol as the extractant by modified fractional maceration in three extractors (raw material-to-solvent ratio of 1:5), followed by a final thermal stage — heating for 30 minutes at 70°C — as described in the invention patent by V.A. Kurkin [11]. The resulting extract was kept for 48 hours at 8 °C in a light-protected environment, then filtered through "Red Ribbon" ashless filter paper into a dark glass bottle and sealed with polyethylene stoppers and screw caps.

Further, the hexane and chloroform fractions of the amounts of substances that were analyzed were obtained from alcohol-water extraction. The choice of fractions is determined by the literature data that volatile organic compounds, which may be part of the studied plants, are soluble in these extractants [12–14].

For this, 10.0 mL of the aqueous-ethanol extract was placed in a separatory funnel (30–50 mL volume), and 5 mL of *n*-hexane (or chloroform) was added. The mixture was shaken for 2 minutes and then left to separate completely. The upper hexane (or lower chloroform) phase was filtered through "Red Ribbon" filter paper into a 25 mL volumetric flask. The extraction was repeated two more times with 5 mL portions, filtering each into the same volumetric flask. The final volume was adjusted to the mark with *n*-hexane (or chloroform), and the solution was mixed.

GC-MS analysis

The analysis was performed under the conditions described earlier [15, 16]. The chromatographic conditions of the analysis are presented in Table 1.

Component identification was performed by calculating linear retention indices and comparing both the results and full mass spectra with reference databases (NIST 2.4 mass spectral library) and published literature. Only components with identification probabilities above 90% were considered. The relative content of each component was calculated by internal normalization using peak area percentages on the total ion current chromatogram [17, 18].

Numerical Quality Indicators

Numerical quality indicators of wild carrot fruits characterize their good quality, compliance with the rules of harvesting and primary processing, transportation and storage conditions, as well as the accumulation of certain secondary metabolites, in particular essential oil, concentration of macro- and microelements, heavy metals, etc. One of the stages in the formation of regulatory documentation on quality and the justification of the MPRM specification is the experimental establishment of normalized indicators and ranges of acceptable values.

Wild carrot fruits are processed both in whole and crushed form, and can also be included in the composition of medicinal products as an active component in powder form, which served as the basis for establishing most of the standardized numerical indicators. The analysis was carried out in accordance with the GPhM.1.5.1.0007 "Fruits", GPhM.1.5.3.0010.15 "Determination of essential oil content in medicinal plant raw materials and medicinal herbal preparations"[6], GPhM.1.5.3.0006 "Determination of the content of extractive substances in medicinal plant raw materials and medicinal herbal preparations"[7], GPhM.1.2.2.2.0013 "Total ash"[8], GPhM.1.5.3.0005.15 "Ash insoluble in hydrochloric acid"[9], GPhM.1.2.2.2.0014 "Sulphate ash"[10], GPhM.1.2.2.2.0012 "Heavy metals"[11], GPhM.1.2.1.0010 "Loss in weight during drying"[12] of SPh RF.

[6] GPhM.1.5.3.0010.15 "Determination of essential oil content in medicinal plant raw materials and medicinal herbal preparations". State Pharmacopoeia of the Russian Federation. Available from: https://pharmacopoeia.regmed.ru/pharmacopoeia/ izdanie-14/1/1-5/1-5-3/opredelenie-soderzhaniya-efirnogo-masla-v-lekarstvennom-rastitelnom-syre-i-lekarstvennykh-rastitelny/

[7] GPhM.1.5.3.0006 "Determination of the content of extractive substances in medicinal plant raw materials and medicinal herbal preparations". State Pharmacopoeia of the Russian Federation. Available from: https://pharmacopoeia.regmed.ru/pharmacopoeia/izdanie-15/1/1-5/1-5-1/opredelenie-soderzhaniya-ekstraktivnykh-veshchestv-v-lekarstvennom-rastitelnom-syre-i-lekarstvennykh/

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[10] GPhM.1.2.2.2.0014 "Sulphate ash". State Pharmacopoeia of the Russian Federation. Available from: https://pharmacopoeia.regmed.ru/ pharmacopoeia/izdanie-15/1/1-2/1-2-2/1-2-2-2/sulfatnaya-zola/

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RESULTS

Macroscopic, microscopic, microchemical and histochemical analyses

During the analysis of raw materials, a direct correspondence was established with the morphological characteristics described earlier in the literature [9]. Mericarps of fruits are oval or oblong in shape, tapering towards the apex. The color of the fruits is brown or light brown (Fig. 1, A). Highly protruding veins of different sizes are localized on the surface of each mericarp. The number of veins is stable and amounts to 7 on each mericarp (Fig. 1, B–G). Their sizes differ. Thus, it is customary to distinguish furrowed veins in the number of 2; lateral primary veins in the number of 2; dorsal secondary veins in the number of 2 and the middle primary vein. In addition, from the side of the carpophore, it is customary to distinguish the commissural (connecting) vein, as well as two marginal primary veins (Fig. 1, D 1–6). However, they are hardly noticeable visually in the structure of the mericarp. Because of this, they are not used as a morphological feature. It is obvious that the number and nature of the expression of the ribs is a sign of raw materials and, as scientists interpret it, a systematic feature in the *Apiaceae* L. family [9].

The dorsal secondary and commissural primary ridges are anatomically similar. In cross-section, they appear irregularly triangular. The surface is covered with a thin-walled exocarp layer. The mesocarp, consisting of hollow cells, forms the base of the ridge wall. Staining with 1% ethanol solution of Sudan III revealed pink to dark brown coloration of the exo- and mesocarp cell walls, indicating suberization. The dorsal and commissural ridges are hollow, with a schizogenous duct occupying the cavity and mirroring the angular shape of the ridge. This cavity is lined with small epithelial cells. The protoplasts of these epithelial cells appear dark brown due to wall staining and amorphous protoplasm. Under UV light excitation (330–400 nm), epithelial cells show pale brown fluorescence. The resinous secretions within the ducts exhibit pale yellow to blue fluorescence (Fig. 2A–F).

When dissecting the fruit and preparing crushed preparations, a combination of schizogenous receptacles can be observed from the surface. These structures are not easily visible under classical light microscopy; therefore, fluorescence microscopy was used. Due to their darker fluorescence relative to surrounding tissues, the ducts can be readily identified within the exocarp (Fig. 2G–I).

On surface view, septa within the ducts are also observable, serving as an additional diagnostic trait for this species[13]. These septa lack cellular structure and resemble cell wall composition. Under UV excitation at 330–400 nm, they fluoresce blue, and at 420–550 nm, pale yellow (Fig. 2J–L).

It is worth noting that the majority of monoterpenes are not detected in this spectral range, suggesting that the observed fluorescence may be associated with secondary metabolites of phenolic nature, possibly flavonoids.

The fluorescence of the mesocarp tissue appears bright blue, attributed to simple phenolic compounds of the C_6–C_1 and C_6–C_2 series. According to some literature, coumarin derivatives have been reported in *Daucus* species [14, 15].

To compare fluorescence patterns, the fluorescence of a coumarin reference standard (RS) was investigated. Under UV light at 330–400 nm, coumarin exhibits pale blue fluorescence (Fig. 3A–D). This similarity suggests the presence of structurally related compounds in the pericarp of wild carrot fruits. A working reference standard of quercetin was also used as an example of a flavonoid secondary metabolite. Under UV excitation at 330–400 nm quercetin fluoresces bright orange, resembling the fluorescence of epithelial cells surrounding the resin ducts (Fig. 3E–H). This fluorescence pattern indirectly indicates the presence of flavonol-class flavonoids in these cells.

The bast fibers are lignified, as confirmed by yellow staining with 10% sulfuric aniline solution. Closer to the center, phloem cells and pairs of xylem vessels are located, forming a collateral vascular bundle. Two schizogenous ducts are located on the dorsal side of the mericarp. A cavity is also present between the endocarp and the seed coat (Fig. 4D–F). The commissural ridge is often lost during dissection.

In the seed tissue, a well-developed parenchymatous endosperm is typically visible, characteristic of the *Apiaceae* Lindl. family[14]. The parenchymal cells of the endosperm are isodiametric, sometimes slightly elongated and oval. Cell walls are noticeably thickened and composed of cellulose. The protoplast is amorphous and unpigmented.

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[14] Ostroumova TA, et al. Analysis of Carpological Features of the *Umbelliferae* Family in the Flora of Russia. In: Systematic and Floristic Studies of Northern Eurasia; 2019. Vol. 2. 168 p. Russian

Table 1 – Chromatographic Conditions for Determining the Composition of Volatile Organic Compounds by GC–MS

Chromatograph	Gas chromatograph "MAESTRO 7820"
Column	Capillary quartz column HP-5, 30 m × 0.25 mm × 0.25 µm (stationary phase: 5 %-diphenyl-95 %-dimethylsiloxane)
Detector	Mass spectrometer Agilent 5975
Injection mode	Auto-injector
Carrier Gas	Helium
Flow rate	1 mL/min
Programming the temperature of the column thermostat	Isothermal at 40°C for 5 min; ramp to 80°C at 3°C/min; to 180°C at 4°C/min; to 280°C at 8°C/min; isothermal at 280°C for 15 min; injection without split
Injector temperature	270°C
Ion source temperature	150°C
Quadrupole temperature	230°C
Transfer line temperature	280°C
Injection volume	1 µL

Table 2 – Component composition of volatile organic compounds in hexane and chloroform fractions of aqueous-ethanol extract from Wild Carrot Fruits

Retention Time, M ± SD, min	Compound	Relative Content, M ± SD, %	
		Hexane	Chloroform
11.725 ± 0.012	2-Thujene	0.09 ± 0.03	0.07 ± 0.02
12.106 ± 0.042	α-Pinene	16.62 ± 2.49	3.66 ± 0.55
12.939 ± 0.013	Camphene	1.22 ± 0.18	0.37 ± 0.11
14.238 ± 0.012	Sabinen	4.98 ± 0.75	2.03 ± 0.30
14.426 ± 0.006	β-Pinene	1.34 ± 0.20	0.52 ± 0.16
15.189 ± 0.01	β-Myrcene	2.51 ± 0.38	1.15 ± 0.17
17.108 ± 0.009	o-Cymene	2.15 ± 0.32	2.02 ± 0.30
17.254 ± 0.007	D-Limonene	1.66 ± 0.25	0.75 ± 0.22
17.342 ± 0.016	β-Phellandrene	0.13 ± 0.04	0.00 ± 0.00
17.663 ± 0.022	trans-β-Ocimene	0.09 ± 0.03	0.00 ± 0.00
18.66 ± 0.29	γ-Terpinene	0.24 ± 0.07	0.33 ± 0.10
19.338 ± 0.342	cis-Sabinene hydrate	0.03 ± 0.01	0.24 ± 0.07
20.76 ± 0.121	n.i.	0.02 ± 0.01	0.19 ± 0.06
20.98 ± 0.013	Linalool	0.15 ± 0.04	0.92 ± 0.28
22.003 ± 0.03	Menth-2-en-1-ol	0.06 ± 0.02	0.03 ± 0.01
22.201 ± 0.01	Neo-allo-ocimene	0.09 ± 0.03	0.06 ± 0.02
22.308 ± 0.014	α-Campholenal	0.14 ± 0.04	0.48 ± 0.14
22.846 ± 0.014	Pinocarveol	0.22 ± 0.06	1.00 ± 0.30
23.109 ± 0.015	cis-Verbenol	0.54 ± 0.16	3.72 ± 0.56
23.726 ± 0.186	Pinocarvone	0.09 ± 0.03	0.42 ± 0.13
23.786 ± 0.023	Sabina ketone	0.03 ± 0.01	0.00 ± 0.00
24.244 ± 0.008	Verbenyl, ethyl ether	0.06 ± 0.02	0.00 ± 0.00
24.571 ± 0.014	4-Terpineol	0.03 ± 0.01	0.31 ± 0.09
24.806 ± 0.015	α-Thujenal	0.02 ± 0.00	0.00 ± 0.00
25.015 ± 0.012	p-Cymene-8-ol	0.08 ± 0.02	0.43 ± 0.13
25.251 ± 0.014	Myrtenol	0.11 ± 0.03	0.61 ± 0.18
25.309 ± 0.012	Myrtenal	0.08 ± 0.02	0.56 ± 0.17
25.819 ± 0.014	Verbenone	0.32 ± 0.10	1.44 ± 0.22
26.239 ± 0.013	Carveol	0.09 ± 0.03	0.58 ± 0.18
26.577 ± 0.014	Myrtenyl acetate	0.04 ± 0.01	0.12 ± 0.04
27.289 ± 0.014	Carvone	0.07 ± 0.02	0.29 ± 0.09
28.529 ± 0.011	Borneol, acetate	0.66 ± 0.20	1.02 ± 0.15
30.766 ± 0.014	α-Terpinyl acetate	0.06 ± 0.02	0.22 ± 0.07

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Retention Time, M ± SD, min	Compound	Relative Content, M ± SD, %	
		Hexane	Chloroform
31.15 ± 0.023	Nerol acetate	0.00 ± 0.00	0.00 ± 0.00
31.373 ± 0.024	trans-Myrtanol	0.03 ± 0.01	0.09 ± 0.03
31.715 ± 0.012	β-Gurjunene	2.30 ± 0.34	0.15 ± 0.05
31.82 ± 0.02	Geranyl acetate	0.33 ± 0.10	0.24 ± 0.07
31.997 ± 0.044	n.i.	0.14 ± 0.04	0.00 ± 0.00
32.128 ± 0.019	beta-Elemen	0.09 ± 0.03	0.00 ± 0.00
32.489 ± 0.01	α-Zingiberene	0.10 ± 0.03	0.08 ± 0.02
32.894 ± 0.009	α-Bergamotene	1.26 ± 0.19	0.16 ± 0.05
33.07 ± 0.013	Santalen	0.44 ± 0.13	0.05 ± 0.02
33.17 ± 0.021	Caryophyllene	2.88 ± 0.43	0.28 ± 0.08
33.366 ± 0.013	Elixene	0.06 ± 0.02	0.00 ± 0.00
33.524 ± 0.016	α-Farnesene	1.94 ± 0.29	0.28 ± 0.08
33.897 ± 0.03	Isocaryophyllene	0.21 ± 0.06	0.49 ± 0.15
33.996 ± 0.023	Epi-β-Santalene	0.10 ± 0.03	0.06 ± 0.02
34.182 ± 0.02	(E)-β-Famesene	1.63 ± 0.24	0.10 ± 0.03
34.249 ± 0.021	β-Sesquiphellandrene	2.66 ± 0.40	0.24 ± 0.07
34.395 ± 0.023	cis-β-Farnesene	2.25 ± 0.34	0.31 ± 0.09
34.505 ± 0.013	γ-Muurolene	0.13 ± 0.04	0.03 ± 0.01
34.992 ± 0.043	β-Cubebene	0.83 ± 0.25	0.65 ± 0.20
35.204 ± 0.021	n.i.	0.69 ± 0.21	0.04 ± 0.01
35.467 ± 0.009	β-Selinene	0.18 ± 0.06	0.00 ± 0.00
35.676 ± 0.003	α-Selinene	0.12 ± 0.04	0.11 ± 0.03
35.851 ± 0.011	Chamigren	0.47 ± 0.14	0.06 ± 0.02
35.965 ± 0.014	β-Bisabolene	1.75 ± 0.26	0.54 ± 0.16
36.187 ± 0.038	Sesquicineole	0.07 ± 0.02	0.09 ± 0.03
36.482 ± 0.006	n.i.	0.59 ± 0.18	0.00 ± 0.00
36.993 ± 0.082	Humulene	0.14 ± 0.04	0.14 ± 0.04
38.421 ± 0.018	Caryophyllene oxide	1.54 ± 0.23	2.65 ± 0.40
39.137 ± 0.116	Carotol	25.9 ± 3.9	30.10 ± 4.52
39.384 ± 0.059	Humulene oxide II	0.32 ± 0.09	0.13 ± 0.04
39.708 ± 0.009	Cedrenol	0.28 ± 0.08	0.34 ± 0.10
40.233 ± 0.008	Daucol	1.02 ± 0.15	3.27 ± 0.49
40.568 ± 0.01	β-Eudesmol	0.10 ± 0.03	0.54 ± 0.16
41.083 ± 0.009	n.i.	0.38 ± 0.11	1.02 ± 0.15
41.127 ± 0.001	Elemol	0.30 ± 0.09	0.00 ± 0.00
41.811 ± 0.008	Eudesm-7(11)-en-4-ol	0.20 ± 0.06	0.51 ± 0.15
47.683 ± 0.006	n.i.	1.04 ± 0.16	1.13 ± 0.17
47.863 ± 0.006	Hexadecanoic acid, ethyl ester	0.78 ± 0.23	3.61 ± 0.54
47.923 ± 0.006	n.i.	0.42 ± 0.13	0.74 ± 0.22
48.256 ± 0.006	n.i.	0.78 ± 0.23	1.28 ± 0.19
50.326 ± 0.006	Ethyl Oleate	3.37 ± 0.51	9.47 ± 1.42
50.628 ± 0.033	Stearic acid, ethyl ester	0.38 ± 0.11	0.58 ± 0.17
51.313 ± 0.007	n.i.	2.54 ± 0.38	5.06 ± 0.76
52.336 ± 0.012	Bicyclo[4,4,0]dec-5-ene, 1,5-dimethyl-3-hydroxy-8-(1-methylene-2-hydroxyethyl-1)	5.19 ± 0.78	11.84 ± 1.78
	Component Classes (average values):		
	Monoterpenes:	29.0	8.94
	Monoterpenoids:	3.17	12.2
	Sesquiterpenes:	19.6	3.74
	Sesquiterpenoids:	29.7	37.6
	Aromatic compounds:	2.23	2.45
	Fatty acids:	4.53	13.7
	Other:	5.19	11.8

Note: “n.i.” — not identified; SD — standard deviation.

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Table 3 – Results of pharmacognostic testing of Wild Carrot Fruits

Indicator	Method of analysis	Characteristic	
		Whole raw material	Powdered raw material
Loss on drying, %	Gravimetric	5.8	5.8
Essential oil content (30 g + 500 mL H_2O, 4 hours), %	Hydrodistillation	0.17	0.88
Extractives (70 % ethanol), %	Gravimetric	6.6	15.2
Total ash, %	Gravimetric	6.9	7.2
Acid-insoluble ash (in 10% HCl), %	Gravimetric	3.2	–
Sulfated ash, %	Gravimetric	9.8	9.2
Heavy metals, %	Visual colorimetry	Not more than 0.01	Not more than 0.01

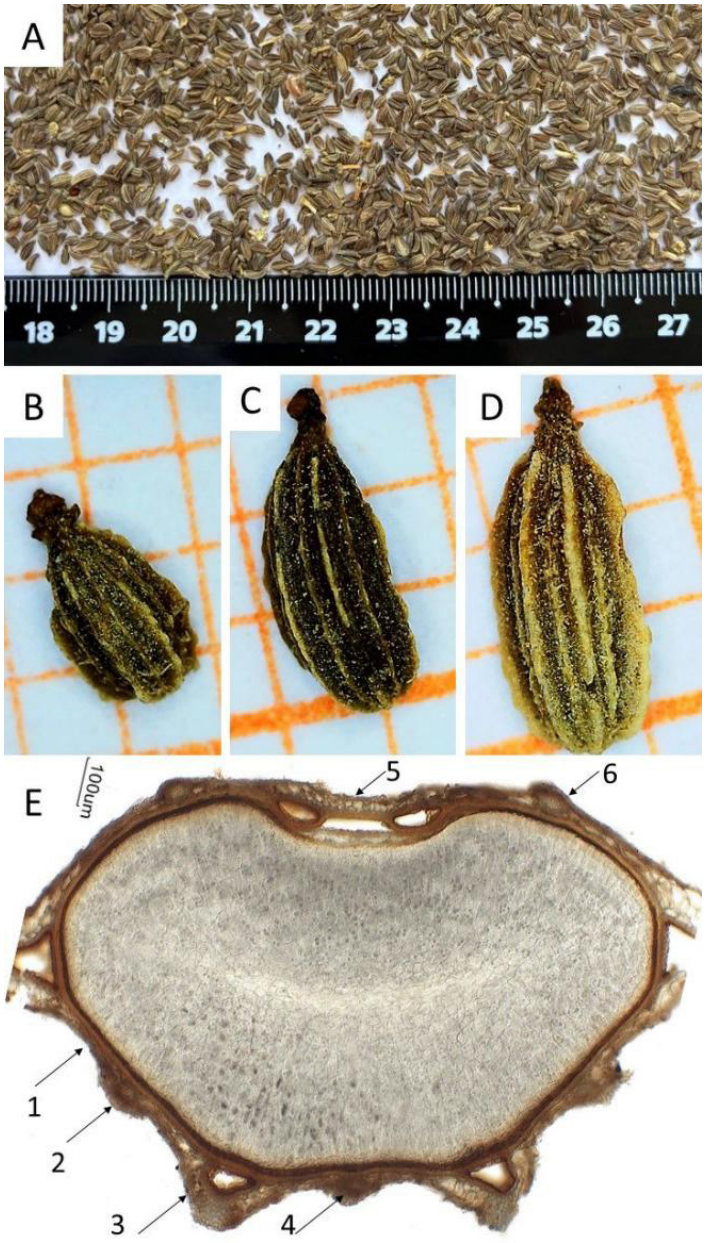

Figure 1 – External view and morphological features of Wild Carrot Fruits.
Note: A — general view of the raw material (industrial sample); B — mericarp up to 2 mm; C — mericarp up to 3 mm; D — mericarp over 3 mm; E — transverse section of mericarp (× 100): 1 — furrowed ridge; 2 — lateral primary ridge; 3 — dorsal secondary ridge; 4 — median primary ridge; 5 — commissural (connecting) ridge; 6 — marginal primary ridge.

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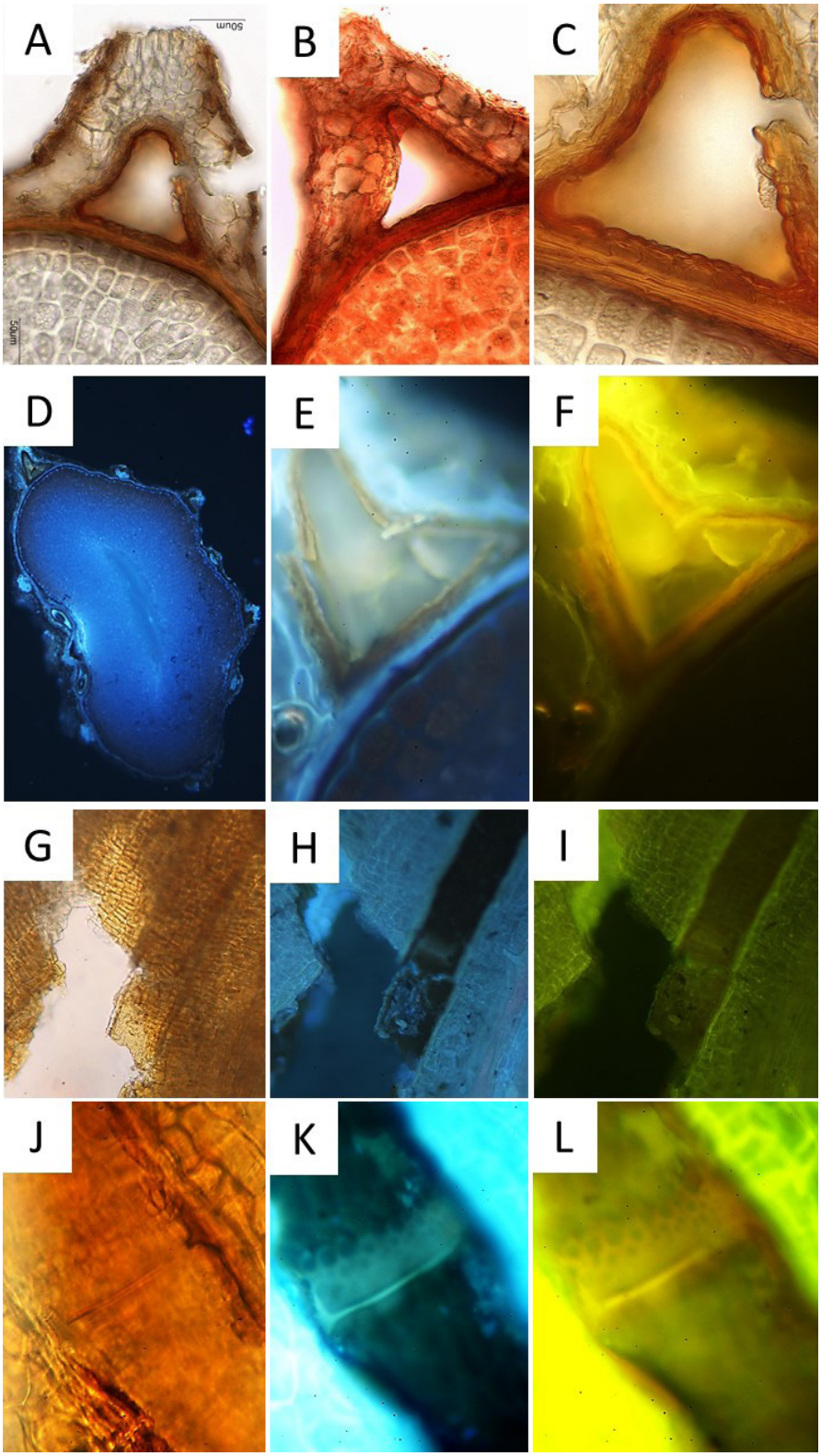

Figure 2 – Histological features of pericarp ridges in cross-section and squashed preparations

Note: A — mesocarp of hollow cells in pericarp ridge (× 400); B — exo- and mesocarp cell walls stained with 1% Sudan III (× 400); C — schizogenous duct in pericarp ridge (× 1000); D — fluorescence of marginal pericarp ridges and embryo tissues under UV light at λ = 330–400 nm (× 40); E — fluorescence of marginal ridge under UV light at λ = 330–400 nm (× 400); F — fluorescence of resinous secretion in marginal ridge under UV light at λ = 420–550 nm (× 400); G — schizogenous ducts in squashed preparation (× 400); H — fluorescence of resin ducts under UV light at λ = 330–400 nm (× 400); I — fluorescence of resin ducts under UV light at λ = 420–550 nm (× 400); J — duct septum under visible light (× 400); K — duct septum fluorescence under UV light at λ = 330–400 nm (× 400); L — duct septum fluorescence under UV light at λ = 420–550 nm (× 400).

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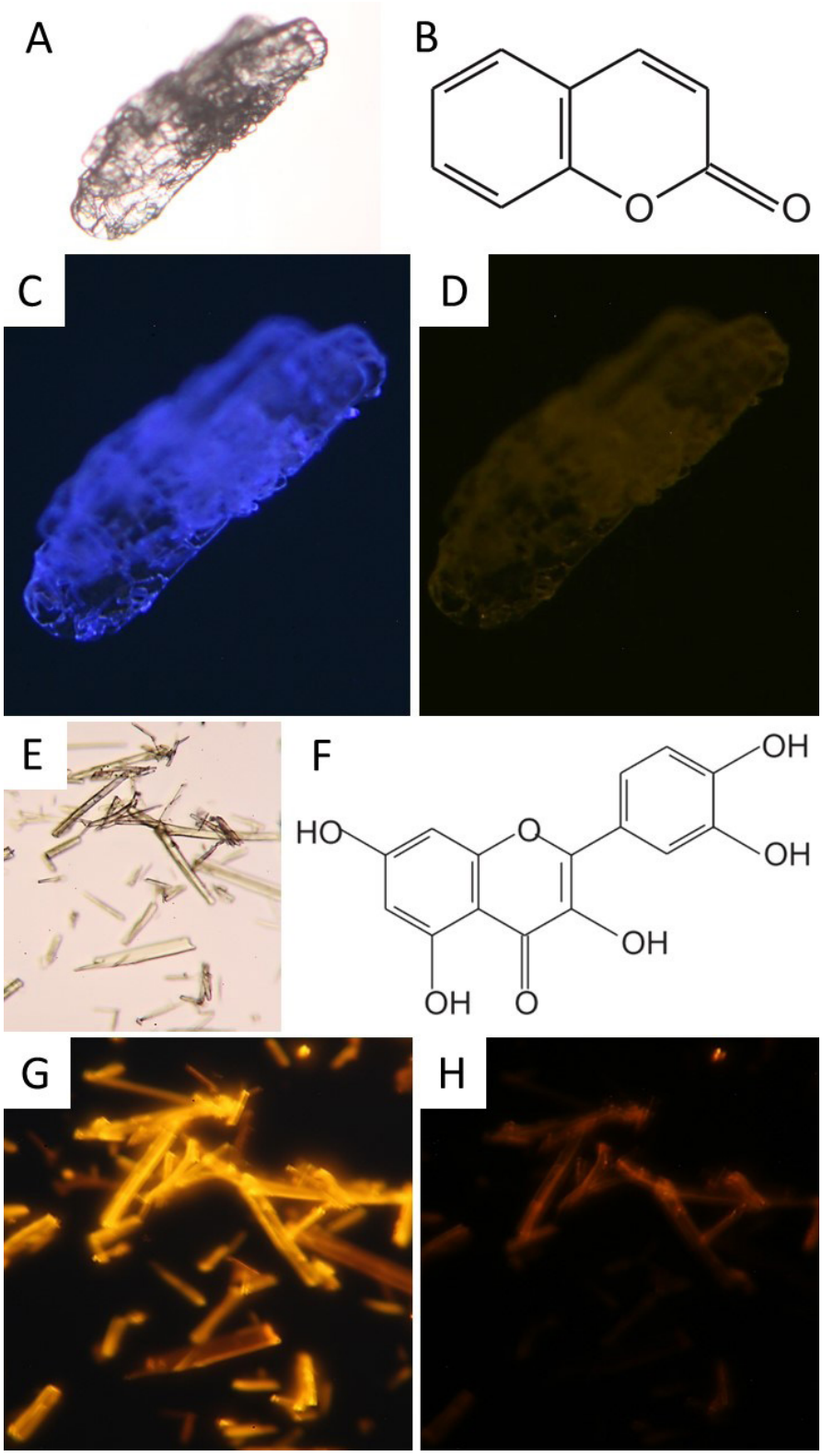

Figure 3 – Cross-section of Wild Carrot Fruit

Note: A — coumarin crystals under visible light (× 400); B — structural formula of coumarin; C — coumarin fluorescence under UV light at λ = 330–400 nm (× 400); D — coumarin fluorescence under UV light at λ = 420–550 nm (× 400); E — quercetin crystals under visible light (× 400); F — structural formula of quercetin; G — quercetin fluorescence under UV light at λ = 330–400 nm (× 400); H — quercetin fluorescence under UV light at λ = 420–550 nm (× 400).

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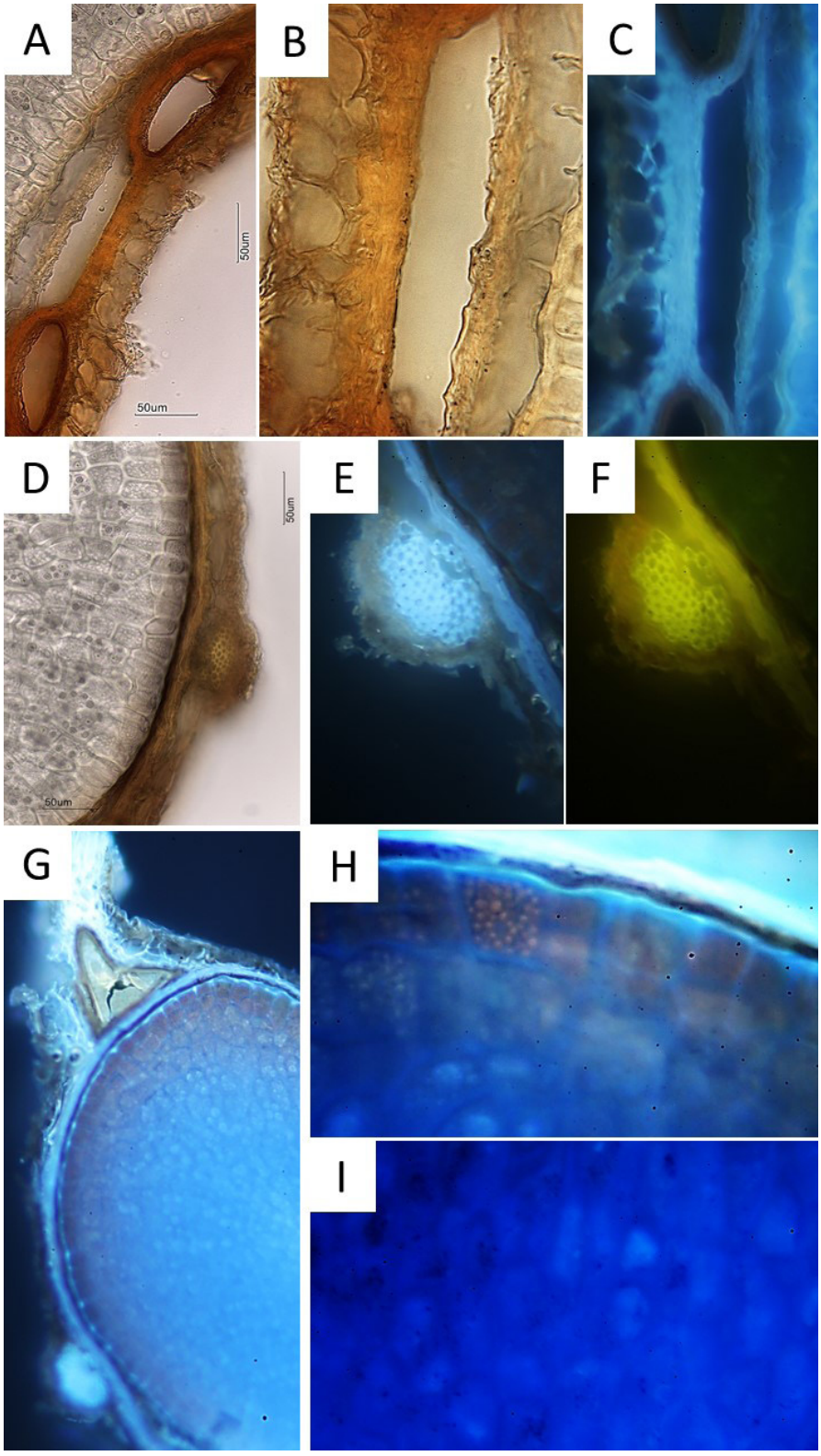

Figure 4 – Secondary pericarp ridges and histology of the dorsal mericarp side (transverse section)

Note: A — schizogenous ducts on the dorsal side of the mericarp (× 400); B — lignified walls treated with 10% sulfuric aniline solution (× 1000); C — fluorescence of connective ridge (carpophore) tissues under UV light at λ = 420–550 nm (× 400); D — bast fibers of the lateral primary ridge (× 400); E — fluorescence of ridge tissue under UV light at λ = 330–400 nm (× 400); F — fluorescence of ridge tissue under UV light at λ = 420–550 nm (× 400); G — fluorescence of embryo protoplasts under UV light at λ = 330–400 nm (× 100); H — red fluorescence of plastids in peripheral endosperm protoplasts under UV light at λ = 330–400 nm (× 400); I — absence of druse fluorescence under UV light at λ = 420–550 nm (× 400).

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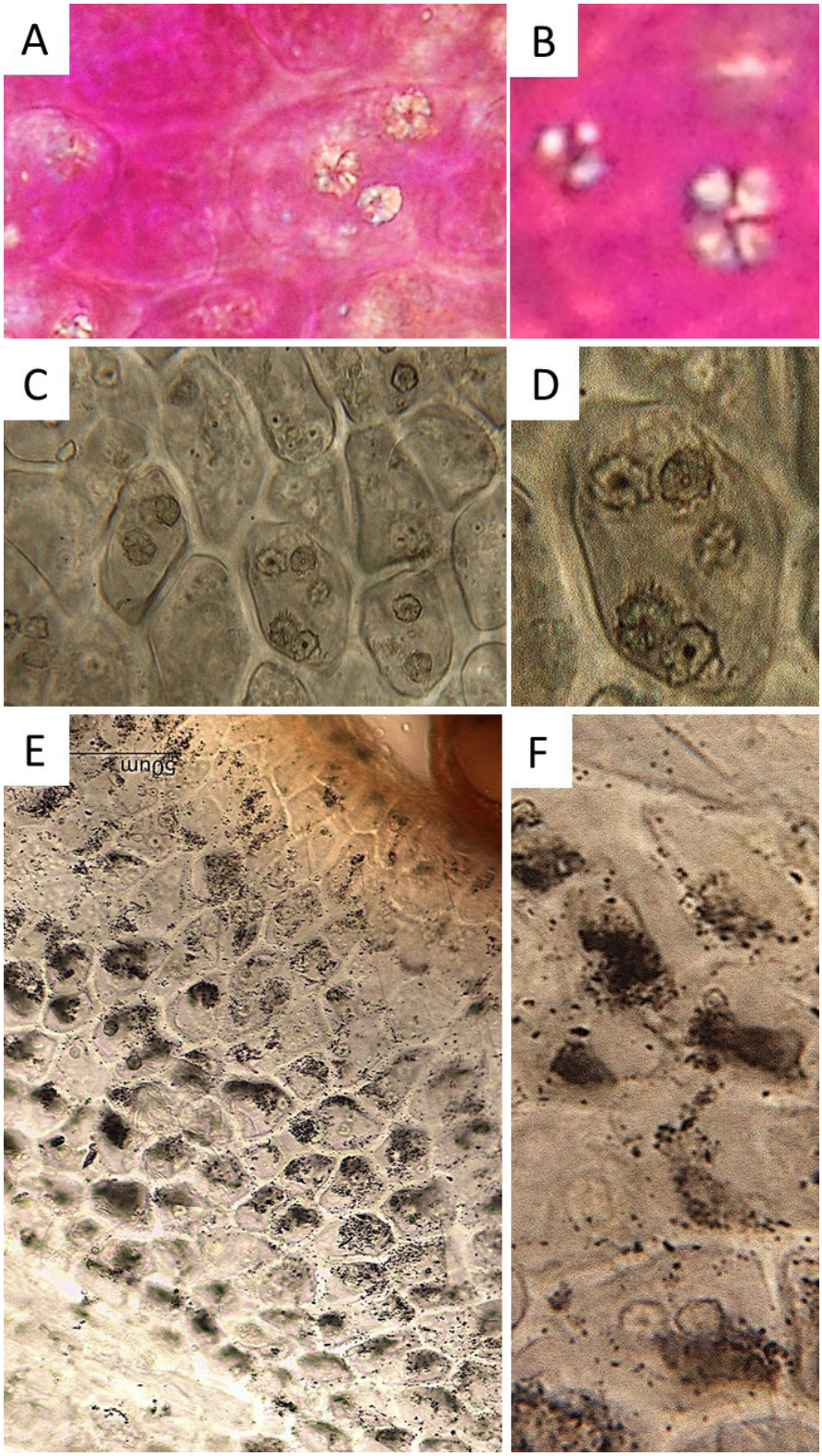

Figure 5 – Crystalline inclusions in embryo tissues

Note: A — small druses in seed endosperm cells under polarization microscopy (× 1000); B — four-rayed druse structure (× 1000); C — view of druses using dark-field condenser (× 1000); D — structure of individual druses (× 1000); E — starch inclusions in embryo parenchyma after Lugol's solution staining (× 400); F — individual parenchyma cells with starch inclusions (× 400).

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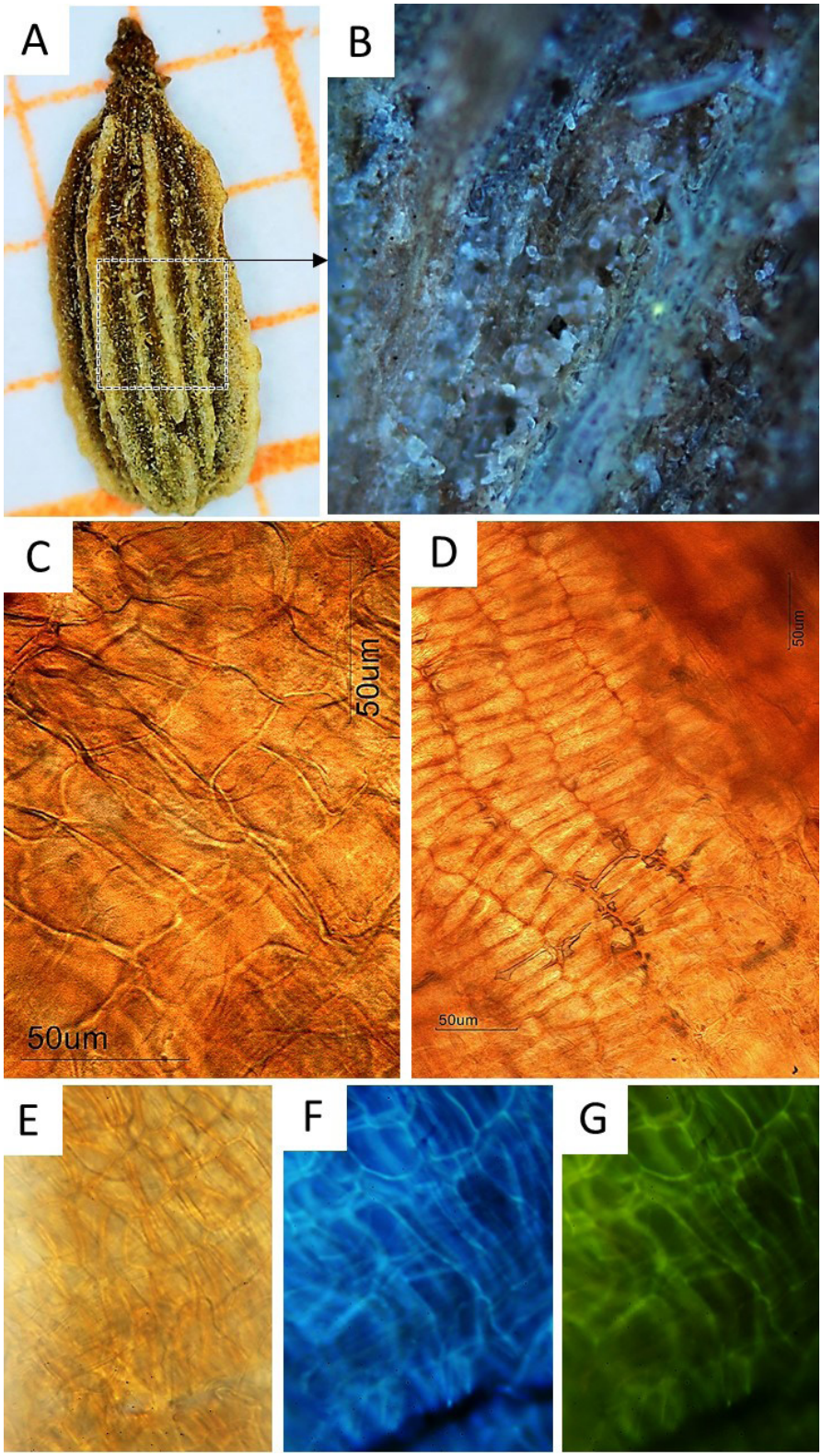

Figure 6 – Exocarp surface of Wild Carrot Fruits

Note: A — absence of pubescence on the exocarp (× 20); B — alternating fluorescence of the exo-/mesocarp and schizogenous ducts under UV light at λ = 330–400 nm (× 400); C — exocarp cells (× 400); D — "parquet endocarp" cell arrangement (× 400); E — surface of exocarp cells under visible light (× 400); F — fluorescence of exocarp cells under UV light at λ = 330–400 nm (× 400); G — fluorescence of exocarp cells under UV light at λ = 420–550 nm (× 400).

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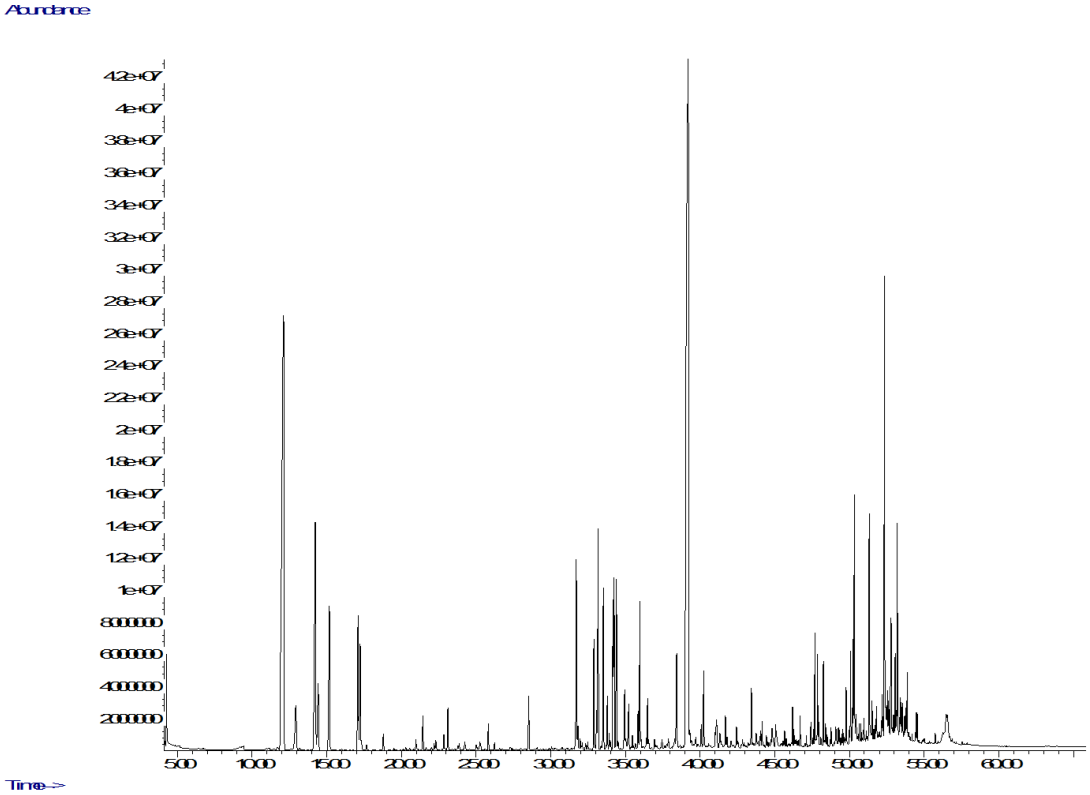

Figure 7 – GC–MS Chromatogram of the hexane fraction of the aqueous-ethanol extract from Wild Carrot Fruits

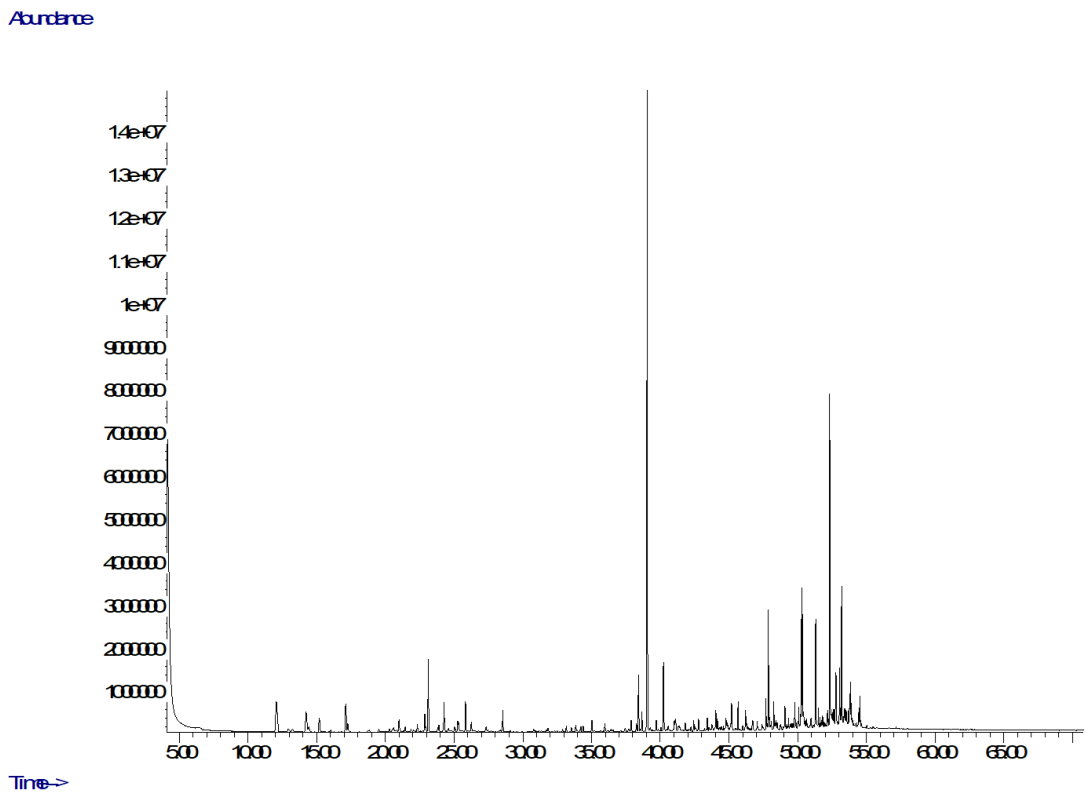

Figure 8 – GC–MS Chromatogram of the chloroform fraction of the aqueous-ethanol extract from Wild Carrot Fruits

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Under UV excitation at 330–400 nm, the druses do not fluoresce. However, the amorphous body of the protoplast emits bluish-blue fluorescence. Toward the periphery of the endosperm, round structures are observed within the protoplasts, showing red fluorescence likely associated with carotenoids and chlorophylls (Fig. 4G–I).

Druses are poorly visible under classical light microscopy (Fig. 5C–D), so polarization microscopy with a dark-field condenser and polarizing filter was used. Under these conditions, numerous druses become clearly visible in nearly every cell of the seed tissue. They are small, round, and up to 10 µm in diameter. Notably, the druses exhibit a unique star-shaped morphology with four symmetrical rays. A distinct central division into four segments is also observed (Fig. 5A–B). This specific druse structure is a valuable diagnostic marker and may be used for taxonomic identification and authentication of “Wild Carrot Fruits” raw material.

Upon treating transverse mericarp sections with ethanol-based Lugol’s solution, small round inclusions stained from dark blue to nearly black are revealed, characteristic of starch (Fig. 5E–F). However, due to their small size and non-specific structure, these inclusions are not suitable as diagnostic features.

When examined under a × 5 or × 10 hand lenses, the surface of the wild carrot fruit appears glabrous (non-pubescent). Observation of the dry fruit surface under UV excitation at 330–400 nm reveals alternating blue fluorescence of the exo- and mesocarp with brown fluorescence of the schizogenous ducts (Fig. 6A–B).

At higher magnification (× 400), the exocarp cells covering the fruit appear thin-walled, parenchymatous, and angular — almost rectangular in shape — with no intercellular spaces. The protoplasts are not detectable.

Upon focusing deeper into the tissue or examining squashed preparations, the endocarp structure becomes visible. The endocarp is composed of elongated, thick-walled cells tightly packed and aligned side-by-side. This distinctive pattern is commonly referred to as a “parquet endocarp” and serves as an important diagnostic feature for species identification. Under visible light, the exocarp surface cells show weak yellow to pale orange coloration. Their fluorescence under UV light is consistent with the previously described cross-sectional fluorescence (Fig. 6C–G).

Chromatographic–Mass Spectrometric Analysis

The GC–MS analysis of wild carrot fruit extracts allowed the identification of the volatile organic compound (VOC) profile. The component composition of the hexane and chloroform fractions of the aqueous-ethanol extract is presented in Table 2. Chromatograms are shown in Figures 7 and 8.

According to the data in Table 2, the predominant component in both fractions was carotol, with an average relative content of 25.9% in the hexane extract and 30.1% in the chloroform extract. Significant differences were noted in the component composition of the two extracts. The hexane extract was rich in monoterpenes (such as α-pinene, sabinene, β-myrcene) and sesquiterpenes (caryophyllene, β-sesquiphellandrene, β-gurjunene). In contrast, the chloroform extract was dominated by oxygenated compounds of essential oils (alcohols, ketones, and esters of monoterpenes and sesquiterpenes).

Numerical Quality Indicators

Determination of numerical quality indicators was performed for wild carrot fruit herbal raw material (MPRM) in two forms: whole and powdered. The loss on drying was preliminarily calculated and amounted to 5.8%. The test results presented in Table 3 allow us to establish criteria for the quality of raw materials.

The given numerical indicators made it possible to establish differences in the content of essential oil and extractive substances in whole and crushed raw materials, which will serve as a justification for choosing approaches to standardization and developing a draft regulatory document “Wild Carrot Fruits”.

DISCUSSION

A morphological and anatomical study of wild carrot fruits confirmed the literature data on the structure of lop-sided plants [9]. The main diagnostic features are the shape and size of mericarps, the number of longitudinal ribs, their color and prominence. We have described for the first time crystalline inclusions in the cells of the embryo parenchyma, represented by druses of a special stellate shape, having four distinct symmetrical rays. In the center of the druse, there is a visually formed division into four parts. The specific structure of the druze is diagnostic in nature and can be used in the process of taxation and authentication of raw materials of “Wild carrot fruits”. Studies of the luminescence of

wild carrot fruit tissues were carried out for the first time. The specificity of the luminescence of secretory canals, seed coat, embryo parenchyma has been proven, and the connection of luminescence with the chemical composition of protoplasts, as well as the release of secretory tissues, has been predicted. The data obtained allow us to develop the sections "Identification. External signs" and "Identification. Microscopic signs" in the draft FS "Wild Carrot Fruits".

According to the results of our research, the predominant components are carotol, α-pinene, daukol, sabinene, β-sesquifellandren. The results of the gas chromatographic analysis generally correspond to the known scientific literature data on the component composition of the essential oils of the studied fruits. The components present in *D. carota* L., include quercetin, pyrrolidine, daucine, daucosterol, tiglinic acid and essential oils, the main components of which are carotene, daucol, copaenol, gerenol, citric acid, pinenic acid and cineolic acid, while for the first three compounds the corresponding percentages are 30.55%, 12.60% and 0.62% [21].

R. Chizzola in the fruits of samples grown in Vienna, found that the predominant components are α-pinene (from 23.5% to 30.4%), sabine (from 21.5% to 46.6%), geranyl acetate (from 3.9% to 28.1%), β-pinene (3–13.1%), α-thuyene (1–8,8%), γ-terpinene (0.3–4.1%), myrcene (3.4–3.9%), carotene (1.2%) [22]. The main components of wild carrot essential oil samples from Morocco were α-pinene (22.2–23.5%), β-azarone (15.1–16.7%), sabinene (1.2–12.4%), β-bisabolene (4.0%) [23, 24];

The fruits of Daucus carota ssp. carota growing in Uzbekistan were dominated by carotene (69.8%), daucene (9%), trans-α-bergamotene (4.7%), trans-β-farnesene (3.7%), in Portugal — geranyl acetate (28.7–65%), α-pinene (13–27.1%), 11aH-himachal-4-en-1-β-ol (0.5–9.4%), Limonene (1.2–9%), β-pinene (2.3–4.5%). The fruits of other subspecies of *Daucus carota* L. contain such components as geranyl acetate (ssp. major, gummifer, maximus), α-pinene (ssp. major, halophilus, gummifer, maximus), α-azarone (ssp. maximus), and carotol (ssp. gummifer) [25].

The variety of main components and their relative content may be due to the peculiarities of obtaining essential oils, as well as the time and place of harvesting, the influence of climatic conditions, soil chemistry, and other factors. Differences in the component composition can lead to changes in the pharmacological activity of raw materials harvested at different times and in different geographical regions. Nevertheless, it is possible to identify the components that were found in most samples: α-pinene, sabinene, carotol, which can act as markers of MPRM, which allows the development of sections "Identification. Determination of the main groups of biologically active substances" and "Tests. Quantitative definition" in the PhA draft.

The established characteristics of numerical commodity indicators (weight loss during drying, essential oil content, extractive substances content, total ash, ash insoluble in 10% hydrochloric acid, sulfate ash, heavy metal content) indicate the quality of the MPRM and allow the development of the "Tests" section.

Study Limitations

To develop a draft of the PhM "Wild Carrot Fruit" and clarify the quality of raw materials, it is necessary to determine numerical quality indicators and evaluate the component composition of biologically active substances in samples harvested in various geographical regions and climatic zones.

CONCLUSION

Morphological and anatomical analysis performed using modern approaches to microscopic analysis has established the characteristics of carrot fruits: the oval shape of the fruit, narrowed to the tip, brown in color with seven veins; angular schizogenic receptacles with non-cellular partitions; endosperm parenchyma of isodiametric, oval cells; parquet-type endocarp of elongated, thick-walled, tightly closed cells stellate druses in the tissues of the embryo with four symmetrical rays. Luminescence in the excitation range of 330–400 nm mesocarp − bright blue; Epithelial cells are light yellow; tarry secretions are blue.

Chromatography-mass spectrometric study of hexane and chloroform fractions extracted from wild carrot fruits determined the profile of volatile compounds. It was found that the predominant component in both fractions is sesquiterpene alcohol carotol (25.9% and 30.1%, respectively), which is a marker compound in the composition of the studied MPRM.

The identified microscopic features, the main biologically active substance and numerical quality indicators are of practical importance for inclusion in the draft pharmacopoeial monograph "Wild carrot fruits".

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CONFLICT OF INTERESTS

The authors declare no conflict of interest.

AUTHORS CONTRIBUTION

Vera V. Artemyeva — research conducting, draft writing and editing; Gada M. Dandash — research conducting, preparing raw materials, draft writing and editing; Ifrat N. Zilfikarov — developing the concept, scientific guidance, draft writing and editing; Denis I. Shishkalov — research conducting, correction of photographic materials, draft writing and editing Vitaly M. Ryzhov — conducting research on anatomy and morphology, preparing and correcting photographic materials, draft writing and editing; Tatyana K. Ryazanova — conducting chemical composition research, processing primary data, draft writing and editing; Inna I. Bochkareva — research conducting, draft writing and editing; Ziyarat A. Guseinova — research conducting, draft writing and editing; Artur K. Arutyunov — research conducting, draft writing and editing. All authors confirm that their authorship meets the international ICMJE criteria (all authors have made significant contributions to the development of the concept, research and preparation of the article, read and approved the final version before publication).

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Results of the study of antiviral activity of ASD substance in experimental infection of mice with influenza viruses

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The aim. To evaluate the antiviral efficacy of ASD drug on a model of lethal influenza pneumonia in mice against influenza A viruses of subtypes H3N2, H5N2 and H5N8.

Materials and methods. Balb/c mice were infected with influenza viruses. The decrease of mortality and improvement in the weight dynamics of infected animals (15 animals per group) were evaluated, and the viral load in the lung tissue was determined on day 3 after infection (6 animals per group). Oseltamivir was used as a comparator medicine, and placebo was administered to negative control group animals. The medicines were administered according to a therapeutic-prophylactic or therapeutic regimen.

Results. The maximum weight loss with the H5N8 virus infected mice (placebo group) was 16.8% on day 12 (therapeutic-prophylactic regimen) and 14.8% on day 9 (therapeutic regimen). ASD worsened the dynamics of weight loss, up to 21% on day 12 (therapeutic-prophylactic regimen) and up to 28.4% on day 9 (therapeutic regimen). The maximum weight loss with the H5N2 virus infected mice was 34.6% on day 8 (therapeutic-prophylactic regimen) and 31.2% on day 7 (therapeutic regimen). The ASD medicine reduced the dynamics of weight loss: up to 29.9% on day 8 (therapeutic-prophylactic regimen) and up to 26.3% on day 7 (therapeutic regimen). The maximum weight loss with the the H3N2 virus was 38.4% on day 11 (therapeutic-prophylactic regimen) and 33.9% on day 9 (therapeutic regimen). The ASD medicine improved the dynamics of weight loss to 18.5% on day 11 (therapeutic-prophylactic regimen), but with the therapeutic regimen, it worsened the dynamics to 34.1% on day 9. A decrease of the survival rate of mice infected with the H5N8 virus using ASD (therapeutic regimen) was revealed. Mice receiving ASD and infected with the H3N2 virus (therapeutic-prophylactic regimen) or H5N2 (therapeutic regimen) showed a tendency towards a protective effect of the drug. No effect of the ASD medicine on the level of viral load was noted.

Conclusion. The ASD medicine exhibits antiviral properties when administered orally according to a therapeutic-prophylactic regimen against influenza A virus (subtypes H3N2 and H5N2).

Keywords: influenza virus; antiviral activity; in vivo testing; lethal influenza pneumonia

Abbreviations: ASD — antiseptic-stimulator Dorogova, fraction 2; ED50 — median effective dose — the dose of the drug that improves the survival rate of animals by 50%; PI — protection index — calculated indicator of drug effectiveness; MLD50 — 50% mouse lethal dose of the virus — the dose of the virus that causes the death of half of the mice in the group; OD — optical density; SOP — standard operating procedure; ALS — average life span; TCID50 — 50% tissue infectious dose — the dose of the virus that causes infection of 50% of cells; ANOVA — Analysis Of Variance; DPBS — Dulbecco's Phosphate-Buffered Saline; PBS — Phosphate-Buffered Saline.

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Результаты исследования противовирусной активности субстанции АСД при экспериментальном заражении мышей вирусами гриппа

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Цель. Оценка противовирусной эффективности препарата АСД на модели летальной гриппозной пневмонии у мышей в отношении вирусов гриппа А подтипов H3N2, H5N2 и H5N8.

Материалы и методы. Мышей линии Balb/c инфицировали вирусами гриппа. Оценивали снижение смертности и улучшение динамики веса зараженных животных (по 15 особей в группе), также определяли вирусную нагрузку в легочной ткани на 3 сут после заражения (по 6 особей в группе). Препаратом сравнения служил осельтамивир, животным из группы отрицательного контроля вводили плацебо. Препараты вводили по лечебно-профилактической или лечебной схеме.

Результаты. При инфицировании вирусом H5N8 максимальная потеря веса (группа плацебо) составила 16,8% на 12 сут (лечебно-профилактическая схема) и 14,8% на 9 сут (лечебная схема). АСД ухудшал динамику снижения веса, до 21% на 12 сут (лечебно-профилактическая схема) и до 28,4% на 9 сут (лечебная схема). При инфицировании вирусом H5N2 максимальная потеря веса составила 34,6% на 8 сут (лечебно-профилактическая схема) и 31,2% на 7 сут (лечебная схема). Препарат АСД уменьшал динамику снижения веса: до 29,9% на 8 сут (лечебно-профилактическая схема) и до 26,3% на 7 сут (лечебная схема). При инфицировании вирусом H3N2 максимальная потеря веса составила 38,4% на 11 сут (лечебно-профилактическая схема) и на 33,9% в на 9 сут (лечебная схема). Препарат АСД улучшал динамику снижения веса до 18,5% на 11 сут (лечебно-профилактическая схема), но при лечебной схеме ухудшал динамику до 34,1% на 9 сут. Обнаружили снижение выживаемости мышей, зараженных вирусом H5N8, при использовании АСД (лечебная схема). У мышей, получавших АСД, и зараженных вирусом H3N2 (лечебно-профилактическая схема) или H5N2 (лечебная схема) отметили тенденцию к протективному действию препарата. Влияния препарата АСД на уровень вирусной нагрузки не отметили.

Заключение. Препарат АСД проявляет противовирусные свойства при пероральном введении по лечебно-профилактической схеме в отношении вируса гриппа А (подтипы H3N2 и H5N2).

Ключевые слова: вирус гриппа; противовирусная активность; тестирование *in vivo*; летальная гриппозная пневмония

Список сокращений: АСД — антисептик-стимулятор Дорогова, фракция 2; ЕД50 — средняя эффективная доза — доза препарата, которая улучшает выживаемость животных на 50%; ИЗ — индекс защиты — расчетный показатель эффективности препарата; МЛД50 — 50% мышиная летальная доза вируса — доза вируса, вызывающая гибель половины мышей в группе; ОП — оптическая плотность; СОП — стандартная операционная процедура; СПЖ — средняя продолжительность жизни; ТИД50 — 50% тканевая инфекционная доза — доза вируса, вызывающая заражение 50% клеток; ANOVA — Analysis Of Variance, анализ достоверности различий между группами; DPBS — фосфатно-солевой буферный раствор по протоколу Дульбекко; PBS — фосфатно-солевой буферный раствор.

INTRODUCTION

The influenza virus causes a highly contagious respiratory disease, which is the cause of annual epidemics, affecting up to half of the world's population. Every year, about 1 billion cases of seasonal influenza are registered in the world, 3–5 million of which are severe. From 290 to 650 thousand people die annually from respiratory pathologies caused by influenza viruses [1]. Influenza is of particular importance due to the high virulence of the pathogen, the ability to pandemic spread, viral variability, and the development of complications. The most common complication of influenza is pneumonia, which accounts for 65% of all complications and often ends in death [2]. In addition, influenza can cause a number of non-respiratory complications, including febrile seizures, Reye's syndrome, and myocarditis [3–5].

According to a large meta-analysis conducted

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by K.E. Lafond et al. (2021), influenza viruses cause more than 5 million hospitalizations worldwide each year. They also found out that influenza accounts for an average of 14.1% of hospitalizations for acute respiratory diseases among the adult population, with no significant differences between age groups [6].

Despite the fact that severe influenza can be observed at any age, children are the most vulnerable. The incidence rate remains consistently high in the pediatric population [7, 8]. Every year, about 870,000 children under the age of 5 are hospitalized worldwide due to influenza [9]. A meta-analysis conducted by H. Nair et al. (2011) found that from 28,000 to 111,500 deaths among children under the age of 5 are associated with causes related to influenza morbidity, and the vast majority of them occur in developing countries [10]. P.J. Gill et al. (2015) assessed which children are at increased risk of developing complications from influenza. It was found out that prematurity, neurological disorders, sickle cell anemia, immunosuppression, diabetes and age under 2 years are risk factors for hospitalization. In addition, having more than one of these factors increased the risk of hospitalization from 52 to 74% [11].

Risk groups for severe influenza also include elderly people (aged ≥ 60 years), pregnant women, people with chronic diseases or congenital/acquired immunodeficiency [12].

This work is devoted to the study of the antiviral activity of the drug ASD-2 *in vivo* on a model of lethal influenza pneumonia in Balb/c mice.

In this work, a model of lethal influenza pneumonia was used, which is generally accepted in studies of the protective activity of chemotherapeutic agents intended for the treatment and prevention of influenza infection [13]. At the same time, the main indicators by which the effectiveness of drug samples was assessed were the dynamics of mortality of experimental animals and the dynamics of weight indicators. The viral load in the lung tissue was also assessed to confirm the specificity of the drug's action against the virus.

THE AIM. To evaluate the protective efficacy *in vivo* of the drug ASD on a model of lethal influenza pneumonia in Balb/c mice against influenza A viruses of subtypes H3N2, H5N2 and H5N8.

Research tasks:

1. Adaptation to Balb/c mice of influenza virus A/common duck/Uvs Nuur lake/26/2016 A (H5N8);
2. Adaptation to Balb/c mice of influenza virus A/Duck/Potsdam/1402-6/86 (H5N2);
3. Adaptation to Balb/c mice of influenza virus A/Aichi/2/68 (H3N2);
4. Determination of the dynamics of animal death when the test drug is administered according to a therapeutic and prophylactic regimen against influenza viruses A/common duck/Uvs Nuur lake/26/2016 (H5N8), A/Duck/Potsdam/1402-6/86(H5N2), A/Aichi/2/68(H3N2);
5. Determination of the dynamics of animal death when the test drug is administered according to a therapeutic regimen against influenza viruses A/common duck/Uvs Nuur lake/26/2016 (H5N8), A/Duck/Potsdam/1402-6/86(H5N2), A/Aichi/2/68(H3N2);
6. Determination of the dynamics of changes in body weight of animals when the test drugs are administered according to a therapeutic and prophylactic regimen against influenza virus A/common duck/Uvs Nuur lake/26/2016 (H5N8), A/Duck/Potsdam/1402-6/86(H5N2), A/Aichi/2/68(H3N2);
7. Determination of the dynamics of changes in body weight of animals when the test drugs are administered according to a therapeutic and prophylactic regimen against influenza virus A/common duck/Uvs Nuur lake/26/2016 (H5N8), A/Duck/Potsdam/1402-6/86(H5N2), A/Aichi/2/68(H3N2);
8. Determination of the viral load in the lung tissue of infected animals on the 3rd day after infection.

MATERIALS AND METHODS

Study design

The study was conducted on the basis of the Smorodintsev Research Institute of Influenza from December 2021 to June 2022.

The study drug ASD (antiseptic-stimulator Dorogova, fraction 2), manufacturer LLC "AVZ S-P", Sergiev Posad. The control drug is oseltamivir (Tamiflu®) LaRoche, Switzerland (positive control). Date of manufacture: 04.2016. Series: M1030. Expiry date: 04.2023. Dosage form: capsules of 75 mg. Storage conditions: in the refrigerator at a temperature of 2–8°C. Placebo (negative control) — saline solution.

The study was conducted on 423 female Balb/c mice aged 5–7 weeks weighing 19.2 8± 0.29. The choice of this line is fully consistent with the methodological documents used in the work[1, 2, 3]. When determining

1 Guidelines for conducting preclinical studies of drugs. Part one. Mironov AN, editor; Moscow: Grif and K; 2012. 944 p. Russian
2 GOST 33215-2014. Guidelines for the care and maintenance of laboratory animals. Rules of equipment of premises and organization of procedures; Introduced on 1.07.2016; Moscow: Standartinform; 2016. 12 p. Russian
3 Ivanov YuI, Pogorelyuk ON. Processing of the results of biomedical research on microcalculators according to programs. Moscow: Meditsina Publ.; 1990. 217 p. DOI: 10.19163/2307-9266-2025-13-5-385-402. Russian

the protective activity (reducing mortality and improving the weight dynamics of infected animals), 15 individuals were used in the group, to determine the viral load in the lung tissue — 6 individuals in the group. Mice were obtained from the "Stolbovaya", Moscow region. During the period of acclimatization and experiment, mice were placed in polycarbonate cages BENEX a.s., Czech Republic, type T3A, S = 1200 cm^2, in groups of 3 individuals (at the first stage) and 15 individuals (at the second stage), on bedding (wood granules); the cages are covered with steel lattice lids with a feeding recess. Granulated feed for keeping mice (LLC "Laboratorkorm", Moscow) was given ad libitum in the feeding recess of the steel lattice lid of the cage. The animals were given water purified by reverse osmosis on a MilliporeRiOs 30 water purification unit. Water in standard drinkers with steel lids-spouts was given ad libitum. Wood granules were used as bedding. The animals were kept in separate rooms of the vivarium of the Smorodintsev Research Institute of Influenza in controlled conditions (18–24°C and relative air humidity 50–80%). The photoperiod was 12 hours night / 12 hours day with artificial lighting with fluorescent lamps. Animal care and maintenance was carried out in accordance with SOP No. B/004/01, adopted at the Smorodintsev Research Institute of Influenza.

When determining the protective activity (reducing mortality and improving the weight dynamics of infected animals), 15 individuals were used in the group, to determine the viral load in the lung tissue — 6 individuals in the group.

Distribution by groups

Animals were distributed into groups by simple randomization according to body weight (individual body weight was within the range of variation ±10 % of the average value of the indicator). This range was chosen to ensure the homogeneity of experimental groups and exclude the influence of individual characteristics of animals on the outcome of the experiment.

For the purpose of identification, each animal in the group was marked with an individual number, which is recorded on the cage card. For marking, a biological dye (brilliant green "Lekker" BZ-3), safe for mice, was used. The following marking rules were followed: 1: left upper paw, 2: left side, 3: left lower paw, 4: head, 5: back, 6: tail, 7: right upper paw, 8: left side, 9: left lower paw, 10: no coloring, 11: left and right upper paws, 12: left and right side, 13: left and right lower paws, 14: head and tail, 15: strip from head to tail.

Laboratory animals were kept for 5 days before the start of the study for adaptation with group housing in cages. During this period, the clinical condition of the animals was monitored every day by visual examination; coat condition, presence of skin lesions, and mobility were assessed. Animals with abnormalities detected during the examination were not included in the experimental groups.

Euthanasia and ethics approval

At the end of the experiment, the animals were subjected to planned euthanasia by overdose of CO_2. Euthanasia was completed by displacement of the cervical vertebrae (cervical dislocation). All procedures with animals in the study were reviewed and approved by the Bioethics Commission of the Smorodintsev Research Institute of Influenza for compliance with regulatory acts (Minutes of the meeting of the Bioethics Commission of the Smorodintsev Research Institute of Influenza No. 49 dated March 04, 2022).

Infectious cultures

The following influenza virus strains were used for the work: A/common duck/Uvs Nuur lake/26/2016 (H5N8), A/Duck/Potsdam/1402-6/86(H5N2), A/Aichi/2/68(H3N2). All strains were obtained from the influenza virus collection of the Laboratory of Chemotherapy of Viral Infections of the Smorodintsev Research Institute of Influenza.

To determine the viral load in the lungs of animals, the MDCK (Madin-Darby canine kidney) cell culture of the spaniel kidney was used, as the most sensitive and permissive to various human influenza viruses [14]. The source of the cell culture is the MDCK London Line cell line (passage 8 / 8) was obtained from the Influenza Reagent Resource, CDC&P, Atlanta, Georgia, USA (cat. No. FR-58). The cells were cultured on a nutrient medium of the following composition: Alpha MEM medium (Biolot, Russia) with the addition of 5% fetal bovine serum (Biowest, USA) and a mixture of penicillin / streptomycin antibiotics at a concentration of 1% (Biolot, Russia).

Experimental design for adaptation of influenza viruses (stage 1)

To adapt the virus to animals, 5 passages were carried out through the lungs of mice. For each virus, the adaptation process was carried out as follows: three animals were infected with the corresponding strain in a dilution of 10:1, on the 3 day after infection, the animals were euthanized and the lungs were taken,

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homogenized, and the resulting suspension was used for subsequent infection.

The influenza virus after adaptation was propagated in the allantoic cavity of 10-day-old developing chicken embryos, after which the liquid was collected, clarified by centrifugation (Eppendorf 5424 centrifuge [Eppendorf, Germany], 5 min at 3000 rpm) and packaged into 1 ml aliquots. All aliquots were made from a single stock of allantoic fluid and frozen simultaneously at –80 °C. A preliminary titration of the virus on Balb/c mice was carried out to determine the 50 % mouse lethal dose of the virus (MLD50) (Tables 1 and 2).

Experimental design for determining antiviral activity (Stage 2)

For the experiment, the drug was diluted according to the following scheme: 0.1 mL is diluted in 39.9 mL of warm water. This dilution scheme allows to obtain the required concentration of the solution when using the minimum amount of the test. The dose was 0.8 mL of the resulting solution per mouse weighing 20 g. The specified volume corresponded to the calculated dosage of the drug, providing the required load of the active substance on the body weight of the animal and a sufficient level of exposure for the implementation of the pharmacological effect. The test drug, or placebo, (depending on the randomization group) was administered fractionally orally using a probe for oral administration 2 times/day with an interval of 6 hours in a volume of 0.4 mL.

All drugs were administered according to a *therapeutic and prophylactic regimen* (10 days before infection, then: for 10 days after infection) and according to a therapeutic regimen (for 10 days after infection) 2 times/day with an interval of 6 hours. These administration regimens were developed and used in the framework of this study to achieve the experimental objectives. Observation of the animals was carried out for 14 days after infection.

The oseltamivir was used to control the specificity of the pathological process. Oseltamivir is a neuraminidase inhibitor with proven clinical efficacy in the treatment and prevention of influenza A and B. The drug is recommended for use in influenza by international health organizations, including the World Health Organization (WHO)[4] and the US Centers for Disease Control and Prevention (CDC)[5]. In clinical guidelines for the treatment of influenza[6, 7], in the Russian Federation, oseltamavir is also recommended for the treatment of influenza in adults and children. It has been proven that oseltamivir shortens the duration of the disease, reduces the viral load and the risk of complications, especially with early initiation of therapy [15, 16]. The effectiveness of the drug in influenza has been confirmed in large systematic reviews and meta-analyses [17, 18].

Oseltamivir was administered to animals orally using a gastric probe (Fisherbrand, USA) in a volume of 0.2 mL at a dosage of 20 mg/kg/day, according to a similar scheme as the test drugs.

In the placebo groups, animals were administered sterile saline solution orally.

Mice under light ether anesthesia were infected intranasally with the virus in a volume of 50 µL at a dose of 2 MLD50. Mice were weighed daily and the death of animals was recorded. The number of animals was 45 individuals at the first stage and 378 individuals at the second stage. The number of animals in each group was 21 individuals, of which 15 individuals were intended to assess mortality and weight dynamics during infection, and 6 individuals — to assess the viral load in the lungs. The general scheme of the experiment is presented in Tables 1 and 2, the manipulations performed with the animals are indicated in Tables 3 and 4.

Collection of organs and preparation of homogenates

On the 3 day after infection, 6 animals from each group were euthanized, dissected, their lungs were isolated and weighed. Lung tissue samples were homogenized in Dulbecco's phosphate-buffered saline solution using a TissueLyserII device (Qiagen, USA).

Virus titration on MDCK cell culture

To assess the level of virus reproduction in animal lung tissue samples, its infectious activity was titrated in MDCK cell culture (4 wells of a 96-well plate for each dilution of the tissue sample). MDCK cells were seeded on 96-well plates in a volume of 100 µL of cell

[4] WHO Guidelines for Pharmacological Management of Pandemic Influenza A(H1N1) 2009 and Other Influenza Viruses. Geneva: World Health Organization; 2010. Available from: https://www.ncbi.nlm.nih.gov/books/NBK138515/

[5] CDC. Treating Flu with Antiviral Drugs. Available from: https://www.cdc.gov/flu/treatment/antiviral-drugs.html

[6] The flu. Clinical Guidelines. 2025. Ministry of Health of the Russian Federation. Available from: https://cr.minzdrav.gov.ru/view-cr/249_2. Russian

[7] Clinical recommendations. Flu in adults. 2022. Ministry of Health of the Russian Federation. Available from: https://cr.minzdrav.gov.ru/view-cr/749_1. Russian

suspension with a cell concentration of 1×105 / mL, i.e. the final cell concentration was 1×104 cells / well. Then MDCK cells were incubated for 24 hours in a CO_2 incubator at 37 °C in an atmosphere of 5% CO_2 until a monolayer was formed. After that, the cells were washed for 5 min with alpha-MEM medium with glutamine and used for virus cultivation. A series of 10-fold dilutions (from 10^{-1} to 10^{-7}) were prepared from the homogenate of tissue samples on alpha-MEM medium with glutamine with the addition of trypsin (1 μg/mL), which is necessary for the successful penetration of the influenza virus into cells, and 20 μg/mL of ciprofloxacin (a broad-spectrum antimicrobial agent of the fluoroquinolone group) and introduced them into the wells of the plate with MDCK cells. The plates were incubated for 72 hours at 37 °C in an atmosphere of 5% CO_2.

After the incubation period, 100 μL of culture fluid from each well of the plate was transferred to the wells of 96-well plates with a round bottom for immunological reactions and 100 μL of 1% suspension of chicken erythrocytes in saline solution was added to each well. The suspension of chicken erythrocytes was obtained according to SOP No. LHT/019/01 from whole blood of Leghorn chickens. The plates were incubated for 1 hour at room temperature, after which the presence or absence of hemagglutination in the wells was visually assessed. The virus titer was calculated by the Reed and Mench method [19] and expressed in 50% tissue infectious doses (TCID50) per 100 μL of volume.

Statistical processing

The main criterion for evaluating antiviral activity is a statistically significant difference in the survival rate of mice in the drug group compared to the placebo group.

Secondary criteria for evaluating antiviral activity:

— a statistically significant difference in the weight loss of animals in the placebo group compared to the drug group;

— a statistically significant decrease in the viral load in the lungs of mice in the drug group compared to the placebo group.

The criterion for the adequacy of the experiment is a statistically significant difference in the survival rate of mice in the comparison drug group compared to the placebo group.

The research materials were statistically processing with methods of parametric and non-parametric analysis. Accumulation, correction, systematization of initial information and visualization of the results were carried out in Microsoft Office Excel 2016 spreadsheets. Statistical analysis was performed with free software environment for calculations R v. 4.0.2 in the RStudio Version 1.3.1056 program[8].

Based on the data obtained from measuring the weight of mice after infection with influenza viruses, survival tables were constructed, then the Kaplan-Meier method was used to construct survival curves [20]. Comparison of survival functions by groups was carried out using the Gehan-Wilcoxon criterion and using the Cox-Mantel log-rank criterion [21][9].

The data obtained as a result of measurements were combined into variational series, in which the calculation of arithmetic mean values (M) and standard deviations (SD) was carried out. Quantitative indicators were assessed for compliance with the normal distribution (n = 10), for this purpose the Shapiro–Wilk criterion was used [22, 23]. To determine the significance of differences between group means in samples with a data distribution different from the normal one, the Mann-Whitney criterion was used [24].

The protection index was calculated using formula 1.

$$PI = (Mc — Me) / Mc \times 100\% \quad (1),$$

where Mc and Me are the percentages of animal deaths in the placebo group and the experimental group receiving the drug under study or the control drug, respectively, at the end of the experiment (14 days after infection). This formula is widely used to calculate the protection index in preclinical studies [25, 26].

For graphical representation of data on the relative decrease in body weight of animals, the relative value of body weight in % to the weight on the day of infection (day 0) was calculated for each animal. Then the arithmetic mean for the group and a curve of the dependence of the group average on the day after infection was constructed. To determine the significance of differences between groups on the day of greatest weight loss in the placebo group after infection, one-way ANOVA analysis of variance was used for group comparison, then the Dunnett's criterion for a posteriori pairwise comparisons with the placebo group. Differences between groups were considered statistically significant if the *p-value* did not exceed 0.05.

[8] R Development Core Team (2008). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available from http://www. R-project.org.

[9] Cox DR, Oakes D. Lifetime type data analysis; Finance and Statistics; 1988. Russian

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RESULTS

Dynamics of body weight loss of experimental animals infected with influenza virus A/common duck/Uvs Nuur lake/26/2016 A (H5N8)

The study showed that infection with the influenza virus led to the development of a pathological process in laboratory mice. External signs of the disease were manifested in limiting the mobility of animals, increased breathing, as well as in reducing the consumption of food and water. All of these signs are typical for influenza pneumonia. The dynamics of changes in the body weight of animals with modeled influenza pneumonia is presented in Figure 1.

The maximum weight loss in the placebo group was 16.8 % on the 12 day after infection with therapeutic and prophylactic and 14.8 % on the 9 day with therapeutic regimens of administration.

The comparison drug oseltamivir reduced the weight loss in mice on the 12 day after infection compared to the Placebo group with the therapeutic and prophylactic regimen to 3.1 % and with the therapeutic regimen on the 9 day to 9.9 %.

The test drug ASD in both regimens of administration worsened the dynamics of weight loss in experimental animals, up to 21 % on the 12 day after infection and up to 28.4 % on the 9 day after infection in the therapeutic and prophylactic and therapeutic regimens of administration, respectively.

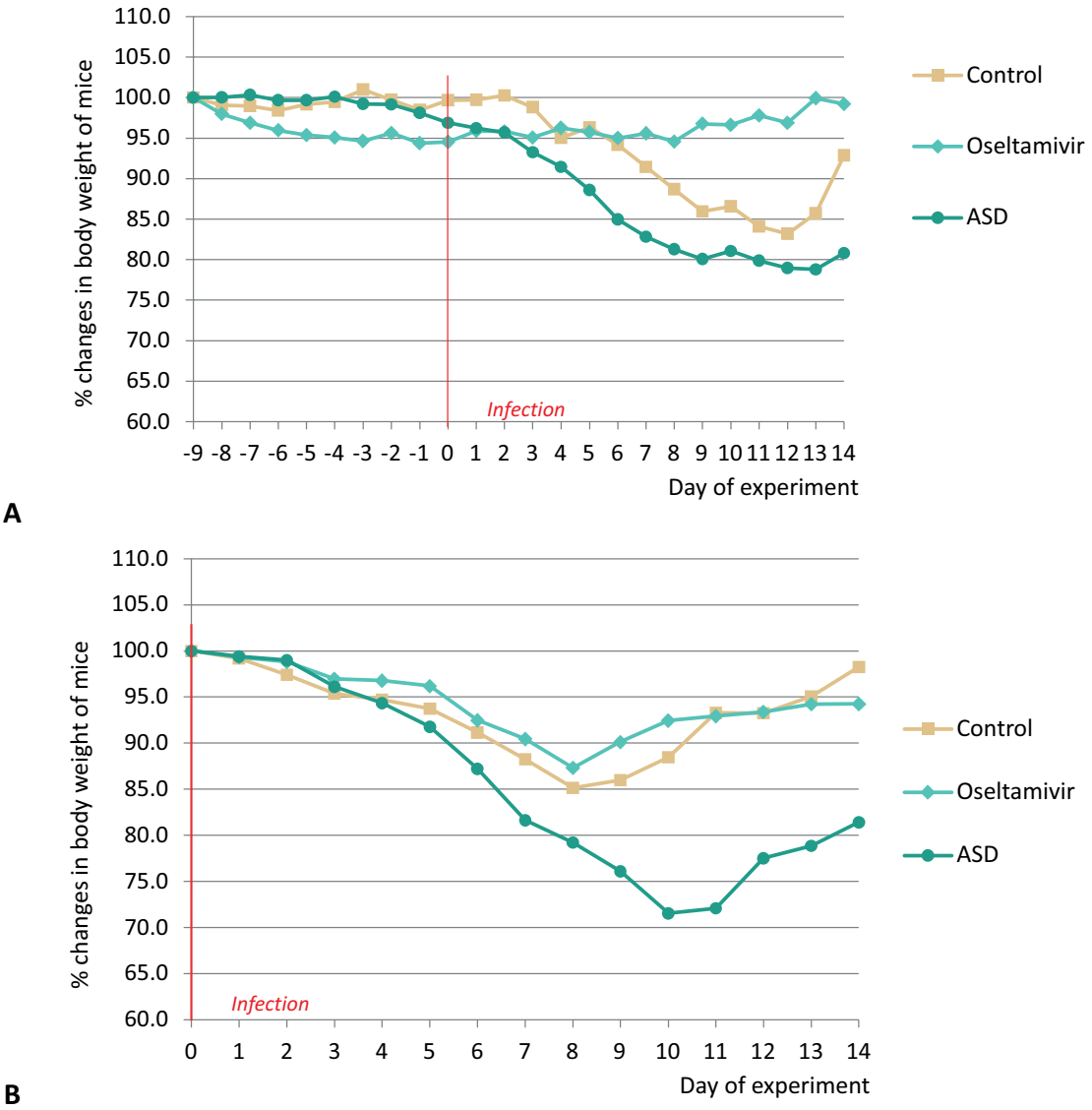

Figure 1 — Dynamics of changes in body weight of Balb/c mice with experimental influenza pneumonia caused by influenza virus A/common duck/Uvs Nuur lake/26/2016 A (H5N8) under conditions of using the test drugs according to a therapeutic and prophylactic regimen (n = 15).

Note: A — prophylactic regimen; B — therapeutic regimen.

Dynamics of body weight loss of experimental animals infected with influenza virus A/Duck/Potsdam/1402-6/86 (H5N2)

The study showed that infection with the influenza virus led to the development of a pathological process in laboratory mice. External signs of the disease were manifested in limiting the mobility of animals, increased breathing, as well as in reducing the consumption of food and water. All of these signs are typical for influenza pneumonia [27, 28]. The dynamics of changes in the body weight of animals with modeled influenza pneumonia is presented in Figure 2.

The maximum weight loss in the placebo group was 34.6 % on the 8 day after infection with therapeutic and prophylactic and 31.2 % on the 7 day with therapeutic regimens of administration.

The comparison drug oseltamivir reduced the weight loss in mice on the 8 day after infection compared to the placebo group with the therapeutic and prophylactic regimen to 24 % and with the therapeutic regimen on the 7 day to 15.5 %.

The test drug ASD in both regimens of administration slightly reduced the dynamics of weight loss in experimental animals, up to 29.9 % on the 8 day after infection and up to 26.3 % on the 7 day after infection in the therapeutic and prophylactic and therapeutic regimens of administration, respectively.

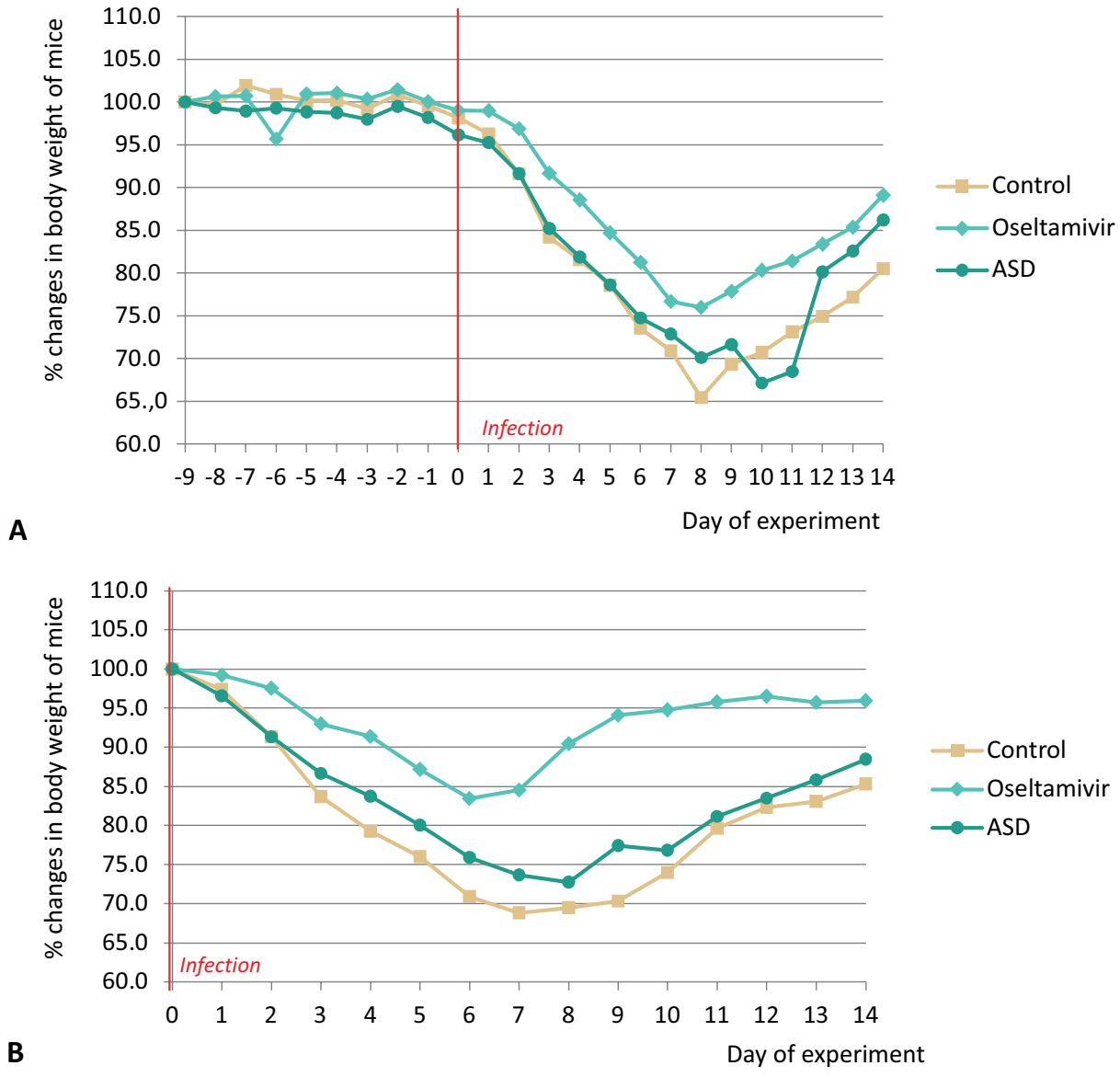

Figure 2 — Dynamics of changes in body weight of Balb/c mice with experimental influenza pneumonia caused by influenza virus A/mallard/Pennsylvania/10218/84(H5N2) under conditions of using the test drugs according to a therapeutic and prophylactic regimen (n = 15).

Note: A — prophylactic regimen; B — therapeutic regimen.

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Dynamics of body weight loss of experimental animals infected with influenza virus A/Aichi/2/68(H3N2)

The study showed that infection with the influenza virus led to the development of typical symptoms of the pathological process in laboratory mice. External signs of the disease were manifested in limiting the mobility of animals, increased breathing, as well as in reducing the consumption of food and water. All of these signs are typical for influenza pneumonia. The dynamics of changes in the body weight of animals with modeled influenza pneumonia is presented in Figure 3.

Infection with the influenza virus H3N2 led to weight loss in animals from all experimental groups. The maximum weight loss in the Placebo group was 38.4 % on the 11 day after infection with the therapeutic and prophylactic regimen and 33.9 % on the 9 day with the therapeutic regimen.

The comparison drug oseltamivir reduced the weight loss in mice on the 11 day after infection compared to the Placebo group with the therapeutic and prophylactic regimen to 9.6 % and with the therapeutic regimen on the 9 day to 14.9 %.

The test drug ASD administration improved the dynamics of weight loss in experimental animals, up to 18.5 % on the 11 day after infection with the therapeutic and prophylactic regimen of administration. With the therapeutic regimen of

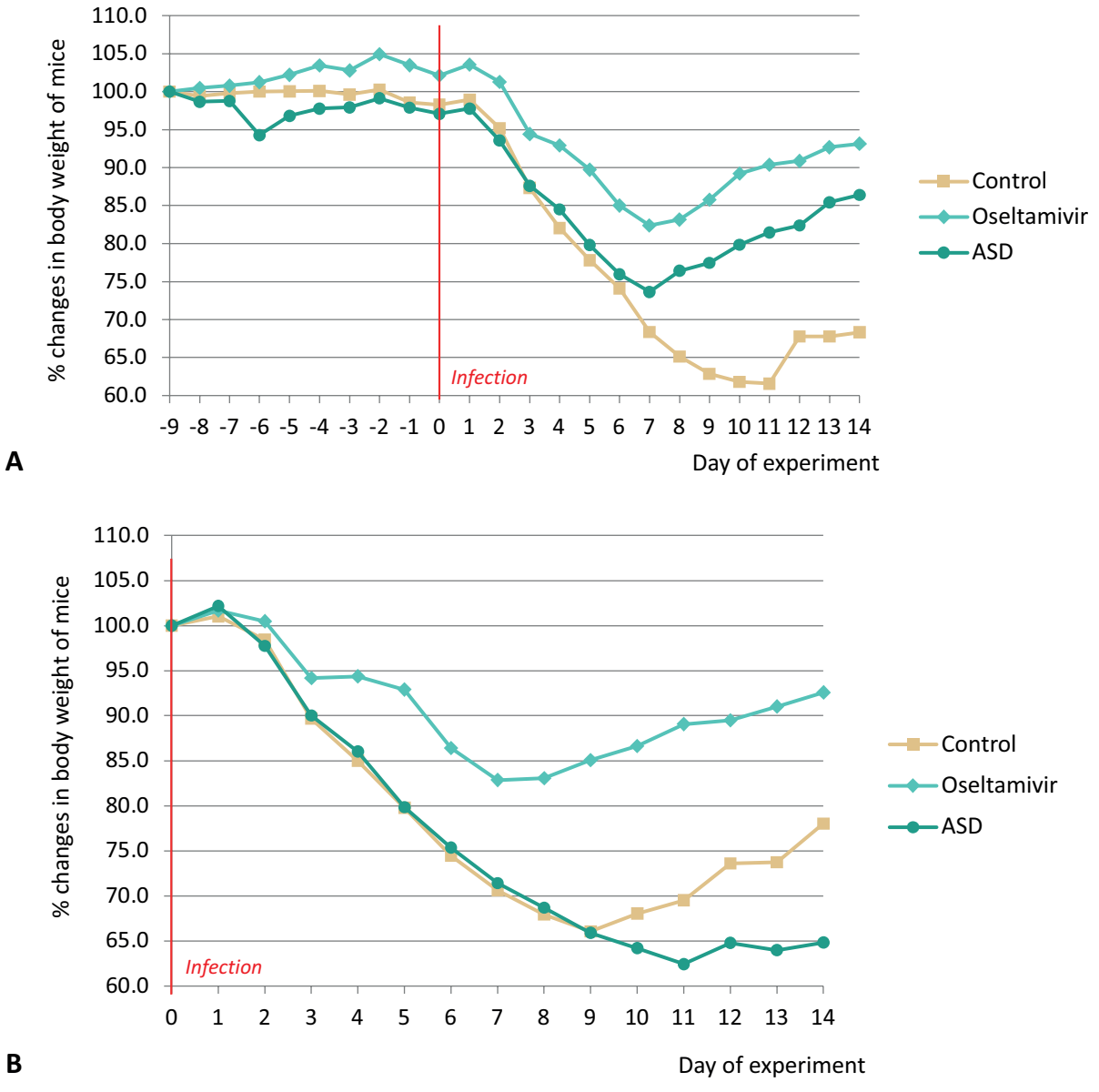

Figure 3 — Dynamics of changes in body weight of Balb/c mice with experimental influenza pneumonia caused by influenza virus A/Aichi/2/68 H3N2 under conditions of using the test drugs according to a therapeutic and prophylactic regimen (n = 15).

Note: A — prophylactic regimen; B — therapeutic regimen.

administration, the drug ASD worsened the dynamics of weight loss to 34.1 % on the 9 day after infection.

Statistical analysis of data obtained from the analysis of survival of mice after infection with influenza virus

In the course of survival analysis, fixed censoring was carried out: for each combination of "virus strain–treatment regimen–drug", a sample of 15 animals was observed for 14 days after infection. The identification of differences between the survival functions of mice receiving drugs and placebo in various experimental groups was carried out using the Gehan-Wilcoxon and Cox-Mantel tests. The proportions of surviving mice are presented in Tables 5–7. The results of comparing survival functions by groups are presented in Table 8, the dynamics of death are presented in Tables 9–11.

As it can be seen from Table 8, statistically significant differences in survival functions in a positive direction were found when compared with the placebo group of oseltamivir in the preventive and therapeutic regimen in the case of infection of mice with the H5N8 virus and in both regimens in the case of infection of mice with the H3N2 virus. In the case of the H5N2 virus, differences were also noted, although they were not statistically significant.

For the ASD, statistically significant differences from the placebo group were noted in the case of the H5N8 virus, when used according to the therapeutic regimen, and towards a worsening of the infectious process.

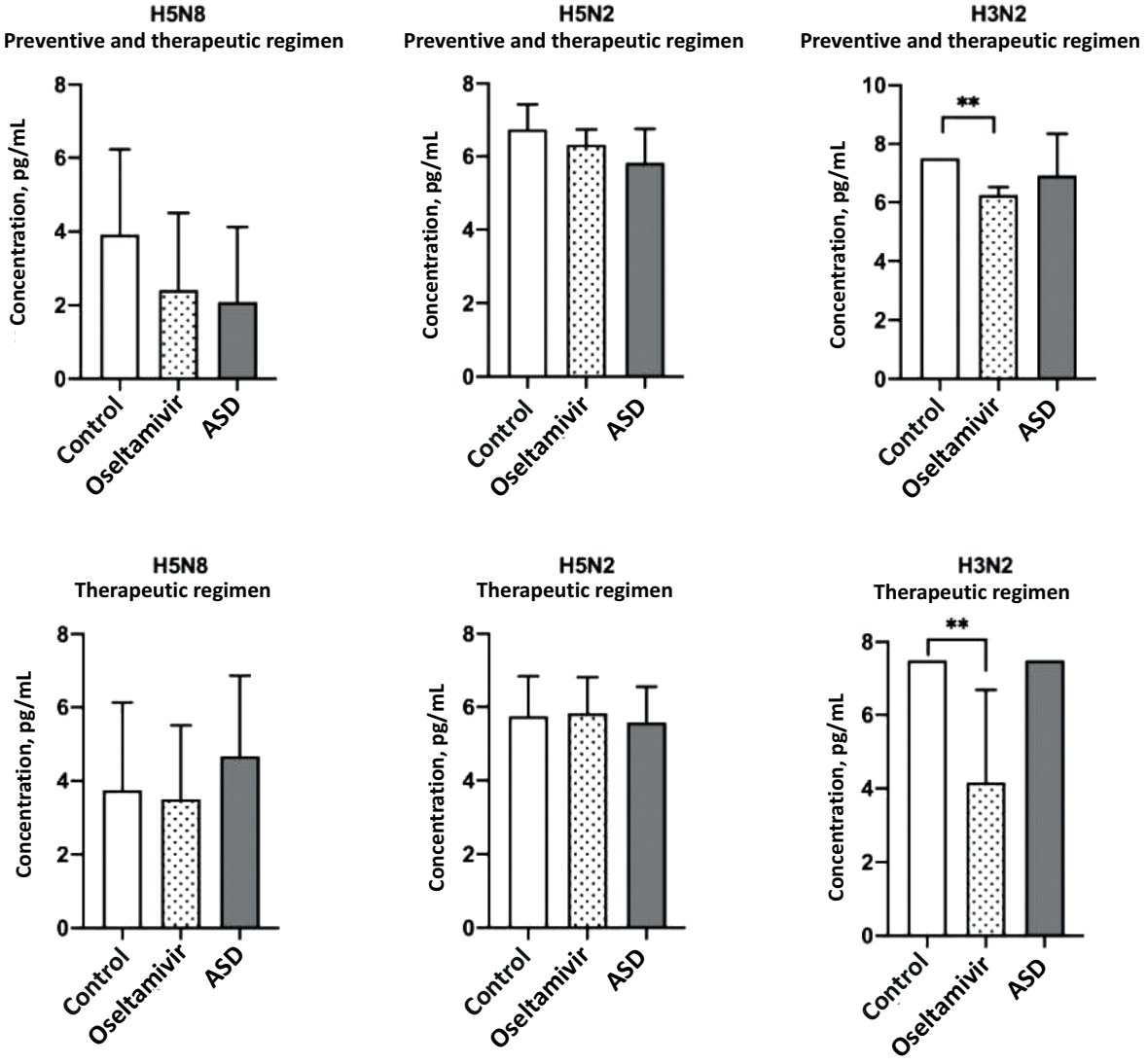

Figure 4 — Viral load in the lung tissue of animals infected with influenza virus (strains H5N8, H3N2, H5N2), determined by titration on a cell culture (n = 6 in each group).

Note: in the diagram, the columns with whiskers indicate the mean ± standard deviation (M ± SD). The P values are indicated above the brackets — the result of applying the one-sided Dunnett's test when compared with the Placebo group.

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Table 1 — Experimental design for adaptation of viruses to Balb/c mice (Stage 1), *n* = 45

Group No.	n of animals	Number of passages	Virus	Virus adaptation
1	15	5	H5N8	Infection with the virus, collection of organs — in 15 mice in the group
2	15	5	H5N2	
3	15	5	H3N2	

Table 2 — Manipulations performed with animals (Stage 1)

Group No.	Manipulations performed																
	-5	-4–-1	0	1–2	3	4	5–6	7	8	9–10	11	12	13–14	15	16	17–18	
1	A, Q	Q	I	E	C	I	E	C	I	E	C	I	E	C	I	E	C
2	A, Q	Q	I	E	C	I	E	C	I	E	C	I	E	C	I	E	C
3	A, Q	Q	I	E	C	I	E	C	I	E	C	I	E	C	I	E	C

Note: A — admission of animals; Q — quarantine; I — infection; C — collection of organs; E — conducting a clinical examination of animals.

Table 3 — Experimental design for determining the antiviral activity of samples on a model of lethal influenza infection in Balb/c mice with therapeutic and prophylactic and therapeutic regimens of administration (Stage 2), *n* = 378

Group No.	No. of animals	Drug	Administration regimen	Virus	Registered indicators
1	21	Placebo (Negative control)	Therapeutic and prophylactic. Administration of the substance for 10 days before infection and 10 days after infection.	H5N8	Lethality (2 times a day), body weight (1 time a day) — in 15 mice in the group, virus titers in lung tissue — in 6 mice in the group
2	21	Oseltamivir (Positive control)			
3	21	ASD substance			
4	21	Placebo (Negative control)		H5N2	
5	21	Oseltamivir (Positive control)			
6	21	ASD substance			
7	21	Placebo (Negative control)		H3N2	
8	21	Oseltamivir (Positive control)			
9	21	ASD substance			
10	21	Placebo (Negative control)	Therapeutic. Administration of the substance after infection for 10 days	H5N8	
11	21	Oseltamivir (Positive control)			
12	21	ASD substance			
13	21	Placebo (Negative control)		H5N2	
14	21	Oseltamivir (Positive control)			
15	21	ASD substance			
16	21	Placebo (Negative control)		H3N2	
17	21	Oseltamivir (Positive control)			
18	21	ASD substance			

Table 4 — Manipulations performed with animals (Stage 2)

Group No.	Manipulations performed								
	-15	-14 –-11	-10 –-1	0	1–2	3	4–10	11–13	14
1	A, Q	Q	B	B, I, C, D	B, C, D	A, E, C, D	B, C, D	C, D	C, D, F
2	A, Q	Q	B	B, I, C, D	B, C, D	A, E, C, D	B, C, D	C, D	C, D, F
3	A, Q	Q	B	B, I, C, D	B, C, D	A, E, C, D	B, C, D	C, D	C, D, F
4	A, Q	Q	B	B, I, C, D	B, C, D	A, E, C, D	B, C, D	C, D	C, D, F
5	A, Q	Q	B	B, I, C, D	B, C, D	A, E, C, D	B, C, D	C, D	C, D, F
6	A, Q	Q	B	B, I, C, D	B, C, D	A, E, C, D	B, C, D	C, D	C, D, F
7	A, Q	Q	B	B, I, C, D	B, C, D	A, E, C, D	B, C, D	C, D	C, D, F
8	A, Q	Q	B	B, I, C, D	B, C, D	A, E, C, D	B, C, D	C, D	C, D, F
9	A, Q	Q	B	B, I, C, D	B, C, D	A, E, C, D	B, C, D	C, D	C, D, F
10	A, Q	Q	Q	I, C, D	B, C, D	A, E, C, D	B, C, D	C, D	C, D, F
11	A, Q	Q	Q	I, C, D	B, C, D	A, E, C, D	B, C, D	C, D	C, D, F
12	A, Q	Q	Q	I, C, D	B, C, D	A, E, C, D	B, C, D	C, D	C, D, F
13	A, Q	Q	Q	I, C, D	B, C, D	A, E, C, D	B, C, D	C, D	C, D, F
14	A, Q	Q	Q	I, C, D	B, C, D	A, E, C, D	B, C, D	C, D	C, D, F
15	A, Q	Q	Q	I, C, D	B, C, D	A, E, C, D	B, C, D	C, D	C, D, F
16	A, Q	Q	Q	I, C, D	B, C, D	A, E, C, D	B, C, D	C, D	C, D, F
17	A, Q	Q	Q	I, C, D	B, C, D	A, E, C, D	B, C, D	C, D	C, D, F
18	A, Q	Q	Q	I, C, D	B, C, D	A, E, C, D	B, C, D	C, D	C, D, F

Notes: A — admission of animals; Q — quarantine; B — administration of the drug/placebo; E — collection of organs; C — weighing; D — recording of mortality; I — infection; F — euthanasia.

Table 5 — Proportions of surviving mice (in percent) infected with the H5N8 virus for two regimens of drug administration

Therapeutic and prophylactic regimen				Therapeutic regimen			
Day after infection	Placebo	Oseltamivir	ASD	Day after infection	Placebo	Oseltamivir	ASD
0	100	100	100	0	100	100	100
1	100	100	100	1	100	100	100
2	100	100	100	2	100	100	100
3	100	100	100	3	100	100	100
4	100	100	100	4	100	100	100
5	100	100	100	5	100	100	100
6	100	100	100	6	100	100	100
7	100	100	93.33	7	100	100	100
8	86.67	100	80	8	100	100	80
9	80	93.33	73.33	9	93.33	93.33	73.33
10	66.67	93.33	46.67	10	86.67	86.67	46.67
11	66.67	93.33	46.67	11	80	86.67	46.67
12	66.67	93.33	46.67	12	80	86.67	40
13	66.67	93.33	46.67	13	80	86.67	40
14	60	93.33	40	14	73.33	86.67	40

Table 6 — Proportions of surviving mice (in percent) infected with the H3N2 virus for two regimens of drug administration

Therapeutic and prophylactic regimen				Therapeutic regimen			
Day after infection	Placebo	Oseltamivir	ASD	Day after infection	Placebo	Oseltamivir	ASD
0	100	100	100	0	100	100	100
1	100	100	100	1	100	100	100
2	100	100	100	2	100	100	100
3	100	100	100	3	100	100	100
4	100	100	100	4	100	100	100
5	100	100	100	5	100	100	100
6	73.33	100	80	6	100	100	100
7	46.67	100	66.67	7	66.67	100	80
8	46.67	100	46.67	8	53.33	100	66.67
9	33.33	100	46.67	9	53.33	100	60
10	20	100	46.67	10	40	100	60
11	13.33	100	46.67	11	33.33	100	60
12	6.67	100	46.67	12	26.67	100	40
13	6.67	100	46.67	13	26.67	100	40
14	6.67	100	46.67	14	26.67	100	40

Table 7 — Proportions of surviving mice (in percent) infected with the H5N2 virus for two regimens of drug administration

Therapeutic and prophylactic regimen				Therapeutic regimen			
Day after infection	Placebo	Oseltamivir	ASD	Day after infection	Placebo	Oseltamivir	ASD
0	100	100	100	0	100	100	100
1	100	100	100	1	100	100	100
2	100	100	100	2	100	100	100
3	100	100	100	3	93.33	100	100
4	100	100	93.33	4	93.33	100	100
5	100	100	93.33	5	93.33	100	93.33
6	100	100	80	6	93.33	100	93.33
7	93.33	100	73.33	7	86.67	93.33	93.33
8	93.33	93.33	73.33	8	80	80	93.33
9	93.33	86.67	73.33	9	73.33	80	86.67
10	86.67	80	73.33	10	66.67	80	80
11	86.67	80	73.33	11	53.33	80	73.33
12	86.67	80	60	12	53.33	80	73.33
13	86.67	80	60	13	53.33	80	73.33
14	86.67	80	60	14	53.33	80	73.33

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Table 8 — Comparison of survival functions with the placebo group in experiments with two drug regimens after infection of mice with three strains of influenza virus

Preventive and therapeutic regimen				Therapeutic regimen			
Virus strain	Drug	Gehan-Wilcoxon criterion, *p-value*	Cox-Mantel criterion, *p-value*	Virus strain	Drug	Gehan-Wilcoxon criterion, *p-value*	Cox-Mantel criterion, *p-value*
H5N8	Placebo	–	–	H5N8	Placebo	–	–
	Oseltamivir	* 0.0380	* 0.0352		Oseltamivir	0.4407	0.3992
	ASD	0.2954	0.2865		ASD	* 0.0331	* 0.0478
H3N2	Placebo	–	–	H3N2	Placebo	–	–
	Oseltamivir	**** < 0.0001	**** < 0.0001		Oseltamivir	**** < 0.0001	**** < 0.0001
	ASD	0.1896	0.0560		ASD	0.3146	0.3269
H5N2	Placebo	–	–	H5N2	Placebo	–	–
	Oseltamivir	0.6549	0.6393		Oseltamivir	0.1989	0.1546
	ASD	0.0927	0.0992		ASD	0.2591	0.2580

Note: the presented P values are the result of applying the Gehan-Wilcoxon and Cox-Mantel tests; significance of differences: * — $p < 0.05$; **** — $p < 0.0001$.

Table 9 — Dynamics of death of Balb/c mice and indicators of protective activity of drugs during experimental lethal influenza pneumonia caused by influenza virus H5N8

Drug	Application scheme	Individuals in group	Mortality by days														Lethality, %	MLD, days	Protection index, %	p*
			1	2	3	4	5	6	7	8	9	10	11	12	13	14				
Placebo	P/T	15								2	1	2				1	40.0	12.9	n/a	n/a
Oseltamivir		15									1						6.6	14.6	83.5	**0.0035**
ASD		15							1	2	1	4				1	60.0	11.7	–	0.2865
Placebo	T	15								1	1	1				1	26.7	13.7	n/a	n/a
Oseltamivir		15								1	1						13.3	14.1	50.2	0.3931
ASD		15								3	1	4		1			60.0	11.7	–	0.0554

Note: * Log-rank (Mantel-Cox) test; MLD — mouse lethal dose; n/a — not available.

Table 10 — Dynamics of death of Balb/c mice and indicators of protective activity of drugs during experimental lethal influenza pneumonia caused by influenza virus H5N2

Drug	Application scheme	Individuals in group	Mortality by days														Lethality, %	MLD, days	Protection index, %	p*
			1	2	3	4	5	6	7	8	9	10	11	12	13	14				
Placebo	P/T	15							1		1						13,3	14,1	n/a	n/a
Oseltamivir		15								1	1	1					20,0	13,8	–	0,6393
ASD		15				1		2	1					2			40,0	12,1	–	0,0992
Placebo	T	15			1			1	1	1	1	2					46,7	11,5	n/a	n/a
Oseltamivir		15							1	2							20,0	13,5	57,2	0,2284
ASD		15					1				1	1	1				26,7	13,3	42,8	0,3607

Note: * Log-rank (Mantel-Cox) test; MLD — mouse lethal dose; n/a — not available.

Table 11 — Dynamics of death of Balb/c mice and indicators of protective activity of drugs during experimental lethal influenza pneumonia caused by influenza virus H3N2

Drug	Application scheme	Individuals in group	Mortality by days														Lethality, %	MLD, days	Protection index, %	p*
			1	2	3	4	5	6	7	8	9	10	11	12	13	14				
Placebo	P/T	15						4	4		2	2	1	1			93,3	8,5	n/a	n/a
Oseltamivir		15															0	15,0	100,0	**<0,0001**
ASD		15						3	2	3							53,3	10,7	42,9	0,0560
Placebo	T	15							5	2		2	1	1			73,3	10,3	n/a	n/a
Oseltamivir		15															0	15,0	100,0	**<0,0001**
ASD		15							3	2	1			3			60,0	11,5	35,7	0,3269

Note: * Log-rank (Mantel-Cox) test; MLD — mouse lethal dose; n/a — not available.

Table 12 — Values of influenza virus titers of strains H5N8, H3N2, H5N2 for two drug regimens (*n* = 6 for each group)

Preventive and therapeutic regimen					Therapeutic regimen				
Virus strain	Drug	M	SD	SEM	Virus strain	Drug	M	SD	SEM
H5N8	Placebo	3.917	2.311	0.943	H5N8	Placebo	3.750	2.382	0.972
	Oseltamivir	2.417	2.084	0.851		Oseltamivir	3.500	2.000	0.817
	ASD	2.083	2.035	0.831		ASD	4.667	2.206	0.901
H5N2	Placebo	6.750	0.689	0.281	H5N2	Placebo	5.750	1.084	0.442
	Oseltamivir	6.333	0.408	0.167		Oseltamivir	5.833	0.983	0.401
	ASD	5.833	0.931	0.380		ASD	5.583	0.971	0.396
H3N2	Placebo	7.500	0.000	0.000	H3N2	Placebo	7.500	0.000	0.000
	Oseltamivir	6.250	0.274	1.429		Oseltamivir	4.167	2.523	1.030
	ASD	6.917	0.112	0.583		ASD	7.500	0.000	0.000

Note: the mean values (M), standard deviations (SD) and standard errors of the mean (SEM) are presented

Table 13 – Values of influenza virus titers (strains H5N8, H3N2, H5N2), Shapiro-Wilk test

Preventive and therapeutic regimen				Therapeutic regimen			
Virus strain	Drug	*W-value*	*p-value*	Virus strain	Drug	*W-value*	*p-value*
H5N8	Placebo	0.9065	0.4140	H5N8	Placebo	0.9551	0.7815
	Oseltamivir	0.8129	0.0765		Oseltamivir	0.9780	0.9410
	ASD	0.7732	* 0.0333		ASD	0.9805	0.9538
H5N2	Placebo	0.8606	0.1912	H5N2	Placebo	0.8672	0.2151
	Oseltamivir	0.8216	0.0911		Oseltamivir	0.9241	0.5353
	ASD	0.8616	0.1948		ASD	0.9124	0.4522
H3N2	Placebo	–	–	H3N2	Placebo	–	–
	Oseltamivir	0.6827	** 0.0040		Oseltamivir	0.8381	0.1257
	ASD	0.4961	**** < 0.0001		ASD	–	–

Note: checking data for normality, W and P values are presented — the result of applying the Shapiro-Wilk test; significance of differences: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$. Dash — calculation is impossible, since all values in the sample are the same.

Table 14 — Values of influenza virus titers (strains H5N8, H3N2, H5N2), Mann-Whitney test

Preventive and therapeutic regimen				Therapeutic regimen			
Virus strain	Drug	*W-value*	*p-value*	Virus strain	Drug	*W-value*	*p-value*
H5N8	Placebo	–	–	H5N8	Placebo	–	–
	Oseltamivir	10	0,2381		Oseltamivir	17,50	0,9827
	ASD	8	0,1190		ASD	13,50	0,5238
H5N2	Placebo	–	–	H5N2	Placebo	–	–
	Oseltamivir	11,50	0,3398		Oseltamivir	16,50	0,8658
	ASD	7	0,0887		ASD	16	0,8030
H3N2	Placebo	–	–	H3N2	Placebo	–	–
	Oseltamivir	0	** 0,0022		Oseltamivir	0	** 0,0022
	ASD	15	0,9999		ASD	18	0,9999

Note: the results of the Mann — Whitney test when comparing the drug and placebo for two drug regimens, *U*- and *p-values* are presented; significance of differences: ** $p < 0.01$.

It should also be noted that in the group infected with the H3N2 virus and receiving the ASD according to the preventive and therapeutic regimen, the *p-value* was borderline (0.056 *vs.* 0.05), which indicates the presence of a pronounced tendency towards a protective effect of the drug. For the H5N2 virus, there was also a tendency to reduce mortality in the group receiving the drug according to the therapeutic regimen, although it did not reach statistical significance.

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From the data presented in the tables, it can be seen that positive values of the PI for the ASD were noted in the groups infected with the H3N2 virus according to both regimens and in the group infected with the H5N2 virus according to the therapeutic regimen.

Statistical analysis of data obtained by measuring influenza virus titers

A study of viral load in lung tissue was carried out by titration on a cell culture. For each combination of "virus strain– treatment regimen–drug", measurements were performed in a sample of 6 animals. The average values of the measured titers of influenza virus (strains H5N8, H3N2, H5N2) for the therapeutic and preventive-therapeutic regimens are presented in Figure 4 and Table 12.

The data were checked for compliance with the normal distribution law in order to justify the choice of the test that identifies differences between measurement groups. The Shapiro-Wilk test was used to check the data for normality, the results of which are presented in Table 13.

As it can be seen from Table 13, with the selected significance level in 9 groups, deviations from the normal distribution are observed in the data. Thus, parametric tests are not applicable, and to identify differences between groups of drugs and the placebo group, we applied the Mann-Whitney U-test, the results of which are presented in Table 14.

As it can be seen from Table 14, with the selected significance level $\alpha = 0.05$, differences were found in the experiment between the oseltamivir and placebo groups in the case of infection with the H3N2 virus for both regimens. No statistically significant effect of the ASD on the level of viral load was noted for any of the studied viruses.

DISCUSSION

Influenza is one of the most significant infections in the world. According to WHO estimates, millions of cases are registered worldwide every year, and mortality from complications can reach up to 650 thousand people[10]. Seasonal influenza vaccination is the most effective tool for protection against infection [29]. However, influenza vaccination has limitations and is not always applicable in patients from risk groups, which include children, people over 60 years of age, as well as patients with chronic pathologies, immunodeficiency states and allergic manifestations [30]. Also, in these groups, vaccination may not be effective enough [31].

As an additional measure of protection in the interval between vaccination and the formation of an adequate post-vaccination immune response, as well as in persons with contraindications to vaccination, the use of antiviral drugs is indicated [32]. However, recently there has been a relative resistance to some traditional antiviral agents, including oseltamivir, zanamivir, amantadine and rimantadine. In this regard, it is critical to develop new strategies and drugs for effective control of viral infection [31].

In some cases, the effectiveness of treatment or prevention of influenza can be increased by using combined therapy consisting of existing and/or new antiviral agents with different mechanisms of action [31]. Ethnopharmacological drugs obtained from various plants or other natural resources that are potentially capable of reducing the severity of clinical symptoms and reducing the risk of complications of influenza and other respiratory infections can be used [33]. According to WHO data, about 21 thousand medicinal plants are known, most of which have an immunomodulatory effect and have the corresponding therapeutic potential [34].

In vivo studies have demonstrated the protective effects of colaviron, a bioflavonoid isolated from the plant *Garcinia kola* Heckel. Acting as a natural antioxidant and anti-inflammatory agent, colaviron is able to inhibit the activity of acetylcholinesterase in the hippocampus and striatum in rats [35]. In an experiment on mice, it was shown that colaviron can delay the appearance of clinical symptoms of influenza through a mechanism that differs from the mechanisms of action of existing drugs against influenza, but is closely related to the antioxidant and immunomodulatory action of this substance [36]. Similarly, it has been shown that an extract of the bark of the roots of the African baobab (*Adansonia digitata* L.; *Malvaceae*) has antiviral activity against avian respiratory virus and may be useful for relieving influenza symptoms [37].

In this study, the protective effect of the drug ASD on a model of lethal influenza pneumonia caused by influenza A viruses was studied. The drug of natural origin, antiseptic-stimulant Dorogova fraction-2 (ASD-F2) is a product of high-temperature dry distillation of meat and bone meal, which has found wide application in veterinary drug in the treatment

[10] Global influenza strategy 2019–2030. Geneva: World Health Organization; 2019. Available from: https://iris.who.int/bitstream/handle/10665/ 311184/9789241515320-eng.pdf?sequence=18

of animals and birds from numerous diseases of viral and microbial etiology, in the prevention of such diseases, as well as in increasing the productivity of cattle and birds. ASD-F2 has neither species nor organ specificity, because distillation ensures the gradual decomposition of organic substances to low-molecular components, which are similar in structure to cell metabolism metabolites. ASD-F2 has a multifaceted effect on the body. It intensifies metabolism, accelerates oxidative processes, increases the reserve alkalinity in the blood, which contributes to the normalization of metabolism in tissues, improves the processes of digestion, absorption of nutrients. The drug causes an improvement in the functional state of the mechanisms of natural resistance, enhances tissue regeneration processes, stimulates immunogenesis, as a result of which resistance to adverse effects, including pathogens of infectious diseases, increases [38].

Previously, the antiviral activity of drugs containing ASD-F2 was demonstrated in mice with infection caused by the influenza virus strain A/California/07/09 (H1N1), and in Syrian hamsters infected with the SARS-CoV-2 virus [39], and *in vitro* studies, its antimicrobial effect was recorded [40], including against Mycobacterium tuberculosis [41]. In this work, positive effects were also observed when using a drug containing ASD-2 in mice infected with influenza virus (strains H3N2 and H5N2). Despite the fact that the effect of the drug on the level of viral load was not revealed, it should be noted a tendency to improve the survival rate of mice infected with influenza (strains H3N2 and H5N2), as well as a decrease in body weight loss against the background of the use of the studied drug. The results obtained are consistent with previously published data indicating the presence of antiviral effects in the drug.

Study Limitations

In this study, the effectiveness of the drug was not studied against influenza virus strains H1N1. Also, the effectiveness of the drug was studied on one type of animal, which does not exclude the manifestation of other pharmacological manifestations.

CONCLUSION

A study of the protective activity of the drug ASD was carried out on a model of lethal influenza pneumonia caused by influenza A viruses A/common duck/Uvs Nuur lake/26/2016 (H5N8), A/Duck/Potsdam/1402-6/86 (H5N2), A/Aichi/2/68, in Balb/c mice with preventive and therapeutic and therapeutic regimens. During the experiments, the dynamics of death and changes in the weight indicators of animals during the pathological process, as well as the viral load in the lung tissue on the 3rd day after infection, were evaluated.

In relation to the influenza virus H3N2, an improvement in the dynamics of weight loss of infected animals was noted when used according to the preventive and therapeutic regimen. A decrease in mortality was observed in both regimens, but it was more pronounced for the preventive and therapeutic regimen.

For the influenza virus H5N2, the effectiveness of the drug was less pronounced, but a protective effect was also noted when used according to the therapeutic regimen.

Thus, based on the data obtained, it can be concluded that the drug ASD has a protective effect against the influenza virus in vivo, and the effect depends on the subtype of the virus and is most pronounced for the H3N2 strain.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS CONTRIBUTION

Sergei V. Engashev, Olga A. Dorogova, Ekaterina S. Engasheva — design, draft editing and final approval of the article; Irina Yu. Merkulova — data processing, writing the text of the article. All authors confirm that their authorship meets the international ICMJE criteria (all authors made a significant contribution to the development of the concept, conduct of the study and preparation of the article, read and approved of the final version before the publication).

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Analysis of preferential drug provision for patients with diabetes mellitus in the Stavropol Region

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The aim. To study the quantitative characteristics of the implementation of drug provision for beneficiaries with diabetes mellitus (DM) in the Stavropol Region in the period from 2020 to 2024.

Materials and methods. The regulatory legal basis of the study was made up of legislative acts of the federal and regional levels on the organization of preferential drug provision (PDP). Clinical and epidemiological monitoring of DM was carried out according to the data of the State Register of DM for the Stavropol Region, data from the Regional Endocrinological Dispensary (Stavropol Region, Russia), as well as reports from executive authorities.

Results. According to the clinical and epidemiological monitoring of DM in the Russian Federation in 2024, the prevalence of DM in the Stavropol Region did not exceed the average Russian indicators (rating 36). A decrease in the number of federal beneficiaries and an increase in the number of regional beneficiaries (with a predominance of DM type II) was noted. 10 municipalities where about 60% of patients with DM live were identified. Therapy for patients with DM type I is based on insulin therapy for 100% of patients. Therapy for patients with DM type II includes the use of insulin for 14,140 (20.63%) patients, oral hypoglycemic agents for 53,404 (77.88%) patients, and dietary adjustments for 1,025 (1.49%) people. Since 2024, in accordance with changes in the legislation of the Stavropol Region, all patients with DM are provided from general funds of the regional budget, with the exception of federal beneficiaries in Lermontov, who are provided at the expense of the program for the provision of necessary medicines (PNMs). The structure of expenditures from the federal and regional budgets for the purchase of funds for the treatment of DM is presented. The dynamics of the growth of expenses per one beneficiary with DM is shown. It should be noted that in 2024 there was an equalization of the amount of expenses per one federal and regional beneficiary.

Conclusion. The results obtained can be used by other researchers to take solutions to improve the availability of drug provision for beneficiaries with DM both in the Stavropol Region and in other subjects of the Russian Federation.

Keywords: diabetes mellitus; regional drug provision; provision of necessary medicines; Stavropol Region

Abbreviations: DM — diabetes mellitus; SSDs — socially significant diseases; PDP — preferential drug provision; RDP — regional drug provision; VED — list of vital and essential drugs; PNMs — provision of necessary medicines; SLDs — sugar-lowering drugs; CGM — continuous glucose monitoring.

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Анализ льготного лекарственного обеспечения больных сахарным диабетом в Ставропольском крае

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Цель. Изучение количественных характеристик реализации лекарственного обеспечения льготополучателей с сахарным диабетом (СД) в Ставропольском крае в период с 2020 по 2024 г.

Материалы и методы. Нормативную правовую основу исследования составили законодательные акты федерального и регионального уровней по организации льготного лекарственного обеспечения (ЛЛО). Клинико-эпидемиологический мониторинг СД проведён по данным Государственного регистра СД по Ставропольскому краю, данных ГБУЗ СК «Краевой эндокринологический диспансер», а также отчётов органов исполнительной власти.

Результаты. По данным клинико-эпидемиологического мониторинга СД РФ в 2024 году распространённость СД в Ставропольском крае не превышала среднероссийских показателей (рейтинг 36). Отмечено снижение федеральных и увеличение численности региональных льготополучателей (с преобладанием СД II типа). Выявлены 10 муниципальных образований, в которых проживает около 60% пациентов с СД. Терапия больных СД I типа основана на инсулинотерапии для 100% пациентов. Терапия больных СД II типа включает применение инсулинов для 14140 (20,63%) пациентов, таблетированных сахароснижающих средств для 53404 (77,88%) пациентов и корректировки питания для 1025 (1,49%) человек. С 2024 года в соответствии с изменениями в законодательстве Ставропольского края все больные СД обеспечиваются из единого источника средств краевого бюджета, за исключением федеральных льготополучателей г. Лермонтова, обеспечиваемых за счёт средств программы обеспечения необходимыми лекарственными средствами (ОНЛС). Представлена структура затрат федерального и регионального бюджетов на закупку средств для терапии СД. Показана динамика роста затрат на одного льготополучателя с СД. Следует отметить, что в 2024 году произошло выравнивание сумм затрат на одного федерального и регионального льготополучателя.

Заключение. Полученные результаты могут быть использованы другими исследователями для выработки организационных решений по повышению доступности лекарственного обеспечения для льготополучателей с СД как в Ставропольском крае, так и в других субъектах Российской Федерации.

Ключевые слова: сахарный диабет; региональное лекарственное обеспечение; обеспечение необходимыми лекарственными средствами; Ставропольский край

Список сокращений: СД — сахарный диабет; СЗЗ — социально значимые заболевания; ЛЛО — льготное лекарственное обеспечение; РЛО — региональное лекарственное обеспечение; ЛП — лекарственные препараты; ЖНВЛП — жизненно необходимые и важнейшие лекарственные препараты; ОНЛС — обеспечение необходимыми лекарственными средствами; ССП — сахароснижающие препараты; СНМГ — система непрерывного мониторинга глюкозы.

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disease characterized by a high prevalence [1–3] and leading to a significant deterioration of the condition and physical abilities of a person, disability, as well as high treatment costs [4, 5]. DM (according to ICD-10 code of diseases E10–E14), by Decree of the Government of the Russian Federation No. 715 of December 01, 2004, is classified as a socially significant disease (SSD)[1]. SSDs affect the population of working age, lead to a deterioration in the patient's condition,

[1] Decree of the Government of the Russian Federation No. 715 dated December 01, 2004 "On approval of the list of socially significant diseases and the list of diseases that pose a danger to others" (revised on January 31, 2020; effective from February 11, 2020). Available from: https://normativ.kontur.ru/document?moduleId=1&documentId=356130. Russian

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limitation of his physical capabilities and to the loss of temporary disability [6–8]. All this causes significant socioeconomic damage to the country [9, 10].

In accordance with Federal Law No. 323-FZ[2], for a citizen suffering from SSD, including DM, social support measures are established when receiving all types of medical care: patient has the right to free diagnosis of the disease, specialist monitoring of his health throughout the entire period of the disease. At the outpatient treatment stage, patients with DM have the right to receive medicines under the federal and regional preferential drug provision (PDP) [11–13].

Currently, the categories of citizens entitled to PDP at the expense of the federal budget (federal beneficiaries) are defined by Federal Law No. 178-FZ of 17.07.1999 "On State Social Assistance"[3]. In case of persistent disorders of body functions associated with the duration of DM or severe conditions due to hypoglycemia, ketoacidosis, neuropathies, etc., disability may be established for both a child under 18 years of age and a citizen aged 18 years and older[4,5]. Such persons have the right to a set of social services, receiving the necessary medicines included in the list of vital and essential drugs (VED), formed by order of the Government of the Russian Federation No. 2406-r dated October 12, 2019 (as amended on January 15, 2025)[6,7,8].

A citizen with DM, in accordance with the Decree of the Government of the Russian Federation No. 890 of 30.07.1994, has the right to drug provision at the expense of the regional budget[9], the provision of MPs is also carried out in the amount of the VED list.

At the same time, if a citizen refuses a set of social services, he retains the right to drug provision at the expense of the regional budget. There is some legal conflict regarding the duplication of rights to PDP for patients with DM who have the right to receive medicines at the expense of the federal and regional budgets. This imposes responsibility on healthcare authorities in organizing the provision of MPs to this category of patients, and the rational use of the allocated budget in order to predetermine the availability of drug provision [14–16].

The increase in the number of patients with DM who take medicines for life increases the costs of the federal and regional budgets for PDP for this category of beneficiaries, contributes to the formation of regional characteristics in the PDP for this category of patients and the consolidation of provisions in regional regulatory documents on drug provision for patients with DM [17–19]. At the same time, the rational use of allocated budget funds contributes to ensuring the availability of medicines for this category of patients [20–22].

Content analysis of regulatory documentation, which became the basis for organizing drug provision for citizens with DM in the Stavropol Territory, showed that along with federal regulations and the territorial program of state guarantees[10], the subject adopted and operates regional regulations on social support measures for persons with SSDs[11], as well as the regional program "Fighting against Diabetes Mellitus on the Stavropol Territory", approved by the Decree of the Government of the Stavropol Territory[12].

n order to rationally allocate financial resources and optimize PDP for patients with DM, changes were

[2] Federal Law of the Russian Federation dated November 21, 2011 No. 323-FZ "On the Basics of Public Health Protection in the Russian Federation (with Amendments and Additions): (as amended on September 26, 2024. Available from: https://www.consultant. ru/document/cons_doc_LAW_121895/. Russian

[3] Federal Law No. 178-FZ of 07/17/1999 "On State Social Assistance" (as amended on May 29, 2024; effective from July 01, 2024). Available from: https://normativ.kontur.ru/document?moduleId=1&documentId=473694. Russian

[4] Federal Law No. 181-FZ of November 24, 1995 (as amended on October 29, 2024) "On Social Protection of Persons with Disabilities in the Russian Federation". Available from: https://normativ.kontur.ru/document?moduleId=1&documentId=475963. Russian

[5] Letter of the Ministry of Labor and Social Protection of the Russian Federation dated February 22, 2020 No. 7222.FB77/2020 "On sending clarifications on the issue of conducting a medical and social examination upon reaching the age of 18 to citizens with insulin-dependent diabetes mellitus who were assigned the category of "disabled child". Available from: https://www.garant.ru/products/ipo/prime/doc/73564214/. Russian

[6] Decree of the Government of the Russian Federation No. 10-r dated January 15, 2025 "On Amendments to Decree of the Government of the Russian Federation No. 2406-r dated October 12, 2019". Available from: https://1glv.ru/#/document/97/527333/. Russian

[7] Federal Law No. 206-FZ dated July 13, 2020 "On Amendments to Certain Legislative Acts of the Russian Federation on the Provision of Medicines, Medical Devices and Specialized Medical Nutrition Products to Citizens" (as amended on July 10, 2023; effective from January 01, 2024). Available from: https://normativ.kontur.ru/document?moduleId=1&documentId=457204. Russian

[8] Letter of the Ministry of Health of the Russian Federation dated July 05, 2024 No. 25-1/3081600-7932 "On the provision of medicines". Available from: https://www.garant.ru/products/ipo/prime/doc/409230040/

[9] Decree of the Government of the Russian Federation dated July 30, 1994 No. 890 "On State Support for the Development of the medical industry and improving the provision of medicines and medical products to the Population and Healthcare Institutions" (with Amendments and additions). Available from: https://normativ.kontur.ru/document?moduleId=1&document Id=61472. Russian

[10] Resolution of the Government of the Stavropol Territory dated December 27, 2024 No. 784-p "On approval of the Territorial Program of State guarantees of free medical care to Citizens in the Stavropol Territory for 2025 and the planning period of 2026 and 2027". Available from: http://publication.pravo.gov.ru/document/2600202412280005. Russian

[11] Resolution of the Government of the Stavropol Territory dated December 02, 2021 No. 625-p "On Amendments to the Resolution of the Government of the Stavropol Territory dated April 19, 2006 No. 49-p "On the organization of social support measures for citizens suffering from socially significant diseases and citizens suffering from diseases that pose a danger to others". Available from: https://mz26.ru/activity/sub-52/. Russian

[12] Resolution of the Government of the Stavropol Territory dated April 26, 2024 No. 230-p "On approval of the regional program "Fighting against Diabetes Mellitus on the Stavropol Territory". Available from: https://stav-pravo.ru/postanovlenie/2024/04/26/n-230-p/. Russian

made to regional legislation regarding the assignment of certain state powers to local governments. Thus, the law of the Stavropol Territory dated February 15, 2013 No. 10-kz "On assigning to local governments of the Stavropol Territory urban district of Lermontov certain state powers of the Stavropol Territory in the field of protecting the health of citizens" assigned to local governments state powers in the field of protecting the health of citizens to provide social support measures[13]. In this regard, according to the current regional legislation, there has been a redistribution of funding for drug provision for beneficiaries with DM. Since 2024, the provision of benefits to beneficiaries with DM is carried out from a single source — all regional beneficiaries with DM and patients who have refused a set of social services are provided with medicines from the regional budget, and patients with a disability group living in Lermontov are provided with all necessary medicines at the expense of the federal budget under the program of provision of essential medicines (PEMs).

The regional program approves target indicators, the achievement of which contributes to improving medical and drug care for patients with DM, reducing the level of complications of the disease and improving the quality of life of patients. The study of the epidemiological situation of DM in the Stavropol Territory indicates the relevance of organizing drug provision for patients with DM as part of state social assistance in the Stavropol Territory, both at the expense of federal and regional budgets. In this regard, monitoring the organization of drug provision for patients with DM allows us to assess the implementation of the tasks set.

THE AIM was to study the consumption of hypoglycemic drugs in the implementation of preferential drug provision for patients suffering from diabetes mellitus in the Stavropol Territory.

The following **tasks** were identified and solved:

1. Analyze data on the prevalence of DM in the Stavropol Territory;
2. Assess the dynamics of the number of federal and regional beneficiaries with DM, identify municipalities, urban districts with the largest number of beneficiaries with DM;
3. To study the structure of budget spending aimed at purchasing funds for the treatment of DM in the Stavropol Territory;
4. Determine the structure of consumption of hypoglycemic agents by beneficiaries with DM.

MATERIALS AND METHODS

The regulatory framework of the study consisted of legislative acts of the Russian Federation and the Stavropol Territory on PDP (Preferential Drug Provision) for patients with DM, which were obtained using the Consultant Plus legal reference system,[14] the official website of the Ministry of Health of the Stavropol Territory[15], providing current regulatory documentation. To structure and systematize the data obtained, content analysis was used, which allows determining the principles and parameters of PDP in the Stavropol Territory.

The source of information on the number of patients with DM was the State Register of Diabetes Mellitus in the Stavropol Territory[16], as an interactive report on the number of patients with DM types I and II, materials of annual reports of the State Budgetary Healthcare Institution of the Stavropol Territory "Regional Endocrinological Dispensary" on the implementation of the Federal Project "Fighting against Diabetes Mellitus" and reporting materials on the implementation of preferential drug provision, as well as the State Register of Medicines[17].

Retrospective analysis methods were used to study the dynamics and changes in indicators of the number of patients with DM, including federal and regional beneficiaries; spending budget funds on the purchase of medicines for PDP for the period 2020–2024; ranking quantitative indicators of the number of beneficiaries by municipal and urban districts and identifying districts with the largest number of beneficiaries; comparative analysis of individual group and intragroup indicators; graphical analysis to visualize changes in

[13] The Law of the Stavropol Territory dated February 15, 2013 No. 10-kz "On Granting local self-government bodies of the Stavropol Territory of the city of Lermontov separate State Powers of the Stavropol Territory in the field of public health protection" (as amended on April 28, 2023) (as amended by Laws of the Stavropol Territory dated November 15, 2013 No. 100-kz, dated April 28, 2023 No. 39-kz). Available from: https://base.garant.ru/27130578/?ysclid=m7su9nr93980718211. Russian

[14] ConsultantPlus Legal Reference System. Available from: https://www.consultant.ru/. Russian

[15] Ministry of Health of the Stavropol Territory. Available from: https://www.mz26.ru/. Russian

[16] Databases of clinical and epidemiological monitoring of endocrinopathies in the Russian Federation. Endocrinology Research Centre of the Russian Federation. Available from: https://diaregistry.ru/. Russian

[17] The State Register of Medicines of the Russian Federation. Available from: https://grls.rosminzdrav.ru/GRLS.aspx. Russian

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indicators in the analyzed period. The study of the dynamics of changes in the number of beneficiaries, the costs of the regional and federal budgets for PDP, the costs per beneficiary was carried out on the basis of calculating the rates of change. The study of the structure of consumption of sugar-lowering drugs (SLDs) by type of therapy and depending on the type of DM was carried out according to the State Register of DM in the Stavropol Region for 2024. MS Excel 2021 spreadsheets (Microsoft Corp., USA) were used to work with digital data.

RESULTS

Clinical and epidemiological monitoring of diabetes mellitus (DM) in the Russian Federation is carried out through the State Register of DM, the methodological and organizational center of which is the Endocrinological Research Centre, which contributes to medical and statistical observations. A unified system for monitoring DM based on the "Database of clinical and epidemiological monitoring of DM in the Russian Federation" allows, at the national level, to obtain information on risk factors, the epidemiological and clinical picture of DM, the use of drugs and modern technologies for monitoring glycemia levels, to assess the effectiveness of program implementation, and to coordinate the work of all levels of healthcare at the level of any subject of the Russian Federation [23–25].

As of January 01, 2024, data on the number of patients with DM in the Russian Federation and the Stavropol Territory were extracted (Table 1).

The presented data show that the prevalence of DM in the Stavropol Territory per 100 thousand people is lower than the average Russian indicators, but, nevertheless, the number of patients with DM is large. The published final ranking of regions by DM showed that the Stavropol Region in 2024 ranked 36th among all subjects of the Russian Federation, in 2023 this figure was 41, which indicates an increase in morbidity[18]

Based on the data from the State Register of DM for the Stavropol Territory and the data from the SBIH of the Stavropol Region "Regional Endocrinological Dispensary", an analysis was made of the number of patients with DM, with the allocation of groups of citizens entitled to drug provision (Table 2).

The data presented in Table 2 indicate a slight decrease in the number of patients with an established diagnosis of DM (a decrease of 2 990 people — -3.52 %), while the number of such patients reached 81 827 by 2024. In the analyzed period, the number of federal beneficiaries decreased by 4 246 people (29.72 %), and regional beneficiaries, on the contrary, increased by 13 992 people. At the same time, their slight decrease by 2022 was replaced by an increase — from 61 670 people in 2022 to 77856 in 2024. Beneficiaries under 14 years of age accounted for about 1.5 % of the total number — their number increased from 929 people in 2020 to 1 172 people in 2024.

When studying the structure of DM morbidity among beneficiaries, it was found that patients with type 2 DM predominated, the proportion of which exceeded 90.0 % (93.95% in 2024).

In the Stavropol Territory, 33 municipal and urban districts are identified[19], in which a different number of beneficiaries are registered, based on data on their number, a ranking of municipalities was carried out from the position of decreasing number of resident beneficiaries (Fig. 1).

Grouping of municipalities by the number of beneficiaries made it possible to identify territories with the largest number of beneficiaries, in 10 (30.3 %) municipal and urban districts, almost about 60 % of beneficiaries are registered. The largest number is registered in Stavropol, Shpakovsky district, Nevinnomyssk, Georgievsky urban district, etc. The identified municipalities are a priority for further study of the organization of drug provision, which will allow building a more effective drug routing for this group of patients. At the same time, in the territories with the smallest number of beneficiaries with DM (Turkmensky, Arzgirsky, Stepnovsky and Novoselitsky municipal districts), the formation of the most acceptable ways of drug routing for patients is an important task for the healthcare authorities of the region.

Drug provision for patients with DM is carried out from the federal and regional budgets. The study of the dynamics of financing drug provision made it possible to determine the trend of changes in indicators (Table 3).

[18] Databases of clinical and epidemiological monitoring of endocrinopathies in the Russian Federation. Endocrinology Research Centre of the Russian Federation.

[19] Resolution of the Government of the Stavropol Territory dated May 04, 2006 No. 63-p "On Approval of the Register of Administrative-territorial Units and Territorial Units of the Stavropol Territory (as amended on July 31, 2024)". Available from: https://docs.cntd.ru/document/461505266. Russian

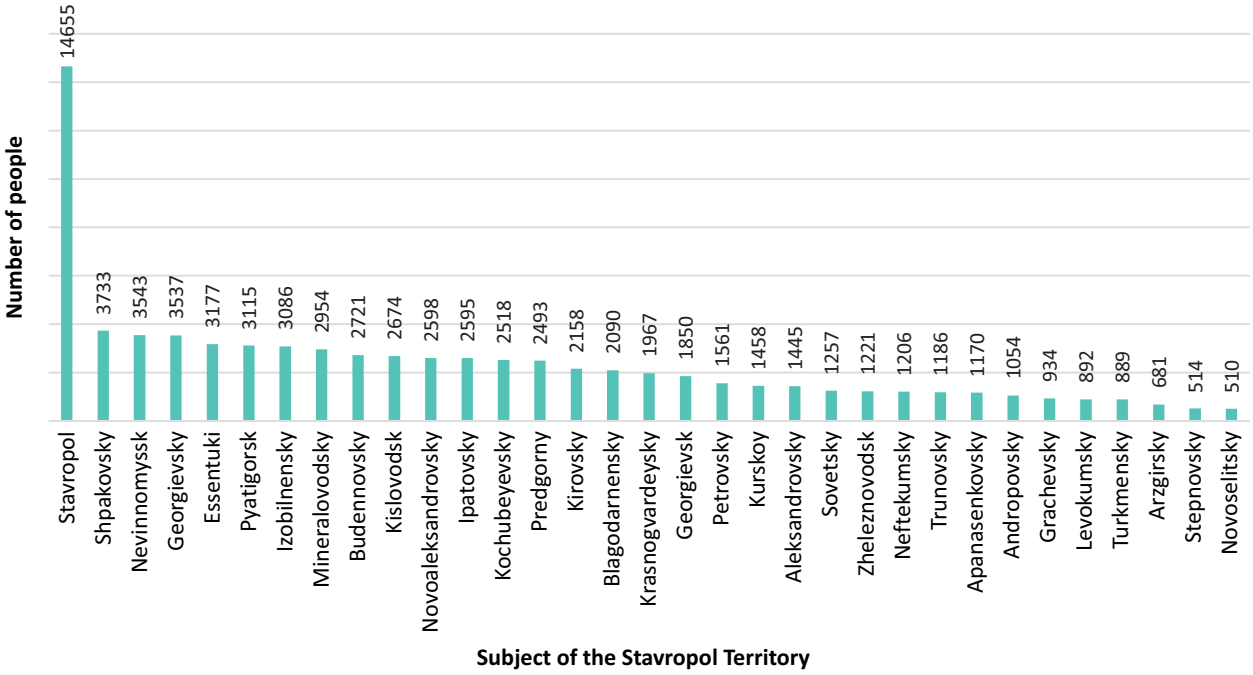

Figure 1 – Ranking of the number of beneficiaries by municipal and urban districts of the Stavropol Territory as of 2024.

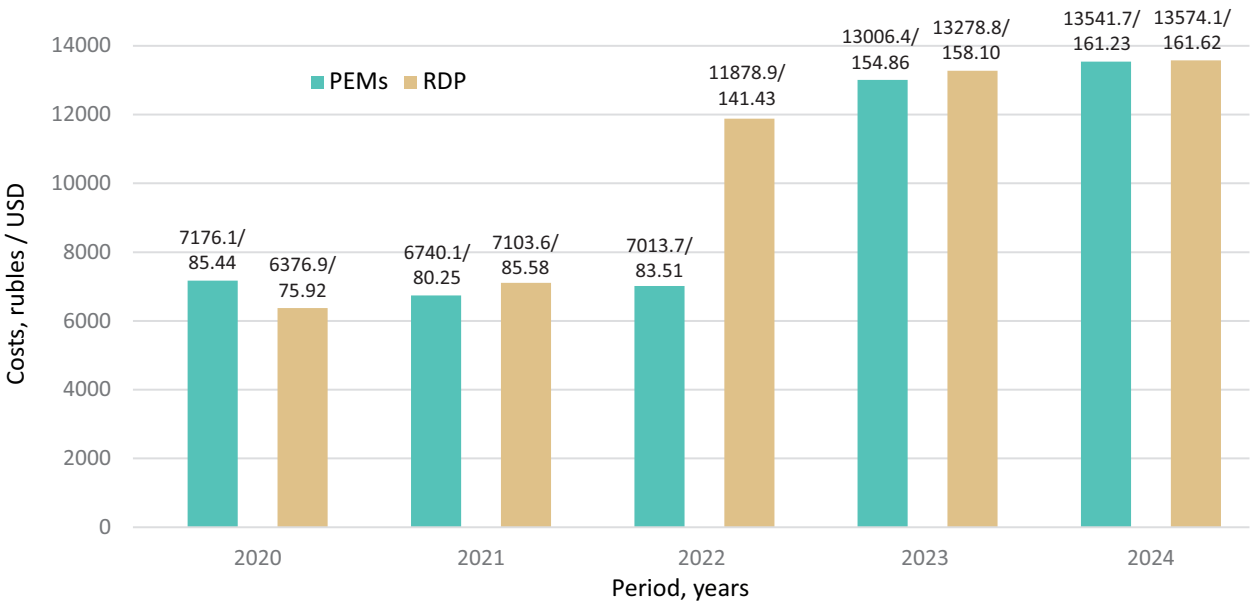

Figure 2 — Costs per beneficiary with Diabetes Mellitus, rubles.
Note: RDP — regional drug provision; PEMs — provision of essential medicines.

Table 1 – Number of patients with DM as of January 01, 2024 according to the State Register of Diabetes Mellitus in the Stavropol Territory

Region	Number of people	Prevalence per 100 thousand people
	Type 1 Diabetes Mellitus	
Russian Federation	290 700	194.2
Stavropol Territory	4 391	152.2
	Type 2 Diabetes Mellitus	
Russian Federation	4 805 659	3 211.2
Stavropol Territory	73 124	2 533.9

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Table 2 – Dynamics of the number of patients with Diabetes Mellitus in the Stavropol Territory

Indicator	Years					Comparison result, 2020 / 2024
	2020	2021	2022	2023	2024	
Total number of patients with DM, people	84 817	84 711	82 224	81 182	81 827	-2990
Rate of change, %	-	99.88	97.06	98.73	100.79	96.47 (-3.52)
Federal beneficiaries with DM, people	14 287	13981	13 450	13 312	10 041	-4246
Rate of change, %	–	97.86	96.20	98.97	75.43	70.28 (-29.72)
Regional beneficiaries with DM, people	63864	63485	61670	62173	77856	+13992
Rate of change, %		99.41	97.14	100.82	122.25	121.91 (+21.91)
Beneficiaries “children category”, people	929	1 109	1 111	1 095	1 172	+243
Specific weight in the total number of beneficiaries, %	1.19	1.43	1.49	1.45	1.43	+0.24

Note: DM — diabetes mellitus.

Table 3 – Costs of federal and regional budgets for drug provision to beneficiaries with Diabetes Mellitus

Indicator	Analyzed period					Comparison results, 2024 / 2020
	2020	2021	2022	2023	2024	
Federal budget funds, million rubles / USD	102.525 / 1.220	94.232 / 1.122	94.334 / 1.123	173.141 / 2.061	53.774 / 0.640	-48.751
Rate of change, %	–	91.91	100.11	183.54	31.06	-47.55
Federal budget funds for PDP of beneficiaries in Lermontov, million rubles / USD	–	5.3 / 0.063	4.07 / 0.048	6.24 / 0.074	7.37 / 0.088	2.07
Rate of change, %	–	-	76.79	153.32	118.11	139.06
Regional budget funds allocated for PDF of patients with DM, million rubles / USD	407.26 / 4.85	450.97 / 5.37	732.54 / 8.72	825.58 / 9.83	1 056.82 / 12.58	649.56
Rate of change, %	–	110.73	162.44	112.70	128.01	259.50

Note: PDF-Preferential Drug Provision; DM — diabetes mellitus.

Table 4 – Structure of budget expenditures for the therapy of Diabetes Mellitus in the Stavropol Territory

Years	“Insulins”		Tablets		Test strips		Syringes + needles		CGM system		Pump	
	million rubles / USD	%	million rubles / USD	%	million rubles / USD	%	million rubles / USD	%	million rubles / USD	%	million rubles / USD	%
2020	305.52 / 3.64	62.00	103.77 / 1.24	21.06	40.29 / 0.48	8.18	13.05 / 0.16	2.65	–	–	–	–
2021	345.58 / 4.11	59.47	95.79 / 1.16	16.48	88.08 / 1.05	15.16	21.54 / 0.26	3.71	–	–	–	–
2022	402.53 / 4.79	54.95	107.88 / 1.28	14.73	70.49 / 0.84	9.62	28.66 / 0.34	3.91	89.39 / 1.06	12.20	–	–
2023	392.67 / 4.68	47.56	135.02 / 1.61	16.35	82.07 / 0.98	9.94	32.14 / 0.38	3.89	140.43 / 1.67	17.01	–	–
2024	457.53 / 5.48	43.29	264.28 / 3.15	25.01	130.45 / 1.55	12.34	38.55 / 0.45	3.65	39.16 / 0.47	3.71	61.96 / 0.74	5.86
Comparison 2024/2020	+152.01	-18.71	+160.51	+3.95	+90.16	+4.16	+25.50	+1.0	–	–	–	–

Note: CGM system — continuous glucose monitoring system.

Table 5 – Structure of expenditure of federal budget funds for the therapy of diabetes mellitus according to the program of providing necessary medicines

Years	"Insulins" million rubles / USD	%	Tablets million rubles / USD	%	Test strips million rubles / USD	%	Syringes + needles million rubles / USD	%	Pump million rubles / USD	%
2020	70.23 / 0.84	68.11	6.01 / 0.07	5.83	4.97 / 0.06	4.82	4.31 /	4.18	17.59 / 0.21	17.06
2021	104.01 / 1.24	67.82	11.41 / 0.13	7.44	14.75 / 0.18	9.62	–	–	23.19 / 0.28	15.12
2022	107.83 / 1.28	55.45	10.48 / 0.12	5.39	12.04 / 0.14	6.19	–	–	64.12 / 0.76	32.97
2023	77.28 / 0.92	44.86	14.66 / 0.17	8.51	4.92 / 0.06	2.86	–	–	75.41 / 0.90	43.77
2024	0.32 / 0.004	0.85	0.06 / 0.0007	0.16	1.56 / 0.019	4.16	–	–	35.59 / 0.42	94.83

Table 6 — Structure of expenditure of the regional budget on the purchase of funds for the therapy of diabetes mellitus

Years	"Insulins" million rubles / USD	%	Tablets million rubles / USD	%	Test strips million rubles / USD	%	Syringes + needles million rubles / USD	%	CGM system million rubles / USD	%	Pump million rubles / USD	%
2020	235.29 / 2.80	62.39	97.75 / 1.16	25.92	35.32 / 0.42	9.37	8.74 / 0.1	2.32	–	–	–	–
2021	241.57 / 2.88	57.41	84.38 / 1.00	20.05	73.33 / 0,87	17.43	21.54 / 0.26	5.12	–	–	–	–
2022	294.70 / 3.51	51.83	97.40 / 1.16	17.13	58.45 / 0,69	10.28	28.66 / 0.34	5.04	89.39 / 1.06	15.72	–	–
2023	315.39 / 3.76	46.01	120.36 / 1.43	17.56	77.14 / 0,92	11.25	32.14 / 0.38	4.69	140.43 / 1.67	20.49	–	–
2024	452.14 / 5.38	45.57	260.63 / 3.10	26.27	141.07 / 1,68	14.22	38.55 / 0.46	3.89	38.54 / 0.46	3.88	61.18 / 0.73	6.17
Comparison 2024 / 2020	+216.85	–16.82	+162.88	–0.35	+105.75	+4.85	+29.81	+1.57	–	–	–	–

The presented data indicate an annual increase in regional budget funds for PDF of patients with DM: for the period from 2020 to 2024, regional budget expenditures increased 2.5 times — from 407.26 / 4.85 to 1056.82 million rubles / 12.58 million dollars, the growth rate was 259.50 %. It should be noted that federal funds allocated for the treatment of DM in the period from 2020 to 2023 tended to increase, while in 2024 there was a decrease in the amount of federal funds by 47.55 %. Federal budget funds were allocated for PNMs (provision of necessary medicines) of beneficiaries with a disability group living in Lermontov, in the analyzed period the funds were increased by 2.07 million rubles / 0.088 million dollars (139.06 %) and in 2024 amounted to 7.37 million rubles.

For the therapy of DM, "insulins" are used in the form of injectable drugs, hypoglycemic tablets and dietary adjustments using developed diets. The study of the data of the state register of DM in the Stavropol Territory for 2024 made it possible to identify the structure of consumption of hypoglycemic tablets by type of therapy and depending on the type of DM. Therapy for patients with DM type I is based only on insulin therapy, which is received by 100 % of patients (4 375 people). Therapy for patients with DM type II includes the use of insulins for 20.63 % (14 140 patients), hypoglycemic tablets for 53 404 people (77.88 %) and for 1.49 % (1 025 people) dietary adjustment is sufficient. It should be noted that the use of oral drugs is based on the combined use of several hypoglycemic tablets. Further study showed that in 2024, for 38.50 % of patients (20 563 people), the treatment regimen includes monotherapy, for 45.76 % (24 436 people) a combination of two drugs is used, and a combination of three hypoglycemic tablets is used in the therapy of 15.74 % of patients (8 405 people).

The results of our analysis of the expenditures of the allocated budget for the purchase of funds for

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drug provision to beneficiaries with DM allowed us to establish its structure. Budget funds are allocated for the purchase of hypoglycemic tablets, injectable forms of insulins depending on the duration of action, necessary means of monitoring blood glucose levels (test strips) and continuous glucose monitoring (CGM) systems, consumables for an insulin pump for children under 18 years. (Table 4).

The presented data demonstrate the predominant expenditure of budget funds on injectable drugs “insulins”. The costs of purchasing these drugs increased by 152.01 million rubles / 1.81 million dollars, but at the same time, the proportion of this group in the analyzed period tends to decrease — from 62. 0% in 2020 to 43.29 % in 2024, which indicates the preservation of leading positions. In our opinion, this may be due to a decrease in the number of federal beneficiaries with type I DM, who are prescribed “insulins”. The costs of oral drugs increased by almost 4.0 % by 2024 — from 103.77 million rubles / 1.24 million dollars in 2020 to 264.28 million rubles / 3.15 million dollars in 2024. It should be noted that CGM system have been purchased for the therapy of patients with DM since 2022: in 2024, 67.96 million rubles / 0.74 million dollars were spent on the purchase of an insulin pump (a device that promotes continuous subcutaneous administration of insulin).

Table 5 presents the structure of expenditures of federal budget funds for drug provision to beneficiaries with DM in the PNMs (provision of necessary medicines).

The analysis of the structure of purchases of funds for beneficiaries with DM in the PEMs in the period from 2020 to 2023 indicates the predominance of “insulins” in the structure, the proportion of which in the analyzed period tends to decrease — from 68.11 % to 44.86 %. During this time, the volume of expenditures on the purchase of hypoglycemic tablets almost doubled — from 6.01 million rubles / 0.07 million dollars in 2020 to 14.66 million rubles / 0.17 million dollars in 2023, the proportion of this group of funds in 2023 amounted to 8.51 %. The volume of expenditures on the purchase of insulin pumps increased from 17.59 million rubles / 0.21 million dollars to 75.41 million rubles / 0.90 million dollars, while there was also an increase in the proportion of expenditures on this group of funds — up to 43.77 % in 2023.

In accordance with the adopted regional regulatory documents on the redistribution of the budget for the purchase of funds for the therapy of DM, since 2024, the subject has decided to provide all citizens with DM, regardless of the presence/absence of a disability group (with the exception of beneficiaries from Lermontov), at the expense of the budget of the Stavropol Region, purchases of drugs at the expense of the federal budget separately for federal beneficiaries in the region have been discontinued. Such a redistribution of funds is a regional feature in the legislation of the Stavropol Region.

The results of the analysis of the distribution of regional budget funds allocated for PDP of beneficiaries with DM are presented in Table 6.

In the analyzed period, funds for the purchase of “insulins” increased by 216.85 million rubles / 2.58 million dollars. In the structure of purchases for regional beneficiaries, “insulins” have a significant downward trend from 62.39 % in 2020 to 45.57 % in 2024, while maintaining a leading position. The costs of hypoglycemic tablets were increased by 162.66 million rubles / 1.94 million dollars, the proportion in the structure of purchases was increased slightly and reached 26.27 % in 2024. An increase in the cost of test strips for beneficiaries was recorded from 35.32 million rubles / 0.42 million dollars in 2020 to 141.07 million rubles / 1.68 million dollars in 2024, the proportion of this group increased by 4.85 % and amounted to slightly more than 14 % in 2024.

It should be noted that since 2022, consumable materials (syringes + needles) have been purchased at the expense of the regional budget, the proportion in the consumption structure is about 4.0%, CGM system in the consumption structure tends to decrease — from 15.72 % in 2022 to 3.88 % in 2024, slightly more than 6.0 % of the funds were allocated for the purchase of insulin pumps and consumable materials for them.

Figure 2 shows changes in the costs per beneficiary, which tend to increase. Thus, the costs per beneficiary with DM in the analyzed period almost doubled, in the PEMs the increase in costs occurred by 88.7 % from 7 176.1 rubles / 85.44 dollars in 2020 to 13 541.7 rubles / 161.23 dollars in 2024, the increase in costs per beneficiary in the regional drug provision (RDP) was 212.9%, and the amount reached 13 574.1 rubles / 161.62 dollars. The presented results indicate an increase in consumption and increased availability of funds for the therapy of DM for beneficiaries.

Furthermore, the redistribution of budgetary funds and the provision of benefits to beneficiaries from a single source made it possible to equalize the costs

per beneficiary in the PEMs and RDP. In 2024, the costs practically corresponded to the same amount, which indicates equal opportunities for both federal and regional beneficiaries.

Traditional insulin therapy is based on the parenteral use of various insulin preparations. Currently, the main devices for insulin administration are syringes, pen injectors, cartridge systems, and insulin pumps. The study determined the proportion of beneficiaries in diabetes therapy who use various devices for insulin administration.

The results of the study indicate the predominant use of insulin in cartridges for patients with type 1 diabetes, both for adults (90.91 %) and for children (81.32%). Insulin therapy for type 2 diabetes is based on the use of insulin preparations in cartridges (87.19 %) and in vials (12.81 %). At the same time, insulin pumps are predominantly provided to children with type 1 DM (18.49 %), which exceeds their use in adults (6.3 %).

DISCUSSION

DM is recognized as one of the main factors of disability and mortality worldwide. According to data published in foreign scientific literature, from 1990 to 2022, the prevalence of DM in the world increased: in 131 countries among women and in 155 countries among men. The healthcare resources of all countries are aimed at optimizing the work of primary health care and healthcare resources for the early detection and effective treatment of DM [26, 27].

In the constituent entities of the Russian Federation, DM is an important medical and social problem an increase in the incidence among the adult population [28–30] and among patients under 14 years of age [31], as well as with the assessment of the loss of working capacity of patients [32] and the organization of drug provision, which has variable regional options.

According to the results of the study, the dynamics of changes in the number of patients with DM in the Stavropol Territory has an upward trend, while there is a decrease in the number of federal beneficiaries and an increase in regional ones. At the same time, state guarantees in the field of drug provision are aimed at ensuring access to drugs for patients with DM, regardless of the category of beneficiary. The implementation of accessibility in drug provision also has regional options. A regional feature of drug provision for beneficiaries with DM in the Stavropol Territory is the redistribution of funding for drug provision for patients with DM, and since 2024, beneficiaries have been provided with drugs from a single source — the regional budget, with the exception of federal beneficiaries in Lermontov (Stavropol Territory, Russia), who are provided at the expense of the PEMs program. In this regard, amendments were made to the regional legislation regarding the delegation of certain state powers to local governments.

From a practical point of view, this made it possible to increase the funds of the regional budget and ensure equal opportunities in drug provision for federal and regional beneficiaries confirmed by the dynamics of costs per beneficiary with DM, which almost doubled in the analyzed period and in 2024 correspond to the same amount — over 13.5 thousand rubles / 0.16 thousand dollars for both federal and regional beneficiaries.

Study Limitations

This study examines certain quantitative indicators of the implementation of drug provision for beneficiaries with DM in the Stavropol Territory. The analysis of the range of self-monitoring devices used in terms of trade names of drugs and nosological forms of DM was not carried out, and the qualitative indicators of the implementation of the drug provision program related to the redistribution of funding for drug provision for beneficiaries with DM since 2024 were not evaluated. All this is a promising area for further scientific research.

CONCLUSION

This study examined the quantitative characteristics of drug provision for beneficiaries with DM in the Stavropol Territory: the dynamics of the number of beneficiaries with DM is presented, municipal and urban districts with the largest number of them are identified, the structure of spending federal and regional budget funds on self-monitoring devices is presented, as well as the dynamics of costs per beneficiary. The results obtained can be used to develop organizational solutions to improve the accessibility of drug provision for beneficiaries with DM both in the Stavropol Territory and in other constituent entities of the Russian Federation. In the future, the authors plan to continue the study of the organization of drug provision for patients with DM in the Stavropol Territory from the point of view of analyzing the range of drugs in the public procurement sector and their possible impact on treatment outcomes.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

Rosa I. Yagudina — idea, conceptualization, methodology, data curation, writing—review & editing. Olga L. Listova — data collection and curation, processing and interpretation of results, writing—original draft. All authors confirm that their authorship meets the international ICMJE criteria (all authors made a significant contribution to the development of the concept, conduct of the study and preparation of the article, read and approved of the final version before the publication).

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Study of the pharmacokinetics of non-immunogenic staphylokinase in patients with acute myocardial infarction and acute ischemic stroke

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The aim. To investigate the pharmacokinetic parameters of non-immunogenic staphylokinase (Fortelyzin®, SuperGene LLC, Russia) in patients with acute myocardial infarction with ST-segment elevation (STEMI) on the electrocardiogram and in patients with ischemic stroke.

Materials and methods. The clinical study was conducted in 50 patients with STEMI after a single intravenous administration of non-immunogenic staphylokinase at a dose of 15 mg and in 50 patients with ischemic stroke after a single intravenous administration of the drug at a dose of 10 mg. The main pharmacokinetic parameters were determined: half-life, initial concentration, volume of distribution, clearance, and area under the pharmacokinetic curve.

Results. As a result of the study of the pharmacokinetics of non-immunogenic staphylokinase, it was found that after a single intravenous administration of the drug at a dose of 15 mg, the initial concentration was 7.1 ± 2.7 μg/mL, the half-life was 5.77 ± 0.72 min, the clearance was 0.33 ± 0.04 l/min, and the area under the pharmacokinetic curve (AUC_{0-t}) was 42.9 ± 3.2 μg/mL*min. After administration of the drug at a dose of 10 mg, the initial concentration was 2.8 ± 0.3 μg/mL, the half-life was 5.11 ± 0.56 min, the clearance was 0.35 ± 0.06 l/min, and the area under the pharmacokinetic curve (AUC_{0-t}) was 28.5 ± 3.6 μg/mL*min. The terminal half-life was 32 min in both dosage regimens.

Conclusion. It was found that non-immunogenic staphylokinase is characterized by a short half-life and high clearance, which ensures the safety of the drug in clinical practice. The peculiarities of the pharmacodynamics of non-immunogenic staphylokinase, associated with its interaction with plasmin in the thrombus and subsequent recirculation of released drug molecules, allow it to be used in low doses, regardless of the patient's body weight, despite the short half-life.

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Keywords: non-immunogenic staphylokinase; pharmacokinetics; thrombolysis; myocardial infarction; ischemic stroke
Abbreviations: MI — myocardial infarction; STEMI — ST-segment elevation myocardial infarction; PE — pulmonary embolism; mRS — modified Rankin scale; TLT — thrombolytic therapy.

Исследование фармакокинетики лекарственного препарата неиммуногенной стафилокиназы у пациентов с острым инфарктом миокарда и ишемическим инсультом

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Цель. Исследовать фармакокинетические параметры неиммуногенной стафилокиназы (Фортелизин®, ООО «СупраГен», Россия) у пациентов с острым инфарктом миокарда с подъёмом сегмента ST (ОИМпST) электрокардиограммы и у пациентов с ишемическим инсультом.

Материалы и методы. Клиническое исследование проведено у 50 пациентов с ОИМпST после однократного внутривенного введения препарата неиммуногенной стафилокиназы в дозе 15 мг и у 50 пациентов с ишемическим инсультом после однократного внутривенного введения препарата в дозе 10 мг. Определялись основные фармакокинетические параметры — период полувыведения, начальная концентрация, объём распределения, клиренс, а также площадь под фармакокинетической кривой.

Результаты. В результате исследования фармакокинетики неиммуногенной стафилокиназы было установлено, что после однократного внутривенного введения препарата в дозе 15 мг начальная концентрация составляла 7,1±2,7 мкг/мл, период полувыведения — 5,77±0,72 мин, клиренс — 0,33±0,04 л/мин, площадь под фармакокинетической кривой (AUC_{0-t}) — 42,9±3,2 мкг/мл*мин. После введения препарата в дозе 10 мг начальная концентрация составляла 2,8±0,3 мкг/мл, период полувыведения — 5,11±0,56 мин, клиренс — 0,35±0,06 л/мин, площадь под фармакокинетической кривой (AUC_{0-t}) — 28,5±3,6 мкг/мл*мин. Терминальный период полувыведения составил 32 мин в обоих вариантах дозирования.

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Заключение. Установлено, что неиммуногенная стафилокиназа характеризуется коротким периодом полувыведения и высоким клиренсом, что обеспечивает безопасность применения препарата в клинической практике. Особенности фармакодинамики неиммуногенной стафилокиназы, связанные с ее взаимодействием с плазмином в толще тромба и последующей рециркуляцией высвобождающихся молекул препарата, позволяют использовать ее в низких дозах независимо от массы тела пациента, несмотря на короткий период полувыведения.
Ключевые слова: неиммуногенная стафилокиназа; фармакокинетика; тромболизис; инфаркт миокарда; ишемический инсульт
Список сокращений: ИМ — инфаркт миокарда; ОИМпST — острый инфаркт миокарда с подъёмом сегмента ST; ТЭЛА — тромбоэмболия легочных артерий; МШР — модифицированная шкала Рэнкина; ТЛТ — тромболитическая терапия.

INTRODUCTION

Thrombolytic therapy (TLT) is a pathogenetically sound method for treating acute myocardial infarction (MI) with ST-segment elevation (STEMI), ischemic stroke, and massive pulmonary embolism (PE), reducing the risk of disability and mortality [1]. TLT is simple to perform, relatively inexpensive, has extensive experience in real clinical practice, and can be used at all stages of care [2]. The introduction of thrombolytic therapy into clinical practice has led to a significant reduction in 30-day mortality in patients with STEMI — from 17–18 to 5–8% [3].

The history of TLT for MI spans more than 60 years. At the Institute of Therapy of the USSR Academy of Medical Sciences (National Medical Research Center of Cardiology named after Academician E.I. Chazov), under the guidance of Academician A.L. Myasnikov, after a large number of experimental studies of fibrinolysin (now understood as plasmin) in thromboses of various localizations, in 1961, E.I. Chazov was the first in the world to use fibrinolysin in a patient with acute MI, followed by heparin therapy [4]. Work on creating effective and safe thrombolytic drugs with a convenient dosing regimen, absence of allergenic properties, and minimal risk of hemorrhagic complications has been carried out in many scientific laboratories. Currently, in the Russian Federation, plasminogen activators (alteplase, tenecteplase, staphylokinase) are most widely used for TLT. It is known that staphylokinase is the most fibrin-selective thrombolytic drug, since it exhibits the highest affinity only to plasminogen adsorbed on a fibrin clot, the so-called γ-plasminogen [5]. Staphylokinase surpasses prourokinase, alteplase, and tenecteplase in its selectivity for fibrin [5]. The plasmin-staphylokinase complex activates the transition of γ-plasminogen to plasmin on the surface of the thrombus. The plasmin formed simultaneously enhances the fibrinolytic activity of staphylokinase, and its excess is rapidly inactivated in the systemic circulation by α2-antiplasmin [6]. The high fibrin selectivity of staphylokinase is confirmed by a minimal decrease in the level of fibrinogen in the blood, on average by 5%, while alteplase causes a drop in the level of fibrinogen to 30% [7]. The absence of systemic fibrinogenolysis explains the high safety of staphylokinase: the risk of bleeding is lower if using other thrombolytics that are non-selective to fibrin.

When the plasmin-staphylokinase complex is inhibited by α2-antiplasmin, an active staphylokinase molecule is released for subsequent recycles. The recirculation of staphylokinase allows reducing the dose used in clinical practice compared to tissue plasminogen activators and makes it independent of the patient's body weight [8]. An expanded kinetic analysis of the reaction of staphylokinase with plasmin showed that its catalytic activity is 1000 times higher than that of alteplase [9].

Unlike the native staphylokinase molecule, three amino acids (Lys74, Glu75, and Arg77) are replaced by alanine in the non-immunogenic molecule, as a result of which the drug does not have antigenic activity [10]. Non-immunogenic staphylokinase is a single-chain molecule with a molecular weight of 15.5 kDa. It has been established that the fibrinolytic activity of non-immunogenic staphylokinase is 40% higher than that of the native staphylokinase molecule [11].

THE AIM. To establish the pharmacokinetic parameters of non-immunogenic staphylokinase in clinical studies in patients with acute myocardial infarction with ST-segment elevation and with ischemic stroke.

MATERIALS AND METHODS

Blood sampling in patients with acute myocardial infarction with ST-segment elevation

A multicenter open randomized comparative study of the efficacy and safety of a single bolus injection of non-immunogenic staphylokinase

(15 mg) and tenecteplase (30–50 mg) in patients with STEMI (FREEDOM-1) based on 11 leading medical institutions in Russia was conducted in the period from October 2014 to August 2016 in accordance with the permission of the Ministry of Health of Russia No. 261 dated May 16, 2014 [12, 13]. Before inclusion in the study, all patients signed an informed consent form. The study was approved by the Ethics Council of the Ministry of Health of Russia (protocol No. 81 dated April 15, 2014) and local Ethics Committees based on research centers.

382 patients were included in the study. Inclusion criteria: diagnosis of STEMI in the first 12 hours from the onset of the disease. *Exclusion criteria*: bleeding, hemorrhagic stroke, ischemic stroke in the preceding 6 months, diseases with an increased risk of bleeding. A complete list of *inclusion* and *exclusion criteria* in the study has been published previously [12, 13]. Criteria for the effectiveness of TLT — the number of patients with a decrease in the ST-segment of the electrocardiogram by 50% from the initial value after 90 min, as well as the number of patients with restoration of coronary blood flow in the infarct-related artery according to TIMI 2 + TIMI 3 (Thrombolysis in Myocardial Infarction) criteria according to coronary angiography. Safety criteria were the number of major bleeding events, intracranial hemorrhages, as well as mortality from all causes, cardiogenic shock, and recurrent myocardial infarction during the hospitalization period.

Patients were randomized into 2 groups (n = 191 each) for the administration of non-immunogenic staphylokinase (Fortelizin®, SuperGene LLC, Russia) or tenecteplase (Metalyse®, Boehringer Ingelheim International, Germany). Randomization was carried out by the method of envelopes. Recombinant non-immunogenic staphylokinase was administered at a dose of 15 mg regardless of body weight as a bolus over 10–15 seconds, tenecteplase — as a bolus at a dose of 30–50 mg depending on body weight, according to the instructions for medical use.

During the clinical study, blood was taken from 50 patients 3, 6, 9, 12, 15, and 20 min after the administration of non-immunogenic staphylokinase in a volume of 2 mL into heparin tubes with the patient's number and study point indicated on the label. The blood tubes were centrifuged using a Liston C 2202 centrifuge for 10 min at a speed of 3000 rpm (Liston, Russia). Blood plasma in a volume of at least 1 mL was transferred to an Eppendorf tube. The choice of study points was based on the results of a preclinical pharmacokinetic study in hamsters, where the half-life was 1.9 min [14].

Blood sampling in patients with ischemic stroke

A multicenter open randomized comparative study of the efficacy and safety of a single bolus injection of non-immunogenic staphylokinase and bolus-infusion administration of alteplase in patients with ischemic stroke (FRIDA) was conducted in the period from March 2017 to March 2019 in accordance with the permission of the Ministry of Health of Russia No. 498 dated July 15, 2016. The study was approved by the Ethics Council of the Ministry of Health of Russia (Protocol No. 125 dated May 24, 2016) and local Ethics Committees based on research centers.

336 patients were included in the study in 19 clinical centers. *Inclusion criteria*: ischemic stroke — no more than 4.5 hours from the onset of symptoms. Exclusion criteria: blood pressure over 185/110 mm Hg, signs of severe stroke (NIHSS stroke scale score >25), active bleeding, hemorrhagic stroke, diseases with an increased risk of bleeding. A complete list of inclusion and exclusion criteria in the study was published previously [15, 16]. The primary efficacy criterion was the number of patients who achieved good functional recovery (0–1 points on the modified Rankin scale [mRS]) at 90 days. Secondary efficacy criteria were the change in the median NIHSS after 24 hours and at 90 days. Safety criteria were mortality from all causes at 90 days, symptomatic hemorrhagic transformation (according to ECASS-III criteria), major bleeding.

Patients were randomized into 2 groups (n = 168 each) for the administration of non-immunogenic staphylokinase (Fortelizin®, SuperGene LLC, Russia) or alteplase (Actilyse®, Boehringer Ingelheim International, Germany). Randomization was carried out by the method of envelopes. Non-immunogenic staphylokinase was administered at a dose of 10 mg regardless of body weight as a bolus over 5–10 seconds, alteplase — as a bolus-infusion at a dose of 0.9 mg/kg (maximum 90 mg) depending on body weight, according to the instructions for medical use.

During the clinical study, blood was taken from 50 patients 3, 6, 9, 12, 15, and 20 min after the administration of non-immunogenic staphylokinase in a volume of 2 mL into a heparin tube with the patient's number and study point indicated on the label. The blood tubes were centrifuged using a Liston C 2202 centrifuge for 10 min at a speed of 3000 rpm (Liston,

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Russia). Blood plasma in a volume of at least 1 mL was transferred to an Eppendorf tube. The choice of study points was also based on the results of a preclinical pharmacokinetic study [14].

Determination of the concentration of non-immunogenic staphylokinase in the blood

To determine the concentration of non-immunogenic staphylokinase in blood plasma, calibration samples were prepared with concentrations of 10; 5; 2.5; 1.25; 0.625; 0.312; 0.156 and 0.078 μg/mL with a buffer solution (20 mM Tris-HCl, 150 mM NaCl, 0.005% Tween-20, pH=8.0) based on literature data on achieving a staphylokinase concentration in the blood of 1.24 ± 0.24 μg/mL after a single administration at a dose of 10 mg [17]. The test samples were diluted 3000 times with a buffer solution. For each sample, 3 wells of a plate with primary mouse antibodies to staphylokinase were allocated. 100 μL of test and calibration samples were added to the wells. 100 μL of buffer solution was added to the negative control wells. The plate was incubated for 60 min at a temperature of 37°C. After incubation, the liquid was removed, and the wells were washed three times with 300 μL of buffer solution. Then, 100 μL of a solution of secondary rabbit antibodies labeled with horseradish peroxidase (20 mM Tris-HCl, 150 mM NaCl, 0.005% Tween-20, pH = 8.0, 2% bovine serum albumin, secondary antibodies diluted 1:1000) was added to each well, and the plate was incubated for 60 min at a temperature of 37°C. After incubation, the liquid was removed from the plate, and the wells were washed five times with 300 μL of buffer solution. Then, 100 μL of substrate solution (150 mM phosphate-citrate buffer, pH = 5.0, 0.07% orthophenylenediamine, 0.06% H_2O_2) was added to each well. Incubated at room temperature for 3–5 min until staining appeared in the negative control sample. 50 μl of stop reagent (10% sulfuric acid solution) was added to each well. Within 15 min after adding the stop reagent, the optical density was measured in each well at 490 nm on a microplate photometer (ImmunoChem-2100, HTI, USA). The average optical density value was calculated in three wells for each sample. The concentration of the drug in the test samples was determined using a calibration curve.

Statistical processing

Sample size calculation

According to the literature, the frequency of achieving the primary efficacy criterion — a decrease in the ST-segment of the electrocardiogram by 50% from the initial level 90 min after thrombolysis — when using tenecteplase is 66% [18], the frequency of achieving the primary efficacy criterion when using non-immunogenic staphylokinase according to a phase II clinical study is 83%. To prove the hypothesis of non-inferiority of non-immunogenic staphylokinase compared to tenecteplase in patients with STEMI, a clinically significant limit of 12.5% was chosen. It was established that a really absent difference between the drugs (the upper limit of the 95% confidence interval [CI] will be lower than the non-inferiority limit) with 80% probability[1] can be detected with a set of 173 patients in each group (n = 347 patients for 2 groups). Taking into account the possible dropout of 10% of patients due to violations of the study protocol, a set of 382 patients is necessary (n = 191 patients in each group). Based on the fact that the study of the pharmacokinetics of the comparison drug tenecteplase was carried out depending on the dose in 20 (50 mg) or 48 (30 mg) patients [19], venous blood was taken from 50 patients to study the pharmacokinetic parameters of non-immunogenic staphylokinase.

To prove the hypothesis of non-inferiority of non-immunogenic staphylokinase compared to alteplase in patients with ischemic stroke, it was established that the frequency of achieving the primary efficacy criterion — the number of patients with good functional recovery at 90 days after thrombolysis (mRS 0–1 points) — according to the SITS-MOST registry is 54.8% [20]. The frequency of achieving the primary efficacy criterion when using placebo averages 37.9% — this value was obtained from the ECASS II [21], Atlantis [22] and ECASS III [23] studies, where the favorable effect when using placebo is 36.6%, 32% and 45.2%, respectively. Thus, the difference between good functional recovery with the use of alteplase and placebo is 16.9%. The non-inferiority limit was set at 16%. When demonstrating at a two-sided significance level of 5% to maintain 80% comparison power, the minimum number of patients included in the study was estimated at 152 patients in each group. Taking into account a possible 10% dropout, the sample size was increased to 336 patients (n = 168 patients in each group). Based on the fact that the study of the pharmacokinetics of the comparison drug alteplase was carried out in 53 patients [19], venous blood was taken from 50 patients to study the pharmacokinetic parameters of non-immunogenic staphylokinase.

[1] Сергиенко В.И., Бондарева И.Б. Математическая статистика в клинических исследованиях. Москва: ГЭОТАР-Медиа, 2006. – 303 с. EDN: QLMSKH

Statistical methods

The pharmacokinetic curve is described by the equation:

$$C(t)=A\times e^{-\alpha t}+B\times e^{-\beta t},$$

where A and B are proportionality coefficients (μg/L); α is the rate constant of drug distribution (1/min); β is the rate constant of terminal elimination (1/min).

The following parameters were calculated: half-distribution period ($t_{1/2\alpha}$), plasma half-life (t1/2β), initial concentration in blood plasma (C_0), volume of distribution (V_1 and V_2), drug clearance (CL_1 and CL_2), area under the pharmacokinetic curve within the duration of observation (AUC_{0-t}), area under the pharmacokinetic curve from zero to infinity ($AUC_{0-\infty}$), area under the curve from zero to ∞ with extrapolation of the final phase (AUMC), mean residence time of the drug in the systemic circulation (MRT), apparent volume of distribution at steady state (V_{ss}).

Statistical analysis was performed using the R program (version 4.2) (The R Foundation for Statistical Computing and Graph Pad Prism 7, Graph Pad Software Inc., USA). For continuous indicators, the following descriptive statistics are presented: number of data excluding gaps (*n*), arithmetic mean (M), standard deviation (SD), median (Me), 25 and 75 percentiles (Q1 and Q3, respectively).

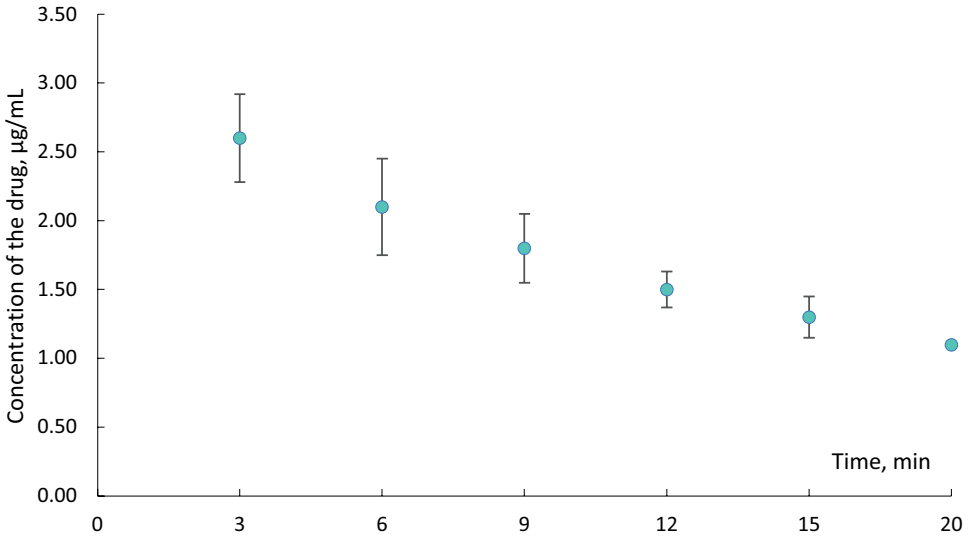

Figure 1 – Pharmacokinetic profile of non-immunogenic staphylokinase at a dose of 15 mg in patients with acute myocardial infarction with ST-segment elevation.

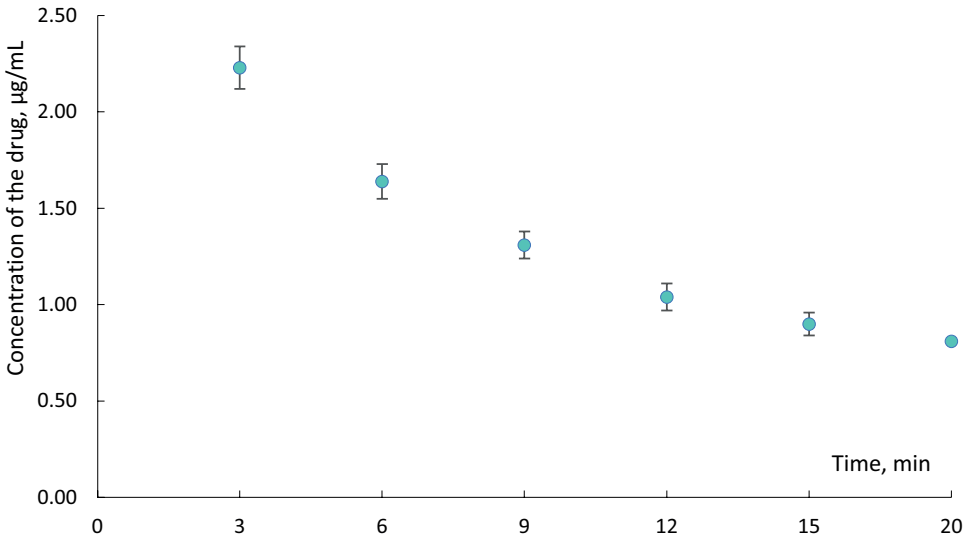

Figure 2 – Pharmacokinetic profile of non-immunogenic staphylokinase at a dose of 10 mg in patients with ischemic stroke.

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Table 1 – Baseline characteristics of patients

Characteristics	Indicator (n = 50)
Gender, male / female, n (%)	38 / 12 (76% / 23%)
Age, M ± SD, years	58.9 ± 9.9
Patients over 75 years old, n (%)	3 (6%)
Weight, Me (Q1; Q3), kg	82 (73; 91)
Body mass index, Me (Q1; Q3), kg/m^2	27.8 (25.3; 31.2)
Arterial hypertension, n (%)	37 (75%)
Dyslipidemia, n (%)	43 (86%)
Smoking, n (%)	20 (40%)
Type II diabetes mellitus, n (%)	7 (14%)
Myocardial infarction, n (%)	6 (12%)
ST-segment elevation, M ± SD, mm	3.58 ± 1.96
SBP, M ± SD, mm Hg	118.6 ± 8.2
DBP, M ± SD, mm Hg	74.7 ± 7.2
HR, M ± SD, bpm	75.9 ± 14.7
STEMI localization, n (%):	
anterior	21 (42%)
inferior	28 (56%)
other	1 (2%)
Time from the onset of symptoms to TLT, M ± SD, h	3.4 ± 1.7

Note: TLT — thrombolytic therapy.

Table 2 – Assessment of the effectiveness and safety of non-immunogenic staphylokinase in patients with acute myocardial infarction with ST-segment elevation

Criterion	Indicator (n = 50), n (%)
Decrease in the ST-segment after 90 minutes by 50%	40 (80%)
Restoration of coronary blood flow according to TIMI criteria:	
0	12 (24%)
1	3 (6%)
2	16 (32%)
3	19 (38%)
2+3	35 (70%)
Death from all causes	1 (2%)
Cardiogenic shock	2 (4%)
Recurrent myocardial infarction	2 (4%)
Major bleeding	0 (0%)
Intracranial hemorrhage	0 (0%)
Allergic reactions	0 (0%)

Table 3 – Pharmacokinetic parameters in patients with acute myocardial infarction with ST-segment elevation

Parameter	Value (M ± SD)	Parameter	Value (M ± SD)
A, μg/L	5.56 ± 2.63	CL_1, L/min	0.33 ± 0.04
α, 1/min	0.32 ± 0.05	V_2, L	3.68 ± 0.74
B, μg/L	1.52 ± 0.14	CL_2, L/min	0.45 ± 0.07
β, 1/min	0.04 ± 0.00	AUC_{0-t}, μg/mL×min	42.9 ± 3.2
$t_{1/2\alpha}$, min	5.77 ± 0.72	$AUC_{0-\infty}$, μg/mL×min	68.02 ± 7.04
$t_{1/2\beta}$, min	32.08 ± 4.11	AUMC, μg/mL×min^2	3742.07 ± 831.14
C_0, μg/mL	7.1 ± 2.7	MRT, min	42.26 ± 5.57
V_1, L	6.62 ± 0.63	V_{ss}, L	10.3 ± 1.05

Table 4 – Baseline characteristics of patients

Characteristics	Indicator (*n* = 50)
Gender, male / female, *n* (%)	32 / 18 (64% / 36%)
Age, M ± SD, years	64.4 ± 9,6
Patients over 80 years old, *n* (%)	2 (4%)
Weight, Me (Q1; Q3), kg	80 (74; 90)
Body mass index, Me (Q1; Q3), kg/m^2	27.1 (24.7; 30.7)
Arterial hypertension, *n* (%)	48 (95%)
Atrial fibrillation, *n* (%)	20 (40%)
Smoking, *n* (%)	13 (26%)
Dyslipidemia, *n* (%)	10 (20%)
Stroke, *n* (%)	7 (13%)
Type II diabetes mellitus, *n* (%)	5 (10%)
Time from the onset of symptoms to TLT, M ± SD, h	2.9 ± 0.8
Median NIHSS before TLT, Me (Q1; Q3), points	11 (8; 14)
Median mRS before TLT, Me (Q1; Q3), points	4 (4; 5)
Focus localization, *n* (%): Right middle cerebral artery Left middle cerebral artery Vertebrobasilar basin	 22 (44%) 24 (48%) 4 (8%)

Note: TLT — thrombolytic therapy.

Table 5 – Assessment of the effectiveness and safety of non-immunogenic staphylokinase in patients with ischemic stroke

Criterion	Indicator (*n* = 50), *n* (%)
Number of patients with mRS 0-1 points at 90 days	25 (50%)
Median NIHSS after 24 hours, points	6 (3–11%)
Median NIHSS at 90 days, points	2 (1–5%)
Death from all causes at 90 days	5 (10%)
Symptomatic hemorrhagic transformation (ECASS-III)	1 (2%)
Major bleeding	0 (0%)
Myocardial infarction	0 (0%)
PE	0 (0%)
Allergic reactions	0 (0%)

Note: PE — pulmonary embolism.

Table 6 – Pharmacokinetic parameters in patients with ischemic stroke

Parameter	Value (M ± SD)	Parameter	Value (M ± SD)
A, μg/L	1.78 ± 0.29	CL_1, L/min	0.35 ± 0.06
α, 1/min	0.4 ± 0.1	V_2, L	3.62 ± 0.42
B, μg/L	1.02 ± 0.1	CL_2, L/min	0.51 ± 0.1
β, 1/min	0.03 ± 0.00	AUC_{0-t}, μg/mL×min	28.5 ± 3.6
$t_{1/2\alpha}$, min	5.11 ± 0.56	$AUC_{0-\infty}$, μg/mL×min	52.94 ± 4.11
$t_{1/2\beta}$, min	32.67 ± 2.12	AUMC, μg/mL×min^2	2424.95 ± 277.57
C_0, μg/mL	2.8 ± 0.3	MRT, min	38.71 ± 2.62
V_1, L	5.79 ± 0.66	V_{ss}, L	9.4 ± 0.8

Table 7 – Comparison of pharmacokinetic parameters of the main thrombolytic drugs

Parameter	Non-immunogenic staphylokinase, 10 mg	Non-immunogenic staphylokinase, 15 mg	Alteplase, 100 mg	Tenecteplase, 30–50 mg
$t_{1/2\alpha}$, min	5.11 ± 0.56	5.77 ± 0.72	3.5 ± 1.4	24 ± 5.5
$t_{1/2\beta}$, min	32.67 ± 2.12	32.08 ± 4.11	72 ± 68	129 ± 87
CL_1, L/min	0.35 ± 0.06	0.33 ± 0.04	0.57 ± 0.13	0.1 ± 0.05

Note: data are presented as M±SD.

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RESULTS

Study of the pharmacokinetics of non-immunogenic staphylokinase at a dose of 15 mg in patients with acute myocardial infarction with ST-segment elevation

The study included 50 patients with STEMI who received non-immunogenic staphylokinase once as a bolus at a dose of 15 mg. Demographic, anthropometric, anamnesis data, clinical characteristics, and time intervals are presented in Table 1.

The average age of patients was 58.9 ± 9.9 years. The proportion of patients with arterial hypertension was 37 patients (75%), previous myocardial infarction — 12%, lipid metabolism disorders — 86%. Inferior infarctions predominated in the study population (56%).

Assessment of the effectiveness and safety of TLT is presented in Table 2.

A decrease in the ST-segment after 90 min by 50% from the initial value was noted in 80% of patients. Restoration of coronary blood flow in the infarct-related artery according to TIMI 2 + TIMI 3 criteria was observed in 70% of patients. Death from all causes was 2%. No intracranial hemorrhages were registered with the use of non-immunogenic staphylokinase. No patients experienced major bleeding or hemorrhagic stroke. No allergic reactions were registered as a result of the study.

The averaged pharmacokinetic profile of non-immunogenic staphylokinase is presented in Figure 1.

Non-immunogenic staphylokinase is a short-lived molecule and is characterized by rapid elimination from the systemic circulation. Table 3 presents the pharmacokinetic parameters of non-immunogenic staphylokinase in patients with STEMI after a single bolus injection.

The initial concentration C_0 of non-immunogenic staphylokinase in blood plasma is 7.1 ± 2.7 μg/mL. The half-life $t_{1/2\alpha}$ of non-immunogenic staphylokinase, administered once as a bolus at a dose of 15 mg in patients with STEMI, is 5.77 ± 0.72 min, the terminal half-life $t_{1/2\beta}$ — 32.08 ± 4.11 min. The mean residence time of non-immunogenic staphylokinase in the systemic circulation (MRT) was 42.26 ± 5.57 min. Thus, non-immunogenic staphylokinase has a very short half-life and is rapidly metabolized to amino acids when passing through the liver.

The clearances of non-immunogenic staphylokinase were CL_1 = 0.33 ± 0.04 L/min and CL_2 = 0.45 ± 0.07 L/min. High clearance rates indicate rapid elimination of the staphylokinase molecule from the systemic circulation. The area under the pharmacokinetic curve within the duration of observation AUC_{0-t} was 42.9 ± 3.2 μg/mL×min, the area under the pharmacokinetic curve from 0 to infinity $AUC_{0-\infty}$ was 68.02 ± 7.04 μg/mL×min. The area under the curve from 0 to infinity with extrapolation of the final phase (AUMC) was 3742.07 ± 831.14 μg/mL×min^2.

The apparent volume of distribution of the drug at steady state (V_{SS}) was 10.3 ± 1.05 L. The magnitude of the apparent volume of distribution is not equivalent to the physiological volume, but reflects the distribution of the drug and the degree of its binding in the body. V_{SS} of non-immunogenic staphylokinase showed an excess of the value over the real volume of circulating blood, which indicates the elimination of the drug from the systemic circulation and probable penetration into the thickness of the thrombus, which is consistent with the available data on the pharmacodynamics of the staphylokinase molecule.

Study of the pharmacokinetics of non-immunogenic staphylokinase at a dose of 10 mg in patients with ischemic stroke

The study included 50 patients with ischemic stroke who received non-immunogenic staphylokinase once as a bolus at a dose of 10 mg. Demographic, anthropometric, anamnesis data, clinical characteristics, and time intervals are presented in Table 4.

The average age of patients was 64.4 ± 9.6 years. The proportion of patients with arterial hypertension was 95%, previous stroke — 13%, lipid metabolism disorders — 20%. The median NIHSS at admission was 11 (8–14) points. The average time from the onset of symptoms to thrombolysis was 2.9 ± 0.8 h

Assessment of the effectiveness and safety of TLT is presented in Table 5.

The number of patients with good functional recovery (0–1 points on the modified Rankin Scale) on day 90 was 50%. 24 hours after thrombolysis, a decrease in the median NIHSS from 11 to 6 points was noted, by the 90 day — to 2 points. Mortality from all causes on the 90th day was 10%. Symptomatic hemorrhagic transformation according to ECASS-III criteria was registered in 1 patient (2%). No major bleeding or allergic reactions were registered.

The averaged pharmacokinetic profile of the medicine in patients with ischemic stroke is presented in Figure 2.

It has been shown that the kinetic profile of non-immunogenic staphylokinase in patients with ischemic stroke is similar to that in patients with STEMI: the drug is characterized by a short half-life and rapid elimination from the bloodstream. Based on the concentration values of non-immunogenic staphylokinase in the blood, pharmacokinetic parameters were calculated when using the drug at a dose of 10 mg (Table 6).

The initial concentration C_0 of non-immunogenic staphylokinase in patients with ischemic stroke in blood plasma was 2.8 ± 0.3 μg/mL. The half-life $t_{1/2\alpha}$ is 5.11 ± 0.56 min, the terminal half-life $t_{1/2\beta}$ — 32.67 ± 2.12 min. The mean residence time of the drug in the systemic circulation (MRT) was 38.71 ± 2.62 min. These values are slightly lower than the indicators obtained in patients with STEMI due to the fact that the drug was administered at a lower dose (10 mg vs 15 mg). The clearances CL_1 and CL_2 were 0.35 ± 0.06 L/min and 0.51 ± 0.1 L/min, respectively, and are generally similar to the clearance values of non-immunogenic staphylokinase in patients with STEMI. The area under the pharmacokinetic curve within the duration of drug observation AUC_{0-t} was 28.5 ± 3.6 μg/mL×min, the area under the pharmacokinetic curve from 0 to infinity $AUC_{0-\infty}$ — 52.94 ± 4.11 μg/mL×min. The area under the curve from 0 to infinity with extrapolation of the final phase (AUMC) was 2424.95 ± 277.57 μg/mL×min^2. The apparent volume of distribution of the drug at steady state in ischemic stroke is slightly lower than in patients with STEMI (9.41 ± 0.8 L vs 10.3 ± 1.05 L), which is probably due to the smaller volume of thrombotic masses in the occlusion of cerebral arteries.

DISCUSSION

This article presents the results of a study of the pharmacokinetic parameters of non-immunogenic staphylokinase after its single bolus administration in patients with STEMI and ischemic stroke. It was established that both at a dose of 15 mg and at a dose of 10 mg, non-immunogenic staphylokinase is characterized by a short half-life (5.11 min and 5.77 min, respectively), the terminal half-life was 32 min in both dosing options, clearance — 0.35 L/min and 0.33 L/min, respectively. It is important to note that these results correspond to the results of a study of the pharmacokinetics of the staphylokinase molecule used at a dose of 10 mg in patients with STEMI [24]: it was shown that its half-life was 6.3 ± 0.6 min; the terminal half-life was 37 ± 15 min. The clearance of the staphylokinase molecule was 0.27 ± 0.1 L/min.

Table 7 shows the main pharmacokinetic parameters of the most commonly used thrombolytic drugs [25]. The half-life of non-immunogenic staphylokinase is the closest to that of the tissue plasminogen activato — alteplase. It is important to note that alteplase is used as a bolus-infusion for 1–2 hours depending on the indication, and the dose is selected individually from the patient's body weight, which greatly complicates its use in emergency medical care and may be the cause of dosing errors. The pharmacodynamic properties of non-immunogenic staphylokinase associated with its interaction with plasmin in the thrombus and subsequent recirculation of released drug molecules allow it to be used in lower doses regardless of the patient's body weight, despite the short half-life. Tenecteplase is a modified alteplase molecule created for single bolus administration. The half-life of tenecteplase is 24 ± 5.5 min, which is significantly higher than that of non-immunogenic staphylokinase and alteplase, however, tenecteplase, like alteplase, requires dose selection depending on the patient's body weight.

The pharmacodynamic and pharmacokinetic properties of the non-immunogenic staphylokinase molecule are reflected in clinical practice. Thus, according to the registry of patients with STEMI "REGION-IM", conducted under the auspices of the National Medical Research Center of Cardiology named after Academician E.I. Chazov, 36% of patients with STEMI receive TLT using non-immunogenic staphylokinase, and at the prehospital stage this figure reaches 42%. The frequency of use of non-immunogenic staphylokinase in primary vascular departments reaches 51% [26]. The pharmacokinetic features of non-immunogenic staphylokinase determine the convenience of its use, especially in emergency medical care. Timely TLT for patients with STEMI using drugs with rapid bolus administration allows accelerating reperfusion, preserving the myocardium, and reducing the burden of complications of cardiovascular diseases, since it is known that every 5% increase in the size of the myocardial infarction zone

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contributes to an increase in one-year mortality from all causes or hospitalization for heart failure within a year by 20% [27]. Monitoring the use of non-immunogenic staphylokinase in real clinical practice includes more than 50 thousand patients, in whom high safety and effectiveness of the treatment are shown [28]. Non-immunogenic staphylokinase is included in the current list of Vital and Essential Drugs, Russian and Eurasian clinical guidelines and standards for the treatment of patients with STEMI.

In 2024, non-immunogenic staphylokinase was included in the clinical guidelines for the treatment of ischemic stroke in the first 4.5 hours from the onset of the disease. A rapid (10 sec) single bolus of non-immunogenic staphylokinase at a single dose of 10 mg in patients with ischemic stroke with any body weight, including those weighing more than 100 kg, has advantages over a one-hour administration of alteplase at a dose of 0.9 mg/kg (maximum 90 mg) in the form of faster reperfusion and a greater number of good functional outcomes.

According to the FORPE study, non-immunogenic staphylokinase, administered as a single bolus at a dose of 15 mg, is effective in the treatment of massive pulmonary embolism (PE) [29]. Currently, a double-blind placebo-controlled clinical trial of non-immunogenic staphylokinase is being conducted in patients with intermediate-high risk PE (permission of the Russian Ministry of Health No. 106 dated March 21, 2024; clinicaltrials.gov No.NCT06362746) [30]. A clinical trial of non-immunogenic staphylokinase is being conducted with its intra-arterial intratrombal administration in patients with thrombosis of the arteries of the lower extremities in comparison with surgical methods of treatment FORAT (permission of the Russian Ministry of Health No. 184 dated March 18.03, 2022; clinicaltrials.gov No. NCT05372718) [31]. It is expected that the pharmacokinetic features of non-immunogenic staphylokinase will contribute to the expansion of indications for safe TLT.

Study Limitations

The pharmacokinetics of non-immunogenic staphylokinase have not been studied with bolus-infusion dosing regimen (10 mg bolus and 5 mg infusion) due to the low prevalence of this regimen. According to observational studies, 96% of patients receive the drug as a single bolus at a dose of 15 mg [28].

CONCLUSION

This article presents the results of a study of the pharmacokinetic parameters of non-immunogenic staphylokinase after a single bolus administration in patients with STEMI and ischemic stroke. The features of the pharmacokinetics and pharmacodynamics of the non-immunogenic staphylokinase molecule are that with a short half-life, high clearance and low dose, the drug has a pronounced fibrinolytic effect, not inferior in effectiveness to other thrombolytic drugs administered bolus and bolus-infusion in significantly higher doses. The data obtained in the course of these studies explain the high efficacy and safety of TLT using non-immunogenic staphylokinase.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS CONTRIBUTION

Sergei V. Ivanov — writing—original draft; Igor P. Beletsky — research design, administration; Marina V. Zakharova — conducting research, statistical data processing; Eduard A. Ponomarev — conducting research; Zhanna Yu. Chefranova — investigation; Sergey L. Konstantinov — investigation; Galina I. Stryabkova — investigation; Yurij A. Lykov — investigation; Ulukpan A. Yelemanov — investigation; Svetlana E. Chuprina — investigation; S.S. Markin — research design, writing-review & editing. All authors confirm that their authorship meets the international ICMJE criteria (all authors have made significant contributions to the development of the concept, research and preparation of the article, read and approved the final version before publication)

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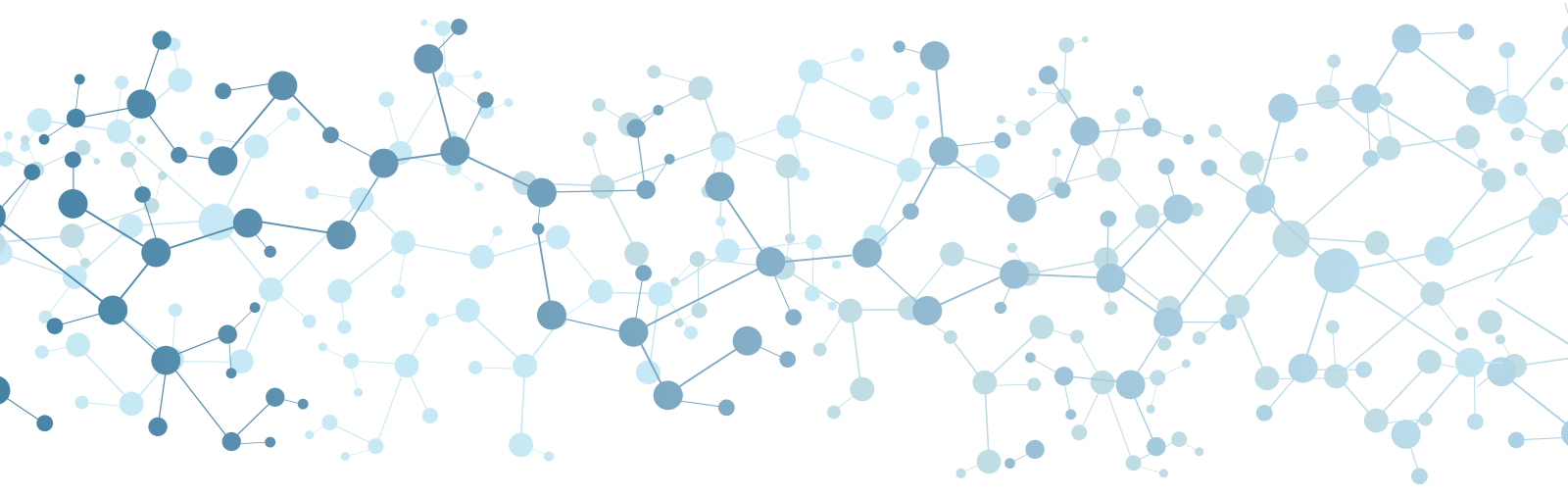

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