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PLGA – THE SMART POLYMER FOR DRUG DELIVERY

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Polymers have become an integral part of novel drug delivery system. One such successful biodegradable polymer is poly lactic-co-glycolic acid (PLGA) which consists of polyesters of lactic acid and glycolic acid. It is one of the FDA-approved biodegradable polymers which is extensively used for therapeutic purposes in recent times.

The aim. To illuminate researchers on the chemistry, novel properties and applications of PLGA in pharmaceutical fields.

Materials and methods. Various internet sources like Science Direct, Scopus, Web of Science, PubMed and google scholar were used as the data source. The key words search was carried out for the following words and combinations: PLGA, Novel drug delivery, PLGA Nano particles, biomedical applications of PLGA.

Results. Pharmaceutical and biomedical industries are flooded with the use of synthetic and natural polymers. The mechanical and viscoelastic properties of the polymers make them suitable for the temporal and spatial delivery of therapeutic agents for an extended period. Employment of copolymerization techniques lead to the modification of water solubility of the polymers and make them suitable for various applications of drug delivery systems. Biodegradable polymers due to their biocompatibility and biodegradable property have attracted their use in novel drug delivery systems. PLGA is one of them. PLGA is versatile as it can be fabricated into any size, shape, and can be used to encapsulate small molecules, tissue engineering, and bone repair, etc.

Conclusion. The sensitivity and biodegradability of PLGA makes it a smart polymer for targeted and sustained delivery of drugs and in various biomedical applications.

Keywords: PLGA; Smart Polymer; Biodegradable; Biocompatible Polymers

Abbreviation: PLGA – poly lactic-co-glycolic acid; PLA – Polylactic acid; PGA – Poly glycolic acid, PEG – Poly ethylene glycol; SNA – spherical nucleic acid; NP – Nano particles; FDA – U. S. Food and Drugs Administration; EMA – European Medicines Agency; CFTR – Cystic Fibrosis Transmembrane Conductance Regulator gene.

PLGA – ПЕРСПЕКТИВНЫЙ ПОЛИМЕР ДЛЯ ДОСТАВКИ ЛЕКАРСТВЕННЫХ СРЕДСТВ

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Полимеры стали неотъемлемой частью новой системы доставки лекарственных средств. Одним из успешных биоразлагаемых полимеров является PLGA, который состоит из сложных полиэфиров молочной и гликолевой кислот. Это один из одобренных «U. S. Food and Drugs Administration» (FDA, США) биоразлагаемых полимеров, который в последнее время широко используется в терапевтических целях.

Цель. Познакомить химиков-исследователей с новыми свойствами и применением PLGA в области фармации.

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Материалы и методы. В качестве источников информации использовались различные базы данных, такие как Science Direct, Scopus, Web of Science, PubMed и Google Scholar. Поиск проводился по следующим ключевым словам и словосочетаниям: PLGA, доставка новых лекарств, наночастицы PLGA, биомедицинское применение PLGA.
Результаты. Фармацевтическая и биомедицинская промышленность переполнены синтетическими и натуральными полимерами. Механические и вязкоэластичные свойства полимеров делают их пригодными для временной и пространственной доставки лекарственных препаратов в течение длительного периода. Применение методов сополимеризации приводит к модификации водорастворимости полимеров и делает их пригодными для различного использования системами доставки лекарственных веществ. Благодаря свойствам биосовместимости и биоразлагаемости полимеры стали использоваться в новых системах таргетной доставки лекарств. Сополимер молочной и гликолевой кислоты PLGA – представитель этих систем. PLGA универсален, так как может использоваться для инкапсуляции малых молекул, в тканевой инженерии, при восстановления костей и т. д., ввиду способности воспроизводить любой размер и принимать любую форму.
Заключение. Сенситивность и способность к биоразложению PLGA делают этот сополимер интеллектуальным полимером для адресной и непрерывной доставки лекарств, а также в различных видах биомедицинского использования.
Ключевые слова: PLGA; перспективный полимер; биоразлагаемый; биосовместимые полимеры
Список сокращений: PLGA – сополимер молочной и гликолевой кислоты; PLA – полимер молочной кислоты; PGA – полигликолевая кислота; ПЭГ – полиэтиленгликоль; SNA – сферическая нуклеиновая кислота; NP – наночастицы; FDA – Управление по санитарному надзору за качеством пищевых продуктов и медикаментов – агентство Министерства здравоохранения и социальных служб США; EMA – Европейское агентство лекарственных средств; CFTR – трансмембранный регулятор муковисцидоза; ЛВ – лекарственное вещество.

INTRODUCTION

Poly lactic-co-glycolic acid or PLGA or PLG, is a copolymer of polylactic acid (PLA) and polyglycolic acid (PGA) approved by FDA and EMA [1–3]. PLGA is a linear copolymer that has immense potential as a carrier for drug, protein, nucleic acid, and peptide delivery and provides a framework for tissue engineering [4]. Biocompatibility, favourable degradation, and sustained release property render it's a popular polymer for drug delivery. The release of embedded drugs from PLGA through stimulation makes it a smart polymer for drug delivery [5]. In recent years it has shown its potential as monolithic injectable implants for sustained delivery of drugs at desirable doses without surgery[6]. The advantages of PLGA are its tuneable physical properties i.e., molecular weight, and the ratio of lactide to glycolide. The hydrolytic biodegradation of PLGA also affects the drug release mechanism based on drug delivery systems either by diffusion or erosion [7].

Literature survey reports various micro and nanoparticulate systems of PLGA showed excellent biocompatibility, sustained and targeted release with high safety profiles, thereby improving the bioavailability and stability of the encapsulated biopharmaceuticals against enzymatic degradation [8]. They are capable to produce local and systemic effects of therapeutic agents and biologics.

The polymer is successfully used to target anti-cancer drugs, helps in organ-specific targeting of drugs especially to the liver, lungs, or brains, and is found to be very effective in delivering therapeutic gene delivery [9, 10].

THE AIM to get a detailed review of the smart and wonder polymer PLGA based on its chemistry, synthesis, properties, and applications in various novel delivery of drugs, vaccine, genes with optimal efficacy.

MATERIALS AND METHODS

Various internet sources like Science Direct, Scopus, Web of Science, Pub Med and Google Scholar were used as the data source within the period of 2018-April 2021. The key words search was carried out for the following words and combinations: PLGA; Novel drug delivery; PLGA Nano particles; Use of PLGA in peptides delivery; biomedical applications of PLGA polymer etc.

RESULTS AND DISCUSSIONS

Chemistry and Synthesis of PLGA

Polylactic acid was first recognized as biodegradable and biocompatible in the year 1966. The same was proved for PGA in 1967 and got introduced in the medical field in the form of surgical sutures. Novel and systematic investigations in the research field merged the components PLA and PGA to evolve a novel copolymer PLGA for delivering the drug in a biocompatible and biodegradable matrix with stimuli sensitive release and controlled properties. Thus it was evolved and recognized as a smart polymer for drug delivery and drug targeting [11–14].

PLGA is an aliphatic polymer[13], contains polylactic acid that has asymmetric α-carbon usually described as the D or L form and sometimes as R and S form [15]. PLGA generally contains D- and L- lactic acid forms in equal ratio [16, 17]

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PLGA is chemically synthesized by the following steps (17) as shown in figure 1.

If glycolic acid is used in a higher ratio than lactic acid the copolymer formed is more hydrophobic due to the increased hydrophobicity of lactic acid[18]. PLGA can be prepared into various shapes and sizes and can sheathe molecules of any size [17–19].

After polymerization, the purification of the polymer is done by dissolution in chloroform followed by precipitation in ethanol. The precipitated crystals are dried under vacuum for 48 h at room temperature. Commercially it is available in either acid or ester form. Depending on the molecular weight different forms of PLGA are available in different viscosity grades. Different grades of PLGA in the commercial name of RESOMER®RG are marketed by Boehringer Ingelheim GmbH Lactel, different ester forms of PLGA are marketed in the brand of DL-PLG by Lactel Absorbable polymers, and another grade of esterified or acidified form is available in the name of Purasorb PDLG by Purac Biomaterials [17].

Properties of PLGA

The physicochemical properties of PLGA mostly depend on the monomer ratio of lactic acid and glycolic acid. Lactic acid has less hydrophilicity compared to glycolic acid. Hence if the proportion of lactic acid increases the degradation rate of PLGA reduces and the reverse is the case, when the monomer units of glycolic acid increases the degradation of PLGA hastens [20, 21]. But the increase in glycolide content leads to the reduction of the tensile strength of the polymer [22]. Hence a 50:50 ratio of PLA and PGA can yield a polymer having good biodegradability and the property of sustaining the drug release with good tensile strength, whereas high lactide content helps to sustain the release of drug with bio erosion. Depending on the proportions of PLA and PGA the properties of the polymer can be customized.

Polymers are always characterized by their crystallinity, molecular weight, and inherent viscosity.

The degree of crystallinity depends on the number of monomeric units of PLA and PGA used in copolymerization and influences the mechanical properties, swelling abilities, and biodegradation of PLGA. They are generally amorphous and show a glass transition temperature between 45–55°C and that confirms the rigidity of the polymer. PLGA gets softened in a wet environment and results in the reduction of glass transition temperature and mechanical properties like tensile strength, young's modulus and % elongation to break etc., of the polymer [23]. The inherent viscosity of the polymer depends on the molecular weight of the polymer. With the increase in molecular weight, viscosity of the polymer increases. PLGA is soluble in a wide range of organic solvents like acetone, benzyl alcohol, chloroform, dichloromethane, ethyl acetate, hexafluoro isopropanol, and tetrahydrofuran. The physico-chemical properties of different grades of polymer with its application is illustrated in table 1.

Biodegradation of PLGA

PLGA undergoes two types of degradation – hydrolytic and autocatalytic, in the biological system. A random hydrolyzation occurs in the polymer backbone within the ester linkages in presence of water, water-soluble fragments of lactic acid and glycolic acid are formed as shown in figure 2. These water-soluble fragments enter the metabolic pathway of the body to yield energy, carbon dioxide, and water.

The monomer ratio of lactic and glycolic acid plays an important role in hydrolytic degradation. PLGA 50:50 thus undergoes degradation at a faster rate than PLGA 85:15 [24].

The autocatalytic degradation of PLGA happens in an acidic environment as shown in figure 3.

Hence the mechanism of release of drug from the matrix of PLGA follows different mechanism as described in table 2.

The assessment of the various factors that are responsible for the degradation of PLGA can help in the synthesis of customized novel copolymers best suited for the effective delivery of the drug. The factors that influence the breakdown of PLGA are listed in table 3.

Current research on PLGA micro and nano particles for drug delivery

Recently PLGA has gained extensive attention as versatile carrier for hydrophilic or hydrophobic drugs, and micro or macro molecules. The polymer is used in several studies for protection of drug from degradation or to control the release of the drug for the improvement of therapy.

Zhang Z. et al., reported the efficacy of paclitaxel loaded PLGA microsphere in the treatment of solid tumours. Solvent evaporation technique was used to prepare these microspheres. A sustained release of drug was achieved and the *in vivo* study reported that the sustained release of drug could cause apoptosis of tumour cell, and reduction in the toxicity of the normal cell [30].

Micronized triamcinolone was encapsulated in PLGA/PLA carriers with an aim to increase the retention time in the joints following intra-articular administration. These microspores were prepared by ultrasonica-

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tion and spray drying technique. *In vivo* rat models revealed the retention of the drug was for 28 days [31].

Abuzar S.M. et al., fabricated oxaliplatin-loaded PLGA microparticles loaded hydrogel in the treatment of colorectal cancer in rats. Double emulsion method was used to prepare the microparticles. The *in vivo* study revealed the prolongation of drug action and hence the bioavailability [32].

Jusu S.M. et al., studied on formulation and evaluation of blend of prodigiosin and paclitaxel loaded PLGA microsphere for the treatment on breast cancer. The microsphere blend was conjugated with Luteinizing hormone releasing hormone. The microspheres were prepared by solvent evaporation technique using PLGA and PEG in 1:1 ratio. The study revealed the in mice the blend was able to sustain the release of the drug for 62 days and they concluded that PLGA -PEG microsphere had the potential for the treatment of triple negative breast cancer [33].

Ryu W.M. and colleagues developed a rapidly dissolving ocular tablet consisting of nano particles of dexamethasone in PLGA in alginate matrix. The retention of the nanoparticles on the preocular surface could increase the ocular bioavailability of the drug [34].

Varga N. et al., developed a nanoparticles of α-tocopherol in PLGA-PLA carriers. The nanoparticles were stabilized using Pluronic F127. And could sustain the release of the drug [35].

Jo A. et al., fabricated and evaluated the PLGA encapsulated large CRISPR–Cas9 plasmid nanoparticles as a revolutionary tool for gene delivery. The delivery of plasmid was aimed to the bone marrow derived macrophages *in vitro*. The particle size was engineered to 160nm. The experimentation showed positive results to induce expression of bacterial Cas9 in murine bone marrow [36].

ROLE OF PLGA IN NOVEL DRUG DELIVERY

Clinical Application of PLGA

Nano drug targeting

To achieve a successful drug delivery through polymeric nanoparticles, the physicochemical characteristics of the polymer play an important role. The polymer should have the following properties so that it can bypass rapid body clearance, and should have an affinity for the target cells. The ideal properties of polymer for a nano targeted delivery are:

- compatibility with the drug;
- high drug loading;
- suitable molecular weight for diffusion or to be absorbed by endocytosis;
- nonimmunogenic, biocompatible and biodegradable.

To achieve these characteristics the polymer should have the ability to allow physical modifications in its structure during the polymerization process, it should allow the formation of copolymers or block polymers to make it suitable for the need of delivery of a particular drug for targeting.

PLGA shows the versatility of modification by changing the lactide to glycolide ratio and can undergo copolymerization with PEG to tailor for specific performance. A block polymer of PLGA with PEG (PLGA-PEG-PLGA) was also studied to modify the release of Docetaxel for targeting bone metastasis [37].

A copolymer of PLGA–Chitosan–PEG nanoparticles were studied for delivery of paclitaxel in human retinoblastoma, breast cancer and pancreatic cell line for effectively targeted delivery to the tumour [38].

Due to its versatility in modifications in polymeric chain, PLGA is being widely studied in various fields of nano-research like delivery of gene, peptides, proteins, and nucleic acids [39, 40]

In gene-drug delivery systems biodegradable PLGA nanospheres have been investigated as nonviral vectors that improve the quality transfection effectiveness [41, 42].

In recent times polymeric spherical nucleic acid (SNA) being a new class of polymeric conjugates has gained its attention. It consists of PLGA nanoparticle (NP) cores. The PLGA-SNA nucleic acid shell showed an increased half-life (>2 h) in foetal bovine serum that could release the nucleic acid in a tuneable manner from the polymer conjugates [43, 44].

Vijet et al reviewed the condition of cystic fibrosis which was due to mutation in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. They reported that ΔF508-CFTR, is a temperature-sensitive common mutation that occurs by trafficking mutant which in turn reduces the chloride transport and exaggerates immune response. For this condition, FDA has approved PS-341 (bortezomib) drug that causes selective inhibition of chymotryptic threonine protease-activity. They have also reported that proteasome inhibitors in PLGA nanoparticle could affect proteostasis and the consecutive processes. Thus, nanoparticle-mediated PS-341 lung-delivery was successful in treating the condition of cystic fibrosis where the drug was loaded with biodegradable PLGA nanoparticle (PLGA-PEG$^{PS\text{-}341}$) and could produce a controlled and sustained drug targeting at the site [45].

Pillai R.R. et al. investigated the development of nafcillin loaded poly DL-lactide-co-glycolide nanoparticles for the targeting of osteoblasts in the treatment of Staphylococcus aureus-mediated osteomyelitis. Single emulsion-solvent evaporation technique was

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used to prepared nanoparticles of nafcillin. The *in vitro* drug release along with cell viability study showed that nanoparticles of nafcillin were effective in the treatment of infected osteoblasts [46].

Kumar R. et. al. studied the enhanced solubility of amphotericin B embedded in PLGA-PEG nanoparticles and also observed the targeting effect of those nanoparticles towards the macrophages of the infected tissues in the treatment of visceral leishmaniasis. Thus, Amphotericin B loaded PLGA-PEG block polymer nanoparticles showed more effectiveness than conventional amphotericin B for the inhibition of amastigotes in the splenic tissue, elicited reduced toxicity, and better therapeutic efficacy than the conventional one [47].

In nano-drug targeting with PLGA the challenges faced are the inconsistencies in particle size that can hinder the clinical success of the formulation the type of organic solvents used to dissolve PLGA and the type of mixing device that is used to disperse the polymeric phase in anti-solvent also affects the physicochemical properties of formulation embedded in PLGA [49].

PLGA Microsphere for targeting

Microspheres are polymeric particles of sizes ranges from 1 to 1000 μm [50]. Use of PLGA in the formulation of drug loaded polymeric microsphere rendered several advantages in drug delivery and targeting.

Feng T. et al. experimented on the sustained release effect of PLGA microsphere of Doxorubicin, and paclitaxel, investigated against B16F10 cells for the treatment of metastatic lung cancer. It was observed that PLGA carrier showed better results in the treatment compared to other carriers [51].

A successful brain targeting was observed by Ozekia et al in animals by incorporating PLGA microspheres in a thermo reversible gelation polymer matrix, for delivery of chemotherapeutic drugs- camptothecin or vincristine. After injection into the brain of the animals, a transformation from sol-gel occurred at body temperature and the microsphere provided a sustained drug release at the target site for glioma therapy with a survival rate of more than 60 days [52].

The complexity of manufacturing PLA/PLGA microspheres creates many challenges in the successful delivery of the drugs [53]. Some of the challenges include degradation of PLA/PLGA that begins after administration due to gastric pH, low drug loading, and poor formulation stability [54]. The improvement of stability can be brought by modifying the carrier system by achieving desirable hydrophilicity in the surface of the polymer. For optimizing the drug loading into PLGA the most effective way is to manipulate the physicochemical properties of PLGA so that there is no penetration of water into the polymer network and drug leaching from the formulation can be avoided [55–57]. Studies have showed that these physicochemical modifications of the polymer can be achieved by using various techniques like electrospinning, radiation, and employing chemical treatment [58].

Protein and peptide vaccine drug delivery

Proteins and peptides are new therapeutics that are used in the treatment of various human ailments in recent times [59]. PLGA particles in humans are one of the promising polymers that have been used in the delivery of proteins and peptides [60].

Allahyari M. et al., identified one of the important criteria to be taken into account during vaccine preparation is the encapsulation of antigen and its stability in the particulate system of polymer. This article has focused on vaccine delivery through PLGA particulate system. Antigenic proteins/peptides can also be encapsulated onto the surface of PLGA particles that show control release of antigenic protein/peptides from the polymeric surface over a while [61].

Jiang et al., reported that polymer-based targeting of oral vaccine with highly porous PLGA microparticles was a successful approach for targeting of peptides [62].

PLGA particles faces some challenge related to protein and peptide delivery considering their stability in acidic or harsh environments [63]. The instability occurs during the process of encapsulation, as the removal of organic solvents is associated with hydration that leads to the partial aggregation of the proteins. The other reason for instability is the hydrolysis of PLGA to PLA and PGA and thus the creation of an acidic microenvironment where the proteins are denatured. By adding stabilizers to the formulation, or by modifying the protein or the polymer proteins are protected from harsh environments [64].

Inhalational therapies

The use of PLGA in inhalational therapies has been extensively used nowadays due to its ability to overcome intracellular and extracellular barriers of lungs which in turn increase the drug deposition in lungs [65]. PLGA is suggested for inhalation therapies for its sustain release properties [51, 66], and is reported to be the most promising polymer in dry powder inhalation formulations [51].

Feng et al. formulated biodegradable PLGA microparticles of doxorubicin into achieve a long-acting release of the drug in pulmonary inhalation treatment. It produced highly porous and effective aerosolization of the drug [51].

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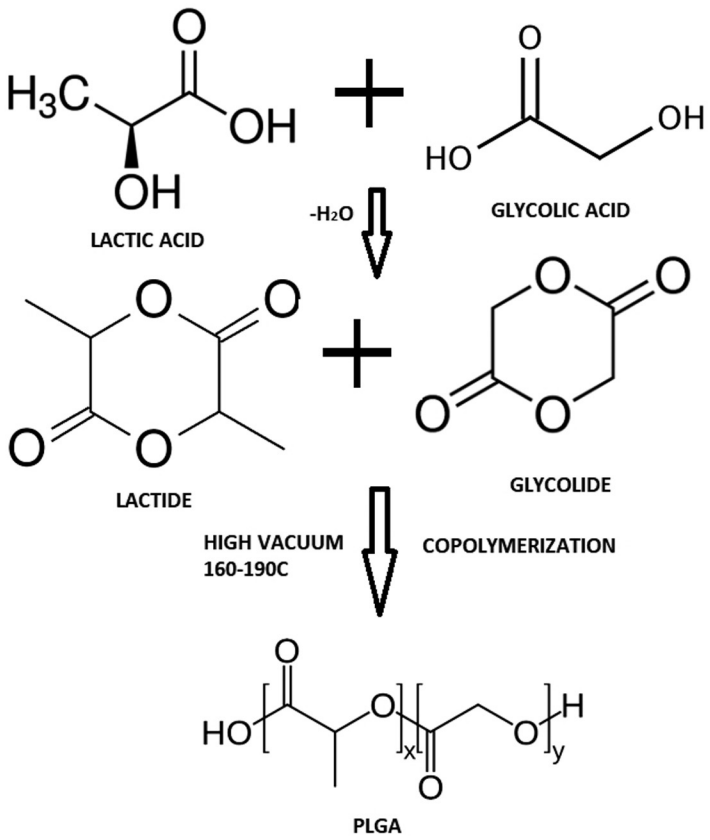

Figure 1 – Synthesis of PLGA

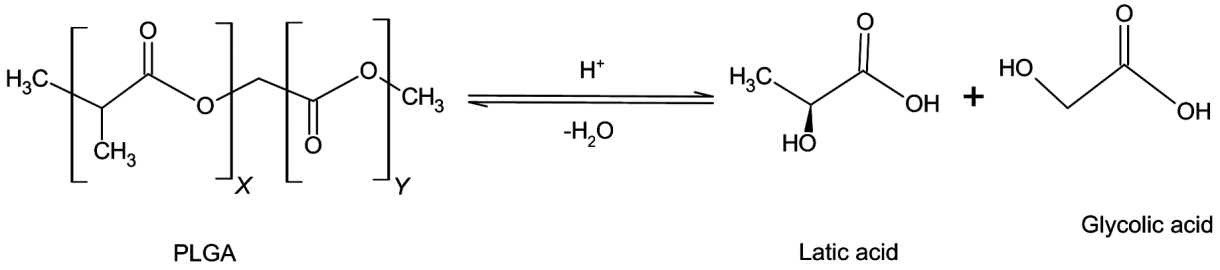

Figure 2 – Hydrolytic degradation of PLGA

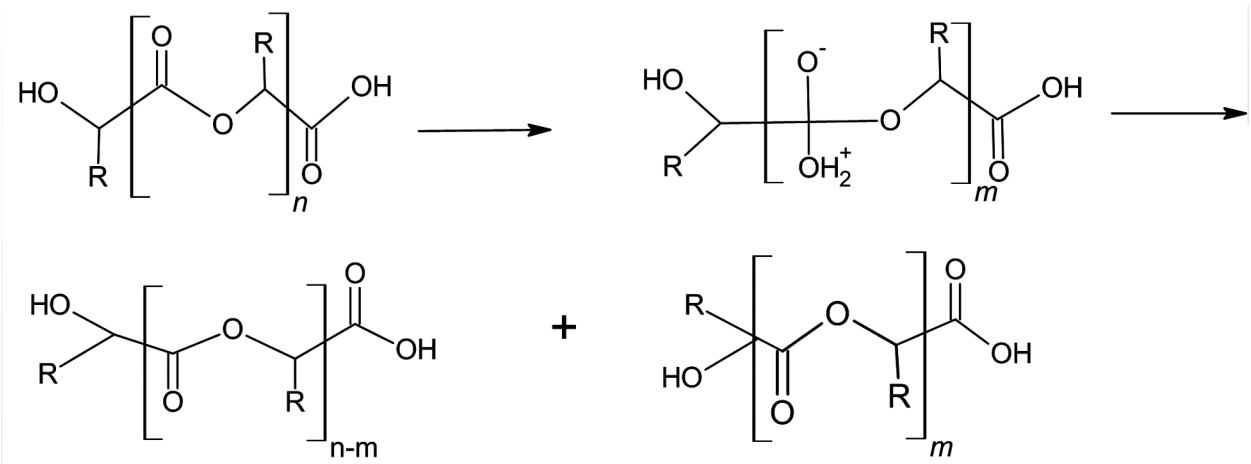

Figure 3 – Autocatalytic degradation of PLGA

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Table 1 – Physicochemical properties and application of different grades of PLGA[1]

Polymer	Type	Viscosity (dl/g)	Glass transition temperature (°C)	Crystalline Melt Transition (°C)	Solubility	Application
RESOMER® RG 502	Poly(D,L-lactide-co-glycolide) 50:50	0.16–0.24	42–46	Amorphous	A, B, C, D, E, F	Controlled release
RESOMER® RG 653 H	Poly(D,L-lactide-co-glycolide) 65:35	0.32–0.44	46–50	Amorphous	A, B, C, D, E, F	Controlled release
RESOMER® RG 752 H	Poly(D,L-lactide-co-glycolide) 75:25	0.14–0.22	42–46	Amorphous	A, B, C, D, E, F	Controlled release
RESOMER® RG 858 S	Poly(D,L-lactide-co-glycolide) 85:15	1.3–1.7	50–55	Amorphous	A, B, C, D, E, F	Controlled release
RESOMER® L 206 S	Poly(L-lactide)	0.8–1.2	60–65	180–185	A, D, E	Medical device

Note: A – Dichloromethane, B – Tetrahydrofuran, C – Ethyl acetate, D – Chloroform, E – Hexachloroisopropanol, F – Acetone

Table 2 – Mechanisms of biodegradation of PLGA

Mechanisms of biodegradation	Consequences
Polymer surface erosion due to the uptake of water	Slow degradation of Crystalline drug and fast degradation of the amorphous drug on release
Breakage of drug-polymer bonding	Release of the drug by the diffusion process
Combination of diffusion and erosion process	Release of the drug in a sustained manner

Table 3 – Factors affecting degradation of PLGA

Factors	Properties for consideration	Effect on degradation rate	References
Composition	Increase in the amount of Glycolic acid, the critical parameter	Increases	[22]
Crystallinity	The crystallinity of Lactic acid	Increases	[25]
Molecular weight	Long polymeric chains	Retards	[26]
pH	Strong acidic and basic media	Increases	[27]
Chemical properties of the drug	The hydrophilicity of the drug	Increases	[28]
Shape and size of the matrix	The ratio of surface area to volume of the device	Increases	[29]

Table 4 – Marketed products

Drug product	Active ingredient	Dosage form / route of administration
Lupron	Leuprolide acetate	Microsphere / Intra muscular[2]
Sandostatin LAR	Octreotide	Microsphere / subcutaneous[3]
Zoladex	Goserelin acetate	Implant / Subcutaneous[4]
Atridox	Doxycycline hyclate	Insitu forming gel / Periodontal[5]
Eligard	Leuprolide acetate	Insitu forming gel / Subcutaneous [87]
Prialt	Zinconotide Acetate	Implant / Intrathecal[6]

[1] RESOMER® Biodegradable Polymers for Medical Device Applications Research (sigmaaldrich.com) – Available from: https://www.sigmaaldrich.com/IN/en/technical-documents/technical-article/materials-science-and-engineering/drug-delivery/resomer. Accessed on 31 March 2021.
[2] LUPRON DEPOT (leuprolide acetate for depot suspension), 2014. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2014/020517s036_019732s041lbl.pdf. Accessed on 31 May 2021.
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Ocular Delivery Systems

The more challenging part of the ocular drug delivery system is to overcome the anatomical and physiological barriers of the eye [67]. In recent times many biodegradable polymers have been used in ophthalmic drug delivery to overcome those challenges. PLGA, is being studied to observe better tissue adherence, prolonged duration of action of the entrapped drug, bioavailability improvement, and toxicity reduction, in targeted delivery of the eye [68].

Chang et al., observed the effect of ocular surface drug targeting of PLGA with Fluorescein labelled albumin and doxycycline. The drugs were encapsulated in PLGA by formulation of w/o/w multiple emulsions. Safety and inflammatory responses were evaluated for the drug loaded PLGA microspheres by administering subconjunctival injections to the rodents. The study reported that the drug release from the microspheres was achieved over controlled periods. Doxycycline loaded PLGA microspheres were found to be efficacious in prevention of corneal barrier disruption in mice [69].

Gupta H. et al. studied a new colloidal system of sparfloxacin PLGA nanoparticles for ophthalmic delivery to improve the residence time and ocular penetration of the drug. The nanosuspension was lyophilized and reconstitution of the formulation, before administration made it more stable than conventional marketed formulations [70].

Biomedical applications

3D Printing technology

3D printing is a constructive way of making a three-dimensional object for medical purposes in digital format [71]. 3D printed medicines were first used in dental implants by Charles Hull in 1984 [72, 73]. Recently FDA has approved a 3D pharmaceutical product in August 2015, Spritam® 3D printed tabled used for the treatment of epileptic seizures[1].

3D printing promises to produce complex biomedical devices based on specific patient anatomical data according to computer design. For use of polymers in 3D printing two main characteristics have to be considered that is biocompatibility and printability [63]. Considering these characteristics PLGA is found to be the best-preferred polymer [74, 75]. PLGA is one of the polymers that can be easily absorbed and eliminated from humans rendering any toxicity [76].

Shim J. H. et al., reported that Osteointegration and bone regeneration are one of the fields of science that mainly require the use of biodegradable polymers, now this use has also led to gain its interest in 3D printing. This polymer was successfully studied for bone regeneration in rabbits that showed better efficacy of this 3D model [77].

Gwak S.J. et al. experimented on 3D printed alginate PLGA copolymer with control release of indomethacin, itraconazole, and gentamicin on the embryonic kidney and stromal stem cell lines of bone of humans and reported a sustained effect of the drugs from the polymer matrix [78].

Researches are reported on the use of methoxy polyethylene glycol and PLGA copolymer in the formation of new bone [79] and regeneration of bone using calcium phosphate cement and PLGA [80].

Miscellaneous applications of PLGA

In periodontitis, the inflammation and the infection associated with the periodontal pockets need an extended release of drug at the inflamed site. Nafea E et al., studied the effect of the mucoadhesive chitosan-PLGA-chitosan copolymer for the entrapment of alendronate sodium with a promising effect in the treatment of periodontitis [81].

Prevention of photodegradation of sunscreen agents and improvement of the penetration of the hair vitalizer tonics can be improved by the use of PLGA nanospheres [82].

PLGA coated drug-eluting stents and PLGA based cardiac implants are found to be an optimal way to control heart diseases for both prolonged and local delivery of drugs to cardiovascular tissues. A revolutionary imaging capability of PLGA micro bubbles in the detection of myocardial defects were studied in a recent study. They have found that the PLGA micro bubbles could withstand the destruction by ultrasound imaging with a significant enhancement in the sound for cardiac contractions [83, 84].

Because of its biodegradable nature, PLGA is considered as a key polymer for drug-eluting medical devices and tissue engineering products. PLGA based nanosystems are suitable for targeting of drugs to the brain, cancerous cell, or tumour cells [85]. Grafting of monoclonal antibodies on the surface of PLGA matrix could able to achieve active targeting of drugs for different types of cancer cells and tumour endothelium cells.

Uptake of corticosteroids loaded PLGA nanoparticles by the ulcerated tissues in the colon or small intestine is found to be beneficial for the treatment of inflammatory bowel diseases. This enhanced uptake is due to the presence of negative charge on the polymer which gets attached by the positive charged protein in ulcerative tissues [86].

Research is still ongoing to discover the wonder effects of this polymer. The presently marketed formulations are listed in table 4.

[1] Spritam. Available from: https://www.spritam.com/#/patient. Accessed on 23 April 2021.

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CONCLUSION

Use of PLGA or PLGA-based polymers in drug delivery has demonstrated countless probabilities for biomedical research. Its adaptable physicochemical properties biocompatibility and biodegradability makes it a unique polymer for drug delivery, drug targeting and making of novel devices. It has become a promising polymer to effectively deliver anticancer drugs, proteins, peptides, and vaccines. It has shown its potential in tissue engineering and microfluidics-based applications for medical devices. The tailor-made modification due to copolymerization of PLGA with other polymers render its efficacy in various applications for controlled and sustained delivery of drugs. Hence it can be concluded that much progress is awaiting in the field of biomedical research involving tissue engineering, stem cell with PLGA for the treatment and diagnosis of various critical diseases.

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CONFLICT OF INTERESTS

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTION

S. Bhattacharyya, N. Surya – Concept, Collection of materials, Writing and Editing of article

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REVIEWS, LECTURES

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HEAT SHOCK PROTEIN HSP70: PREREQUISITES FOR USE AS A MEDICINAL PRODUCT

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Heat shock protein Hsp70 is one of the main cytoprotection components under the action of various external stimuli. The analysis of the literature data shows that nowadays, the researches' overwhelming evidence has proven the role of Hsp70 as a biological target for the drug development; however, the ideas about its use as a drug are often multidirectional.

The aim of the article is to analyze and generalize the literature data on the features of the physiological functions of heat shock protein Hsp 70, and indicate the possibilities of its use for the pharmacological correction of various pathological conditions.

Materials and methods. In the process of selecting material for writing this review article, such databases as Google Patents, Science Research Portal, Google Scholar, ScienceDirect, CiteSeer, Publications, ResearchIndex, Ingenta, PubMed, KEGG, etc. were used The following words and word combinations were selected as markers for identifying the literature: Hsp70, Hsp70 stroke, Hsp70 neuroprotection, Hsp70 cytoprotection, recombinant drugs.

Results. In this review, the pharmacology of one of the key members of this family, Hsp70, was focused on. The literary analysis confirms that this molecule is an endogenous regulator of many physiological processes and demonstrates tissue protective effects in modeling ischemic, neurodegenerative and inflammatory processes. The use of recombinant exogenous Hsp70 mimics the endogenous function of the protein, indicating the absence of a number of typical limitations characteristic of pharmacotherapy with high molecular weight compounds, such as immunogenicity, a rapid degradation by proteases, or a low penetration of histohematogenous barriers.

Conclusion. Thus, Hsp70 may become a promising agent for clinical trials as a drug for the treatment of patients with neurological, immunological, and cardiovascular profiles.

Keywords: Hsp 70; cytoprotection; chaperone; neuroprotection; recombinant drugs

Abbreviations: MPs – medicinal products; ALS – amyotrophic lateral sclerosis; Hsp – heat schock protein; HSF1 – heat protein factor 1 / heat shock factor 1; HSEs – heat shock elements; TNF – tumor necrosis factor; PRRs – pattern recognition receptor; SBDa – sphingolipid binding domain alfa; NBD – nucleotid binding domen; NEF – nucleotide exchange factor; DISC – DISC-death-inducing signaling complex; BAG-1 – BAG family molecular chaperone regulator 1; CHIP – carboxyl terminus of Hsc70-interacting protein; E3 – ubiquitin-protein isopeptide ligase; TRAIL – TNF-related apoptosis-inducing ligand; BID – pro-apoptotic member of the Bcl-2 family; FANCC – Fanconianemia complementation group C; PKR – proteinkinasa R; MCA – middle cerebral artery; 17-DMAG – 17-demetoxigeldanamycin; NF-kB – nuclear factor-kappa B; AIF – apoptosis inducing factor; UPS – ubiquitin- proteasome system; JNK – Jun N-terminal kinases; Hip – hunting interacting protein; Hop – hunting interacting protein; Hsp 70-1 – Heat shock 70 kDa protein 1; DR4 – death receptor 4; DR5 – death receptor 5; p53 – protein p53; rhHsp70 – recombinant human heat schock protein 70; NMDA – N-methyl-D-aspartate receptor; IL-6 – Interleukin 6; TNF-α – Tumor necrosis factor-alpha; IL-1β, – Interleukin 1β; MCP-1 – monocyte chemotactic protein; TLRs – Toll-like receptors; DAMP – damage-associated molecular pattern; FasR – Fas-receptor; SMAC – the second mitochondrial activator of caspase; MAPK mitogen-activated protein kinase; ICAD – inhibitor of caspase-activated DNase; IKK – kappa B inhibitor kinase; Apaf 1 – apoptosis protease activating factor-1; **C**CP – cellular cytosolic protein; MMPs – matrix metalloproteinases; Mcl-1 – myeloid Cell Leukemia differentiation protein 1; ASK1 – apoptosis signal-regulating kinase 1; BBB – blood-brain barrier; Casp 9 – caspase 9; FADD – Fas-associated death domain.

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БЕЛОК ТЕПЛОВОГО ШОКА HSP70 – ПРЕДПОСЫЛКИ ИСПОЛЬЗОВАНИЯ В КАЧЕСТВЕ ЛЕКАРСТВЕННОГО СРЕДСТВА

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Белок теплового шока Hsp70 является одним из основных компонентов цитопротекции при действии различных внешних раздражителей. Анализ литературных данных показывает, что на сегодняшний день исследователями сформированы многочисленные доказательства роли HSP70 в качестве биологической мишени для разработки лекарственных средств, однако, представления о его использовании в качестве лекарственного средства зачастую разнонаправлены.
Цель. Обобщить и проанализировать данные литературы об особенностях физиологических функций белка теплового шока Hsp 70 и обозначить возможности его применения с целью фармакологической коррекции различных патологических состояний.
Материалы и методы. В процессе подбора материала для написания обзорной статьи использовали такие базы данных, как: Google Patents, Science Research Portal, Google Scholar, ScienceDirect, CiteSeer, Publications, ResearchIndex, Ingenta, PubMed, KEGG и др. Параметрами для отбора литературы были выбраны следующие слова и словосочетания: Hsp70, Hsp70 stroke, Hsp70 neuroprotection, Hsp70 cytoprotection, recombinant drugs.
Результаты. В данном обзоре мы сфокусировались на фармакологии одного из ключевых представителей данного семейства – Hsp70. Литературный анализ подтверждает, что данная молекула является эндогенным регулятором многих физиологических процессов и демонстрирует тканезащитные эффекты при моделировании ишемических, нейродегенеративных и воспалительных процессов. Применение рекомбинантного экзогенного Hsp70 имитирует эндогенную функцию белка, свидетельствуя об отсутствии ряда типичных ограничений, характерных для фармакотерапии высокомолекулярными соединениями, таких как иммуногенность, быстрое разрушение протеазами или низкая степень прохождения через гистогематические барьеры.
Заключение. Таким образом, Hsp70 может стать перспективным агентом для клинических испытаний в качестве препарата для лечения пациентов неврологического, иммунологического и кардиоваскулярного профилей.
Ключевые слова: Hsp 70; цитопротекция; шаперон; нейропротекция; рекомбинантные препараты
Список сокращений: ЛС – лекарственные средства; БАС – боковой амиотрофический склероз; Hsp – белки теплового шока; HSF1 – фактор транскрипции – фактора теплового шока 1; HSEs – элементы теплового шока; TNF – фактор некроза опухоли; PRRs – рецепторы распознавания образов; SBDa – сфинголипид связывающий домен альфа; NBD – нуклеотидсвязывающий домен; NEF – фактор обмена нуклеотидов; DISC – комплекс, индуцирующий смерть; BAG-1 – Регулятор семейства молекулярных шаперонов BAG 1; CHIP – карбоксильный конец Hsp70 – взаимодействующего белка; E3 – убиквитин-протеин-изопептидная лигаза; TRAIL – TNF-связанный лиганд, индуцирующий апоптоз; BID – проапоптотический член семейства Bcl-2; FANCC – группа комплементации фанконианемии; PKR – протеинкиназа R; MCA – средняя мозговая артерия; 17-DMAG –17-деметоксигельданамицин; NF-kB – ядерный фактор-каппа B; AIF – фактор вызывающий апоптоз; UPS – убиквитин-протеасомная система; JNK – N-концевая киназа Jun; Hip – хантинг-взаимодействующий белок; Hop – хантинг организующий белок; Hsp70-1t – белок теплового шока 70 кДа белок 1; DR4 – рецептор смерти 4; DR5 – рецептор смерти 5; p53 – белок p53; rhHsp70 – рекомбинантный человеческий белок теплового шока 70; NMDA – N-метил-D аспартат рецептор; IL-6 – интерлейкин 6; FNO-α – фактор некроза опухоли-альфа; IL-1β –интерлейкин 1β; MCP-1 – хемотаксический белок моноцитов; TLR – толл-подобные рецепторы; DAMP – молекулярная структура, связанная с повреждением; FasR – Fas рецептор; SMAC – второй митохондриальный активатор каспазы; MAPK – активируемая митогеном протеинкиназа; ICAD – ингибитор каспазо-активируемой ДНКазы; IKK – киназа ингибитора кВ; Apaf 1 – клеточный цитозольный белок; MMPs – матриксные металлопротеиназа; Mcl-1 – белок дифференцировки миелоидноклеточного лейкоза 1; ASK-1 – киназа, регулирующая сигнал к апоптозу; BBB – гематоэнцефалический барьер; Casp 9 – каспаза 9; FADD – белок, взаимодействующий с доменом смерти Fas-рецептора.

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INTRODUCTION

Protein homeostasis in mammals has been maintained by a multicomponent system of proteins that regulate metabolic processes. That system depends on environmental conditions. The hypothesis of the existence of a heat shock proteins family, the first mention of which dates back to 1962, was put forward on the basis of the discovery of mammalian tissues' tolerance phenomenon to high temperatures after a sharp heating of the same tissue site to sublethal temperatures [1].

Currently, many studies aimed at studying the spatial form, molecular interactions and physiological functions of heat shock proteins, have been carried out [2, 3]. The proteomics of a large family of chaperones, the function of which is traditionally associated with the folding and assembly of proteins, has been described. Molecular chaperones play an important role in proteostasis. In particular, Hsp70 means a lot in protein coagulation, disaggregation, and degradation [4]. Through substrate-binding domains, Hsp70 interacts with a wide range of molecules, providing cytoprotective properties against cellular stresses of various etiologies. The functions variety of heat shock proteins prompts the need to study their behavior in various pathological conditions. In eukaryotic cells, in physiological and pathological terms, there are four main pathways of protein degradation: the ubiquitin-proteasome system and three types of autophagy: macroautophagy, microautophagy, and chaperone-mediated autophagy [5]. Hsp70 provides specificity in the choice of substrate for all types of the above listed processes. In the literature, different variants of this protein name can be found: heat shock protein 70 kDa, Hsp70, chaperone Hsp70, and Hsp73.

The multitude of Hsp70 physiological functions determine the researchers' interest in studying the possibilities of its use in various pathological conditions. Heat shock proteins occupy one of the important positions in all the variety of folding proteins in mammalian bodies. At the same time, the use of these chaperones is hard due to the high cost of their production using producers' bacterial strains. Recombinant drugs are the substances obtained by artificial means based on genetic engineering. At the moment, in the classification of recombinant drugs, pharmacologists distinguish 2 main groups: protein recombinant drugs and hormonal recombinant drugs. With the help of recombinant DNA, more than 400 genes (mainly in the form of cDNA) of various human proteins that are or can become drugs have been cloned. The analysis of the biotechnology market shows that the annual volume of the world drug market based on human proteins is about $ 150 billion and is constantly growing [6]. The advantage of biopharmaceuticals lies in their high targeting action, which is associated with a reduced risk of side effects in comparison with conventional low molecular weight drugs [7]. Biotechnological drugs have found application in the treatment of patients with pronounced adverse reactions to traditional synthetic drugs [8]. Modern methods of creating transgenic animal producers of recombinant proteins open up new prospects for their use aimed at the pharmacological correction of pathological conditions associated with a violation of the structural organization of protein molecules. This mini-review reflects the main mechanisms of functioning and molecular interaction of Hsp70 with effector molecules known to science in various pathological cascades. Taking into account the available literature data, the prospects of using this substance as a drug with neuroprotective and cytoprotective activities, are discussed.

THE AIM of the article is to analyze and generalize the literature data on the features of the physiological functions of heat shock protein Hsp 70, and indicate the possibilities of its use for the pharmacological correction of various pathological conditions.

MATERIALS AND METHODS

In the process of selecting material for writing this review article, the databases of Google Patents, Science Research Portal, Google Scholar, ScienceDirect, CiteSeer, Publications, ResearchIndex, Ingenta, PubMed, KEGG, etc. were used The following words and phrases were selected for the selection of literature: Hsp70, Hsp70 stroke, Hsp70 neuroprotection, Hsp70 cytoprotection, recombinant drugs.

RESULTS AND DISCUSSION

Basic Hsp 70 Biology

The human Hsp70 family of proteins includes 13 molecules that differ from each other in the expression level, a subcellular arrangement, and an amino acid composition. They are encoded by a polygenic family consisting of up to 17 genes and 30 pseudogenes [9]. The functional genes encoding human Hsp70 proteins are associated with several chromosomes. Major stress-induced Hsp70s chaperones include Hsp70-1 (HspA1A) and Hsp70-2 (HspA1B) proteins, referred to as Hsp70 or Hsp70-1 as a whole, differ from each other in only two amino acids. The expression of basal HSPA1A / B mRNA varies in most tissues and exceeds the expression levels of other Hsp70 isoforms in humans. Hsp70-1t (Heat shock 70 kDa protein 1) is a constitutive, non-inducible

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chaperone that is 90% identical to Hsp70-1 [10].

Hsp70 is known to consist of two main domains: the N-terminal nucleotide binding domain (NBD) (45 kDa) and the substrate binding domain (SBD) (25 kDa). The first is a V-shaped structure consisting of two subdomains (lobes) surrounding the ATP binding site. The second one also consists of two: a substrate binding domain beta (SBDβ) and a substrate binding domain alpha – (SBDa) [11].

Later data showed that chaperones perform a dual function in proteostasis, contributing to the implementation of the main stages of protein degradation [12]. The interaction of a particular chaperone with other chaperones or cochaperones determines the fate of the former through one of the common pathways of protein degradation, the ubiquitin-proteasome system (UPS), or autophagy. In eukaryotes, Hsp70 interacts with two cochaperones: J, the domain cochaperone, Hsp40, and the nucleotide exchange factor NEF. Hsp40 is known to stimulate ATP Hsp70 hydrolysis and can participate in the presentation of Hsp70 substrates [13, 14]. NEF promotes the exchange of nucleotides by Hsp70, inducing the release of ADP (Fig. 1) [15].

It has been proven that normally, Hsp70 plays several roles in signaling cascades involved in the cell growth and differentiation. The molecular mechanism of Hsp70 induction regulation depends on the activity of a unique heat shock transcription factor 1 (HSF1), which binds to the 5'-promoter regions of all Hsp genes and triggers the transcription. Under homeostatic conditions, Hsp70 is intracellular and associated with HSF1 [16]. High temperature, ischemia, and other causes for the accumulation of unfolded proteins lead to Hsp70 dissociation from HSF, leaving it free for target proteins to bind. In the stressed cell, dissociated HSF is transported to the nucleus, where it is phosphorylated, possibly by protein kinase C, to form activated trimers. The resulting trimers bind to the highly conservative regulatory sequences of the heat shock gene known as heat shock elements (HSEs). HSEs bind to the promoter region of the inducible gene Hsp70, which leads to an increase in the Hsp70 generation [17]. Through binding to HSF1, Hsp90 can also affect Hsp70: when Hsp90 dissociates with HSF1, the latter is released to bind HSEs and leads to an even greater Hsp70 induction [18].

The newly generated Hsp70 in combination with ATP, Hsp40 and Hsp90 binds to denatured proteins and acts as a molecular chaperone, promoting the repair, clotting and transport of damaged peptides within the cell. Subsequently, a complex is formed, which includes the Hip (hunting-interacting protein) and Hop (hunting-organizing protein) associated with the N and C terminal domains, respectively, due to which clotting and then refolding of denatured structures occurs [19]. If no clotting occurs, BAG-1 binds to the N-terminus of Hsp70, and CHIP E3 ubiquitin ligase binds to the C-terminus of Hsp70. This complex then interacts with the denatured protein and recruits it into proteasome [20]. Thus, Hsp70 is involved in the damaged proteins refolding.

Interaction of Hsp70 with some of the pro- and anti-apoptotic proteins

A stress-induced expression of Hsp70 allows cells to cope with a large number of unfolded and / or denatured proteins resulting from the external stress. Traditionally, such typical pathological processes include inflammation, hypoxia, apoptosis, and tumor growth [21].

Apoptosis, as the body's response to pathological changes, is involved in the pathogenetic links of many diseases, such as strokes, neonatal hypoxia, degenerative retinal diseases, graft rejection, Alzheimer's disease and other neurodegenerative diseases [21, 22].

Caspase-independent and caspase-dependent apoptosis pathways are distinguished. The caspase-dependent pathway of apoptosis is divided into internal and external. Complex signaling pathways occur in the cell from the initiation to the start of a signaling molecules cascade, including many proteins. It is obvious that the impact on any element of this cascade can be a therapeutic target for a pharmacological correction of apoptosis processes. For example, nerve growth factors inhibit apoptosis and appear to meet therapeutic needs in diseases with extensive autolysis. An increase in Bcl-2 expression can inhibit pathological neuronal apoptosis in response to neurotoxic factors. In addition, it has been proven that low molecular weight caspase inhibitors, for example, Z-VAD-FMK, are effective in the treatment of amyotrophic lateral sclerosis in animals [23].

Apoptosis is required to maintain cellular homeostasis. The Hsp70 expression increases the cell survival under stress. Hsp70 knockdown cells are sensitive to autolysis [24], while the Hsp70 overexpression inhibits apoptosis, acting either through the internal Akt / PKB mitochondria-dependent or the external receptor-dependent pathway [25].

External apoptosis is initiated by plasma membrane-bound proteins of the TNF receptor family, which lead to the activation of caspase-8/10 in the death-inducing signaling complex (DISC) [26]. Hsp70 can also inhibit the assembly of the DISC signaling complex, in-

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hibiting apoptosis induced by Fas, TRAIL, and TNF. After TNF-induced DISC formation and activation of caspase 8, Hsp70 can inhibit BID activation [27]. When interacting with an extracellular ligand, membrane receptors transmit death signals to the intracellular space through their cytoplasmic domains. The membrane receptors involved in apoptosis belong to the superfamily of tumor necrosis factor (TNF) receptors, the activation of which depends on two main ligands: TNF and Fas. TNF and its receptors, namely TNFR-1 and TNFR-2, are responsible for initiating the main apoptosis pathway, i.e. the TNF pathway. The interaction between TNF and its receptors has been shown to signal death by recruiting two adaptive proteins: the TNF receptor-associated death domain (TRADD) and the Fas-associated death domain (FADD) protein. A cascade of these processes affects programmed cell death through the action of caspases. FNO ligands form homotrimers that bind to FNO receptors on the membrane [28]. In TNF-α-induced apoptosis, Hsp70 interacts with the FANCC protein (Fanconianemia complementation group C, PKR inhibitor) through its ATP domain and forms a triple complex with FANCC and PKR [29, 30]. It also resists TRAIL-induced apoptosis and the formation of a death-causing signaling complex with death receptors DR4 and DR5 [31]. The Hsp70 function in Fas-induced apoptosis is under-explored, but the adverse effects depend on the cellular context [32].

The internal apoptotic pathway is initiated by the release of various factors from the cell mitochondria. In response to the brain damage and the resulting oxidative stress, a transitional pore of permeability is formed in mitochondria. That leads to the release of cytochrome C into the cytosol, where a number of pro-apoptotic molecules ultimately cause the activation of effector caspases. Among these molecules, there are Bcl-2 family proteins, some of which are pro-apoptotic. These molecules are the main regulators of the mitochondrial membrane. Bcl2 and Bax are targets for the suppressor protein of p53 tumor. In response to DNA damage, Bcl2 transcription is repressed, and Bax is induced [33, 34]. Tumor cells often have mutated p53 that forms a stable complex with Hsp70 / Hsc70. A stress-mediated expression of Hsp70 inhibits nuclear import of p53 [35]. However, the Hsp70 regulation of the NF-kB function is still under-explored. Cytosolic Hsp70 can inhibit the expression of NF-kB, and membrane-bound Hsp70 can induce this transcription factor [36]. In neuronal stem cells, the Hsp70 induction by the recombination plasmid pEGFP-C2-HSP70 significantly blocks caspase-3 and reduces neuronal cytotoxicity, including a neuronal loss and a synapse damage in cocultured cells [37]. After an inflammatory stimulus, oligodendrocyte progenitor cells and mature oligodendrocytes from mice deficient in Hsp70 come into apoptosis caused by the caspase-3 activation [38]. Fig. 2 shows some of the apoptotic proteins that Hsp70 interacts with.

Experience in pharmacological use of recombinant Hsp70

Neuroprotective action

Studies confirming the neuroprotective role of endogenous heat shock proteins [39] have stimulated the development of pharmacological strategies based on the use of recombinant human Hsp70 [40]. Thus, Xinhua Zhan et al. demonstrated that the administration of Fv-Hsp70 2 and 3 hours after focal cerebral ischemia resulted in a 68% decrease in the volume of the infarction zone and significantly improved sensorimotor functions compared to the control group [41]. Similar results were presented in the publication by a Russian scientific group under the guidance of M.A. Shevtsov. The authors demonstrated that both preliminary and postischemic intravenous administration of rhHsp70 dose-dependently reduced the zone. Moreover, a long-term treatment of ischemic rats with rhHsp70 in the form of alginate granules with a sustained protein release further reduced the infarction volume and the apoptosis zone [42].

Similarly, the intranasal rhHsp70 administration resulted in a two-fold decrease in the local ischemia volume in the prefrontal cortex in the study of the mice with a photothrombotic stroke. In addition, the intranasal rhHsp70 administration reduced the level of apoptosis in the ischemic penumbra, stimulated axonogenesis, and increased the number of synaptophysin-producing neurons. In an isolated crayfish mechanoreceptor consisting of a single sensory neuron surrounded by a glial membrane, exogenous Hsp70 significantly reduced photoinduced apoptosis and necrosis of glial cells [43].

Moreover, as a therapeutic agent for slowing down neurodegenerative processes, rhHsp70 also demonstrates a high potential. In the study by David J. Gifondorwa et al., recombinant human Hsp70 delayed the onset of paralysis in a murine model of amyotrophic lateral sclerosis caused by overexpression of the mutant SOD1 gene. When administered intraperitoneally three times a week, starting from the 50th day of life, rhHsp70 led to an increase in life expectancy, a delay in the onset of symptoms, preservation of motor function, and an increase in the number of innervated neuromuscular connections compared with the control tissue [44].

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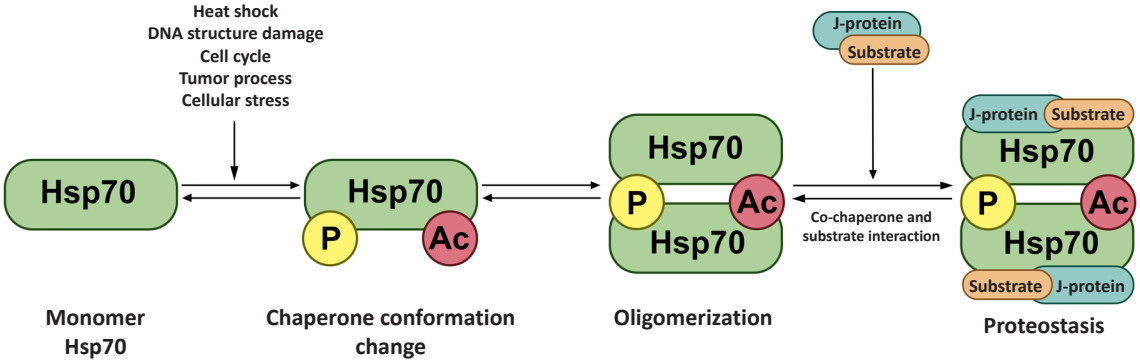

Figure 1 – Model of the Hsp70 oligomerization assembly line
Note: Cellular stress changes chaperone conformation, which facilitates Hsp 70 oligomerization. Co-chaperones and associated substrates bind to Hsp 70 oligomer, forming active chaperone complex

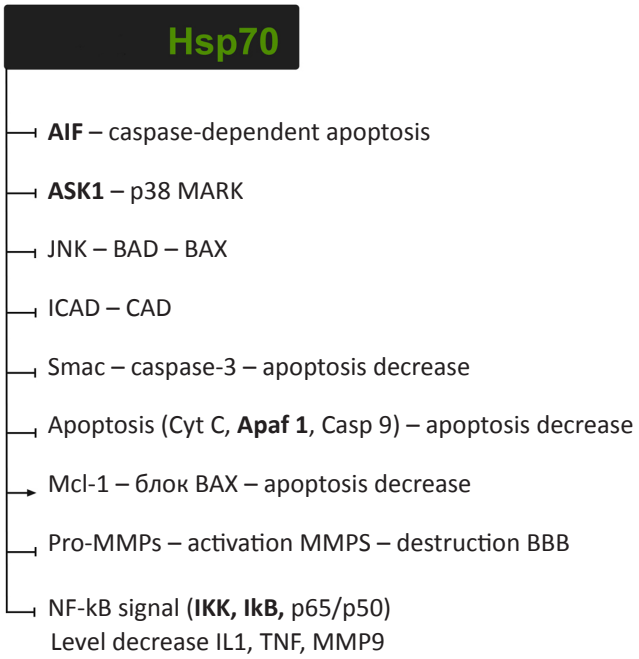

Figure 2 – Interaction of Hsp70 with apoptosis and inflammation regulating proteins

In the transgenic mouse model of Alzheimer's disease and in the mice with bulbectomy, intranasally administered rhHsp70 quickly penetrates the affected areas of the brain and mitigates multiple morphological and cognitive anomalies, normalizing the density of neurons in the hippocampus and cortex of the brain and reducing the accumulation of amyloid-β and amyloid plaques [45, 46].

In addition to the direct cytoprotective activity against neurons, rhHsp70 demonstrates a GABA-ergic effect: a preliminary intracerebroventricular administration of Hsp70 reduces the severity of the seizures caused by NMDA- and pentylenetetrazole. Moreover, traced Hsp70 in neurons was co-localized with NMDA receptors, synaptophysin, and L-glutamic acid decarboxylase [47].

Anti-inflammatory activity

A preventive Hsp70 administration reduced the toxic effect of *E. coli* endotoxin on the rat organism and significantly increased the survival rate of the animals during the experiment [48, 49]. In addition, in the models of sepsis caused by the administration of lipoteichoic acid, it was shown that the prophylactic administration of exogenous human Hsp70 significantly attenuates numerous homeostatic and ehmodynamic disorders and partially normalizes the coagulation system disorders and many biochemical blood parameters, including the concentrations of albumin and bilirubin [50, 51].

It has been shown that both intracellular and extracellular Hsp70 modulates the activation of the key

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pro-inflammatory factor NF-kB [52]. Thus, overexpressed Hsp70 blocks the NF-kB activation and p50/p65 nuclear translocation by inhibiting IKK-mediated phosphorylation of IkB (NF-kB inhibitor). It is of interest to note that the opposite effect occurs when Hsp70 is outside the cell. It is assumed that extracellular Hsp70 can act as a damage-associated molecular pattern (DAMP) through the innate immunity receptors TLR2 and TLR4 and thus trigger pro-inflammatory cascades. [53] An increase in the expression / secretion of NF-kB-dependent pro-inflammatory cytokines, including interleukin IL-1β, interleukin IL-6 and FNO-α, in response to extracellular Hsp70 in human lung cancer cells, dendritic cells and monocytes, was also notified. [54] However, other studies have shown that in the cultures of synoviocytes obtained from the patients with rheumatoid arthritis, extracellular Hsp70 inhibits the NF-kB signaling pathway, decreasing the level of IL-6, IL-8, and MCP-1 [55]. In addition, it has been shown that extracellular Hsp70 reduces the production of proinflammatory cytokines such as FNO-α and IL-6 in monocytes exposed to TLR agonists and contributes to the attenuation of the inflammatory response [56].

Thus, the results of a number of studies indicate that Hsp70 exhibits a predominantly anti-inflammatory activity, but under certain conditions it can activate pro-inflammatory cascades.

Modern methods for producing recombinant forms of Hsp70

Currently, it is known about the creation of Hsp70A1 recombinant forms. In particular, two sources are isolated: its isolation from the biomass of the *E. coli* bacterial culture, expressing it in increased quantities, and from transgenic mice-producers. To analyze its activity, the following parameters are determined: a substrate-binding activity, the analysis of the restoring activity of proteins, the ability to displace endogenous substance from the cells, the ability to reduce endotoxin-induced ROS production, and the ability to stimulate the natural killer cell activity towards the cancer cells *in vitro*. It is known that a protein glycosylation during the production can complicate the result of its administration to patients, especially when the body contains cells expressing the native form, causing an autoimmune response. A modified version of the protein was named rhHsp70.128, which differs fundamentally from the wild-type protein (rhHsp70.135) at five putative N-glycosylation sites: QGDRTTPSY, YFNDAQRQA, DLNKAINPD, KRNSAIPTK, and ILNVAATDK. A chaperone activity of the recombinant Hsp70 was assessed using carboxymethylated lactalbumin as a substrate protein. It was shown that the activity of the modified protein corresponds to the activity of the reference wild-type version and binds denatured lactalbumin with a similar efficiency. The next test consisted in measuring the activity of luciferase after its denaturation and recovery using the Hsp70 preparation in order to analyze its coagulability. The data show that all three tested samples were almost equally active. A series of experiments was also carried out to confirm the ability of the modified recombinant Hsp70 to displace its endogenous analogue from cells. Modified rhHsp70.128 as well as the wild-type probe, entered the cells and displaced endogenous Hsp70. An alternative way to obtain a recombinant Hsp protein, similar to that created in *E. coli,* was the creation of a female producers line expressing it in the mammary glands with a content of 1–2 mg/ml of protein in milk, depending on the animal. The study of its expression was carried out by the method of immunoblotting. It has been shown that the mutant protein can be efficiently isolated using ATP columns, as opposed to the wild type, by reacting to commercial antibodies. Based on the data obtained, it is obvious that the secretory production of the protein is technologically more advantageous in comparison with the cytoplasmic production due to the simplicity of its purification [57].

CONCLUSION

Chaperones are key regulators of cellular homeostasis that perform pleiotropic functions involving a wide range of signaling pathways. At the same time, heat shock proteins, the most studied family of chaperones, have a high pharmacotherapeutic potential for the treatment of a number of diseases associated with inflammation, apoptosis, and accumulation of misfolded proteins. This review was focused on the pharmacology of one of the key members of this family, Hsp70. The literature analysis confirms that this molecule is an endogenous regulator of many physiological processes and demonstrates tissue protective effects in modeling ischemic, neurodegenerative and inflammatory processes. The use of recombinant exogenous Hsp70 mimics the endogenous function of the protein, indicating the absence of a number of typical limitations characteristic of pharmacotherapy with high molecular weight compounds, such as immunogenicity, rapid degradation by proteases, or a low degree of passage through histohematogenous barriers. Thus, Hsp70 may become a promising agent for clinical trials as a drug for the treatment of patients with neurological, immunological, and cardiovascular profiles.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

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METHODS FOR QUANTITATIVE DETERMINATION OF TOTAL FLAVONOIDS IN *QUERCUS ROBUR* L. BUDS

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Currently, the actual task of modern pharmacy is to study the chemical composition and pharmacological properties of plant objects. Within the framework of this concept, it seems interesting to study *Quercus robur* L. buds. One of the promising groups of biologically active compounds of *Quercus robur* L. buds are flavonoids. This group of substances has a wide range of a pharmacological activity, which is significant in the creation of new medicines based on medicinal plant raw materials.
The aim of the article was to work out methods for quantitative determination of total flavonoids in *Quercus robur* l. buds.
Materials and methods. The research materials were aqueous-alcoholic extracts from *Quercus robur* L. buds with 70% ethyl alcohol which were analyzed by differential UV spectrophotometry on spectrophotometer "SF 2000" (Russia).
Results. The methods for quantitative determination of total flavonoids in *Quercus robur* L. buds by differential UV spectrophotometry, has been developed using a standard sample of cynaroside at the analytical wavelength of 400 nm. The optimum parameters for the extraction of total flavonoids from *Quercus robur* L. buds have been determined. They are: the optimum extractant is 70% ethyl alcohol; the "raw material-extractant" ratio is 1:50; the extraction time is 120 min, the degree of atomization is 2 mm.
The content of total flavonoids for *Quercus robur* L. buds has been determined; it varies from 0.27%±0.01 to 0.44%±0.02. These results make possible to recommend the content of total flavonoids for this type of raw materials not less than 0.25% as a lower limit.
Conclusion. The data obtained in the course of the experiment, makes it possible to conclude that a further study of *Quercus robur* L. buds is promising, and it also contributes to the implementation of medicinal plant raw materials "*Quercus robur* L. buds" in the State Pharmacopoeia (Russia).
Keywords: *Quercus robur* L.; buds; flavonoids; cynaroside; differential spectrophotometry; standardization
Abbreviations: BASs – biologically active substances; HPLC – High Performance Liquid Chromatography; SP (Russia), XIVth ed. – State Pharmacopoeia of the Russian Federation, XIVth edition; GM – general monograph; SS – standard sample; UV spectroscopy –ultraviolet spectroscopy; PM –pharmacopoeial monograph; SD – Standard Deviation; RSD – Relative Standard Deviation.

МЕТОДИКА КОЛИЧЕСТВЕННОГО ОПРЕДЕЛЕНИЯ СУММЫ ФЛАВОНОИДОВ В ПОЧКАХ ДУБА ЧЕРЕШЧАТОГО *QUERCUS ROBUR* L.

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ОРИГИНАЛЬНАЯ СТАТЬЯ

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В настоящее время актуальной задачей современной фармации является изучение химического состава и фармакологических свойств растительных объектов. В рамках данного направления представляется интересным изучение почек дуба черешчатого *Quercus robur* L. Одной из перспективных групп биологически активных соединений почек дуба являются флавоноиды. Данная группа веществ обладает широким спектром фармакологической активности, что является значимым при создании новых лекарственных препаратов на основе лекарственного растительного сырья.

Цель. Разработка методики количественного определения суммы флавоноидов в почках дуба черешчатого *Quercus robur* L.

Материалы и методы. Материалом исследования являлись водно-спиртовые извлечения почек дуба черешчатого *Quercus robur* L. на спирте этиловом 70%, которые анализировали методом дифференциальной УФ-спектрофотометрии на спектрофотометре «СФ 2000» (Россия).

Результаты. Разработана методика количественного определения суммы флавоноидов в почках дуба черешчатого методом дифференциальной УФ-спектрофотометрии с использованием стандартного образца цинарозида при аналитической длине волны 400 нм. Установлены оптимальные параметры экстрагирования суммы флавоноидов из почек дуба черешчатого: оптимальный экстрагент – 70% спирт этиловый; соотношение «сырьё-экстрагент» – 1:50; время экстракции – 120 мин, степень измельчения – 2 мм.

Определено **с**одержание суммы флавоноидов для почек дуба черешчатого, которое варьирует от 0,27%±0,01 до 0,44%±0,02. Данные результаты позволяют рекомендовать в качестве нижнего предела содержание суммы флавоноидов для данного вида сырья не менее 0,25%.

Заключение. Полученные в ходе эксперимента данные позволяют сделать вывод о перспективности дальнейшего изучения почек дуба черешчатого, а также способствуют внедрению лекарственного растительного сырья «Дуба черешчатого почки» в Государственную Фармакопею Российской Федерации.

Ключевые слова: Дуб черешчатый; *Quercus robur* L.; почки; флавоноиды; цинарозид; дифференциальная спектрофотометрия; стандартизация

Список сокращений: БАВ – биологически активные соединения; ВЭЖХ – высокоэффективная жидкостная хроматография; ГФ РФ XIV изд. – Государственная Фармакопея Российской Федерации XIV издания; ОФС – общая фармакопейная статья; СО – стандартный образец; УФ-спектроскопия – ультрафиолетовая спектроскопия; ФС – фармакопейная статья; SD – стандартное отклонение; RSD – относительное стандартное отклонение.

INTRODUCTION

The genus *Quercus* L. (*Fagaceae*) is represented by more than 500 species, most of which are the most important producers of broad-leaved and mixed coniferous-broad-leaved forests in the European part of Russia and Western Europe[1,2]. In Russia, 19 species grow wild, and about 60 species have been introduced [1].

Quercus robur L. is a large tree with a wide-pyramidal tent-like crown, reaching more than 50 meters in height[3]. The economic importance of *Quercus robur* L. is quite great, so it is used in many areas: in the furniture and leather industries, in forestry, etc. The bark of *Quercus robur* L. is used in the world medical practice, it is found in such pharmacopoeias as Russian, British, European and others [6, 7]. Its bark is also used in the production of various complex medicines, such as “Stomatophyt”, “Tonsilgon N”, “Dentos” and others[4,5,6,7].

Quercus robur L. is rather widely used in folk medicine as a remedy for the prevention and treatment of gastrointestinal, gynecological, as well as otorhinolaryngological and dermatological diseases [1].

A complex of biologically active substances (BASs), which include flavonoids, is present in plant objects, particularly, in the oak bark. This group of substances is one of the most common groups of all phenolic plants compounds, in the chemical structure of which there is a C_6-C_3-C_6 carbon skeleton [2–6]. These are the substances of a phenolic nature with valuable pharmacological properties such as anti-inflammatory, diuretic, choleretic, antispasmodic, antiviral, antioxidant, antimicrobial, etc., ones[8] [2–6]. The oak bark also contains tannins (gallic acid, ellagic acid), triterpenes (fridelin, fridelinol, 3-friedelanol)[9] and a number of other valuable substances [6–12].

Besides studying *Quercus robur* L. bark, the buds of this plant are of interest as a source of flavonoids. An important concept in the study of *Quercus robur* L. buds and their implementation into pharmaceutical and medical practice, is to solve the problem of standardization of raw materials, as well as the development of methods for the quantitative analysis of BASs in the raw materials. As a type of a medicinal plant raw material, buds are included in SP (Russia), XIVth ed., as a general monograph (GM). It should be noted that the attention of domestic and foreign scientists used to be attracted to the study of some plants’ buds [13–16]. Currently, for the quantitative determination of flavonoids compounds, rath-

[1] State Pharmacopoeia of the Russian Federation. Ministry of Health of the Russian Federation. XIV ed. Vol. 1–4. M., 2018. Available from: http://http://femb. ru/femb/pharmacopea. php

[2] Assessment report on *Quercus robur* L., *Quercus petraea* (Matt.) Liebl., *Quercus pubescens* Willd., cortex EMA/HMPC/3206/2009.

[3] Ibid.

[4] State Pharmacopoeia of the Russian Federation. Ministry of Health of the Russian Federation. XIV ed. Vol. 1–4. M., 2018.

[5] Assessment report on *Quercus robur* L., *Quercus petraea* (Matt.) Liebl., *Quercus pubescens* Willd., cortex EMA/HMPC/3206/2009.

[6] European Pharmacopoeia – 8th. “01/2008:1887 corrected 6.0”. 2013. Available from: http://pharmeuropa.edqm.eu

[7] British Pharmacopoeia 2009. British Pharmacopoeia Herbal Drugs and Herbal Drug Preparations // Oak Bark. 2009;37:203.

[8] Assessment report on *Quercus robur* L., *Quercus petraea* (Matt.) Liebl., *Quercus pubescens* Willd., cortex EMA/HMPC/3206/2009.

[9] State Pharmacopoeia of the Russian Federation. Ministry of Health of the Russian Federation. XIV ed. Vol. 1–4. *M*., 2018.

er a wide list of analytical methods is used. The most frequently used methods are high-performance liquid chromatography (HPLC) and UV spectroscopy [14–17]. The UV spectroscopy method makes the quantitative determination of total flavonoids of biologically active substances in plant objects possible, whereas the HPLC method, as a rule, is used to determine individual components of the studied objects [14, 17].

Thus, the research was conducted on the study of *Aesculus hippocastanum* L. buds, which resulted in the development of a method for the quantitative determination of rhamnocitrin in *Aesculus hippocastanum* L. buds by HPLC [14, 15]. The same scientists studied the chemical composition of *Aesculus hippocastanum* L. buds by differential spectrophotometry, which resulted in the identification of the dominant substance in the raw material [15]. The study of new antimicrobial agents of the plant origin for the suppression of a microbial biofilm formation was also conducted to identify and quantify phenolic compounds extracted from *Populus nigra* and *Populus alba* L. buds. It was also done to evaluate their antimicrobial and antibiotic activity by HPLC [13]. Besides studying *Populus nigra* L. and *Populus alba* L. buds, the research of *Populus balsamifera* L. buds was conducted to determine the optimal way of extraction by a barothermic method, with ethanol and supercritical carbon dioxide, the isolation and purification of flavonoid components of *Populus balsamifera* L. buds [13].

The method of differential UV spectroscopy is widely used for the qualitative and quantitative assessment of BASs in plant raw materials [1, 15, 17–21]. The essence of differential spectrophotometry is the complex formation of aluminum cation, carbonyl and hydroxyl groups of flavonoid resulting in the stable complex formation, due to which the so-called bathochromic shift occurs [1, 19]. The differential spectrophotometry method was used in the development of methods for the quantitative determination of flavonoids in *Leontodon autumnalis* L. raw materials after the formation of a stained complex with an aluminum chloride solution [20]. This method was also used in the process of the development of the quantitative determination method of total flavonoids in *Juglans regia* L. leaves using the rutin standard sample at the analytical wavelength of 416 nm in order to solve the issues of the new type standardization of medicinal plant raw materials [22]. Differential spectrophotometry was used in the development of methods for the quantitative determination of total flavonoids in *Tagetes patula* flowers using a patulitrin standard sample (7-O-β-D-glucopyranoside 3,5,7,3',4'-pentahydroxy-6-methoxyflavone) at the analytical wavelength of 428 nm [22]. As a result of the analysis of the above mentioned studies, it can be concluded that the method of differential spectrophotometry is in demand in modern pharmaceutical practice in the standardization of medicinal plant raw materials [22].

The method of differential spectrophotometry in the quantitative analysis of flavonoids has significant advantages, such as simplicity, availability, accuracy, small amounts of time spent on the analysis. Proceeding from the fact that the method of differential spectrophotometry makes it possible to determine the content of flavonoids, their total or the individual substance in the analyzed raw materials; it is logical to use this method in the development of regulatory documentation on the raw materials – *Quercus robur* L. buds [19].

In the course of the literature review regarding the study of *Quercus robur* L. buds, the data on the research of morphological and anatomical signs of *Quercus robur* L. buds, an important link in the standardization of new medicinal plant raw materials, were found [23]. A study of the alcoholic extracts based on *Quercus robur* L. buds, which revealed an antimicrobial activity against a number of pathogenic strains of microorganisms *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Bacilluscereus, Candida albicans,* had been conducted [24]. A further direction in the study of *Quercus robur* L. buds, is the development of methods for the quantitative determination of the BASs in the raw materials.

THE AIM of the article was to work out methods for the quantitative determination of total flavonoids in *Quercus robur* L. buds.

MATERIALS AND METHODS

The objects of the study were three samples of *Quercus robur* L. buds, harvested in the winter-spring period from late February to early April 2021. Sample No. 1 was collected in the Samara region (Pohvistnevsky district, Pervomaisk village); sample No. 2 – in the Botanical Garden of Samara University (Samara); sample No. 3 – in the Nature Forest Park "Dubki" (Samara, Russia). The species specificity of the analyzed objects was confirmed by the determinants of the central part of Russia [1].

Morphologically, *Quercus robur* L. buds are obovate, multilobed, dense, dark brown topping off in the center at the end of the shoot with one or three apical (terminal) buds [1, 23]. Both vegetative and generative buds from three representatives of this species were selected for the analysis. After harvesting, the buds were crumbled in a thin layer and dried without heating in a well-ventilated room without direct sunlight. The end of drying was determined by the brittleness of the buds. A differential spectrophotometry method was used to develop the methodology, which was carried out in accordance with the Pharmacopoeial Monograph of the State Pharmacopoeia (Russia), XIVth ed. (SP (Russia), XIVth ed.)[10].

A solution of cynaroside in 70% alcohol was used as a standard sample (Fig. 1). The cynaroside standard

[10] State Pharmacopoeia of the Russian Federation. Ministry of Health of the Russian Federation. XIV ed. Vol. 1–4. *M*., 2018.

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sample complies with the requirements of the Pharmacopoeial Monographs (PMs) and was provided for the research by the Research Equipment Sharing Center Center of SamSMU Institute of Pharmacy.

During the analysis of aqueous-alcoholic extractions of *Quercus robur* L. buds and standard samples of cynaroside solutions, the device "SF 2000" (Russia) was used. The aqueous-alcoholic extractions were prepared using 96% alcohol (Trademark OOO "Hippocrates", Russia, Samara, series: 360917). The alcohol concentrations of 40%, 50%, 60%, 70%, 80%, 90% and 96% were obtained by diluting 96% alcohol according to Table No. 5 of Appendix to SP (Russia), XIVth ed.[11].

Method of quantitative determination of total flavonoids in Quercus robur L. buds

Preparation of aqueous-alcoholic extractions from *Quercus robur* L. buds

The analytical sample of the raw material is ground to a particle size, passing through a sieve with a diameter of 2 mm. About 1 g of the crushed raw material (a precisely weighed amount) is placed in a conical heat-resistant flask (Erlenmeyer flask) with a 100 ml slotted volume, and 50 ml of 70% alcohol added. The flask is closed with a stopper and weighed on Sarto GOSM laboratory balance (LV 210-A (Ru-LV-210-A), No. 23425181; 2008; Russia) with an accuracy of ±0.001. The flask is attached to a reflux condenser and heated in a boiling water bath (moderate boiling) for 120 min. Then the flask is cooled for 30 minutes, closed with the same stopper, weighed again and the missing extractant is added to the original flask weight. The extraction is filtered over a paper filter (a red band)[12].

Preparation of test solution

5 ml of the obtained extraction is placed in a 25 ml volumetric flask, 1 ml of a 3% alcohol solution of aluminum chloride is added, the volume of the solution is brought to the mark with 96% alcohol (test solution A), stirred and left for some time (40 minutes) to form a flavonoid complex with aluminum. Then the optical density of the test solution is measured on a spectrophotometer at the wavelength of 400 nm. The solution obtained is used as a reference solution: 5 ml of the extraction (1:50) is placed in a measuring flask with a capacity of 25 ml and the volume of the solution is brought to the mark with 96% alcohol.

Preparation of cyanaroside standard sample solution

About 0.01 g (a precisely weighed amount) of cyanaroside is placed in a 50 ml volumetric flask, dissolved in 30 ml of 70% alcohol and heated in a water bath. The use of 70% alcohol provides the best dissolution of a cynaroside standard sample. After cooling the contents of the flask to room temperature, its volume is brought to the mark with 70% alcohol (solution A of cynaroside). 2 ml of a cynaroside A solution is placed into a 25 ml volumetric flask, 1 ml of a 3% alcohol solution of aluminium chloride is added, and the volume of the solution is adjusted to the mark with 96% alcohol (cynaroside test solution B). The optical density of the solution B on a spectrophotometer is measured at 400 nm.

Preparation of reference solution

2 ml of the cynaroside A solution was placed in a 25 ml volumetric flask and the volume of the solution was brought to the mark with 96% alcohol (the cynaroside B reference solution). Since 96% alcohol was used to bring a selected aliquot of aqueous-alcoholic extractions of *Quercus robur* L. buds to the mark, the alcohol of this concentration was also used to bring the cynaroside A solution to the mark.

The content of total flavonoids equivalent to cynaroside and absolutely dry raw materials in percent (X), is calculated by the formula:

$$X = \frac{D * m_0 * 50 * 25 * 2 * 100 * 100}{D_0 * m * 5 * 25 * 25 * (100 - W)},$$

where: D – the optical density of the test solution; D_o – the optical density of the cynaroside standard sample; m – the mass of raw materials, g; m_o – the mass of the cynaroside standard sample, g; W – the mass loss in drying, %.

In the absence of a cynaroside standard sample, it is advisable to use the theoretical value of the specific absorption index, 334.

$$X = \frac{D * 50 * 25 * 100}{m * 334 * 5 * (100 - W)},$$

where: D – the optical density of the test solution; m – the mass of raw materials, g; *334* – specific absorbance ($E_{1cm}^{1\%}$) of cynaroside standard sample at 400 nm; W – the weight loss in-drying, %.

The value of the specific absorption index ($E_{1cm}^{1\%}$) for the cynaroside standard sample at 400 nm was calculated experimentally by the formula:

$$E_{1cm}^{1\%} = \frac{D * V_1 * V_2}{100 * q * m_0},$$

where: D – the optical density of the test solution; m_0 – the mass of the cynaroside standard sample, g; V_1 – the volume of flask 1, ml; V_2 – the volume of flask 2, ml; q – the volume of the aliquot, ml;

Validation of analytical methods

Validation of the developed methods was carried out according to the following indicators: specificity, linearity, precision (a repeatability level), intralaboratory precision, correctness in accordance with SP (Russia),

[11] Ibid.
[12] Ibid.

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XIV[th] ed.[13]. Microsoft Excel 2013 software was used for the calculations.

RESULTS AND DISCUSSIONS

In the course of the experiment, a method for the quantitative determination of total flavonoids in *Quercus robur* L. buds has been developed. As a result, the optimum conditions for the extraction have been determined, and the choice of the optimal extractant has been substantiated.

Since at present, the component composition of the buds has not been studied, the total substances (flavonoids) in the studied extracts were determined.

The development of the methods was carried out stage by stage. At the first stage, the absorption spectra of aqueous-alcoholic extractions on the basis of *Quercus robur* L. buds were studied. During the analysis of the obtained extracts by differential spectrophotometry, the absorption maxima of spectral curves characteristic for the substances of the flavonoid nature, were determined (Fig. 2). A bathochromic shift of the electronic absorption spectrum of the aqueous-alcoholic extractions of *Quercus robur* L. buds with an absorption maximum similar to that of the standard sample of a cynaroside solution (400 nm) was recorded (Fig. 3). Therefore, when carrying out the quantitative determination of total flavonoids in the aqueous-alcoholic extractions based on *Quercus robur* L. buds, cynaroside was chosen a standard sample (Fig. 4 and 5). The observed similar picture of spectral absorption curves in the analysis of the studied samples of raw materials and the cynaroside standard sample solution, makes it possible to assert that in aqueous-alcoholic extractions of *Quercus robur* L. buds flavonoids are present, and the method of differential spectrophotometry makes it possible to carry out their quantitative definition.

At the second stage of the methods development, it was found out that the complete extraction of flavonoids from *Quercus robur* L. buds is achieved with the extraction of 70% alcohol. The next stage was an experiment to determine the optimal ratio "raw material-extractant" (1:50). Then the extraction time parameters were determined: it was found out that the maximum extraction of flavonoids from raw materials occurs during 120 minutes. The final step was to determine the degree of atomization of raw materials (2 mm), contributing to the full extraction of flavonoids by the extractant (Table 1).

On the basis of the obtained results, the conditions for the quantitative determination methods have been determined: the extraction of flavonoids from *Quercus robur* L. buds crushed to 2 mm, in 70% ethanol, in the ratio of "raw material-extractant" 1:50, within 120 min in a boiling water bath. The quantitative determination of total flavonoids equivalent to cynaroside, is carried out by differential spectrophotometry at the analytical wavelength of 400 nm, using a standard sample or value of the specific absorption index of the cynaroside standard sample (334).

The criterion for evaluating the analytical methods is the validation assessment. The validation of the methods was performed in accordance with SP (Russia), XIV[th] ed.[14].

The methods specificity was determined by the correspondence of the absorption maxima of the *Quercus robur* L. buds flavonoid complex and the solution of the standard cynaroside sample with aluminum chloride and the differential peak of the standard cynaroside sample.

The methods linearity was determined for a series of 10 cynaroside solutions (with the concentrations ranging from 0.00225 to 0.0225 mg/ml: 0.00225; 0.00325; 0.00425; 0.00525; 0.00625; 0.00725; 0.00825; 0.00925; 0.0125; 0.0225) with aluminum chloride at the wavelength of 400 nm. Based on the data obtained, a dependence graph of the optical density values of cynaroside solutions with aluminum chloride on the concentration of cynaroside was constructed, and then a linear regression equation was calculated (Fig. 6; Table 2).

While studying the linear dependence of the kind of $y = bx + a$, the correlation coefficient made 0,99957, hence, the given methods can be used for the analysis of total flavonoids in *Quercus robur* L. buds equivalent to cynaroside in the specified range of concentrations (Fig. 6; Table 2).

The precision of the methods (a repeatability level) was estimated by analyzing the studied sample of medicinal plant raw materials in a 10-fold replication (Table 3).

To assess the in-laboratory precision, the analysis of the test sample was performed by another analyst on other days using the same equipment (Table 4). For each sample, the studies were carried out in six replications. Table 4 shows that the calculated value of Fisher's F-criterion 1.19 is less than the tabulated value of 5.05. Consequently, the variance of the analysis results of both chemistries are statistically equivalent, and the differences between the values obtained are random. Thus, the developed methods meets the validation requirements for the index of in-laboratory precision.

The correctness of the methods was determined by the addition method. Cynaroside solutions with the known concentration (80%, 100% and 120%) were added to the aliquot of the test sample. The average opening percentage was 100.30±2.12% (Tables 5 and 6). Three determinations were performed for each concentration. The error determined for the samples with additives of the standard samples, was within the error of a single determination, indicating that there was no systematic error. The value of the average opening percentage of the experiment 100,30±2,12% was within the normalized range of values and within 100±5% (Tables 5 and 6).

[13] State Pharmacopoeia of the Russian Federation. Ministry of Health of the Russian Federation. XIV ed. Vol. 1–4. *M*., 2018.

[14] Ibid.

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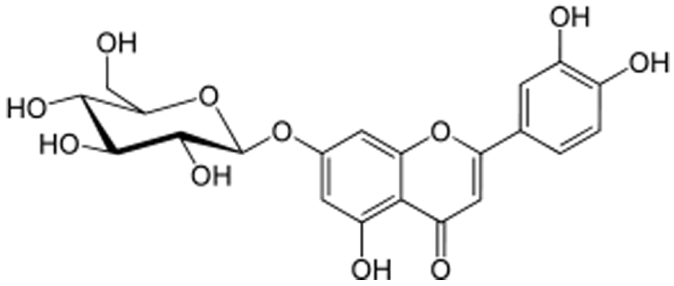

Figure 1 – Cynaroside formula

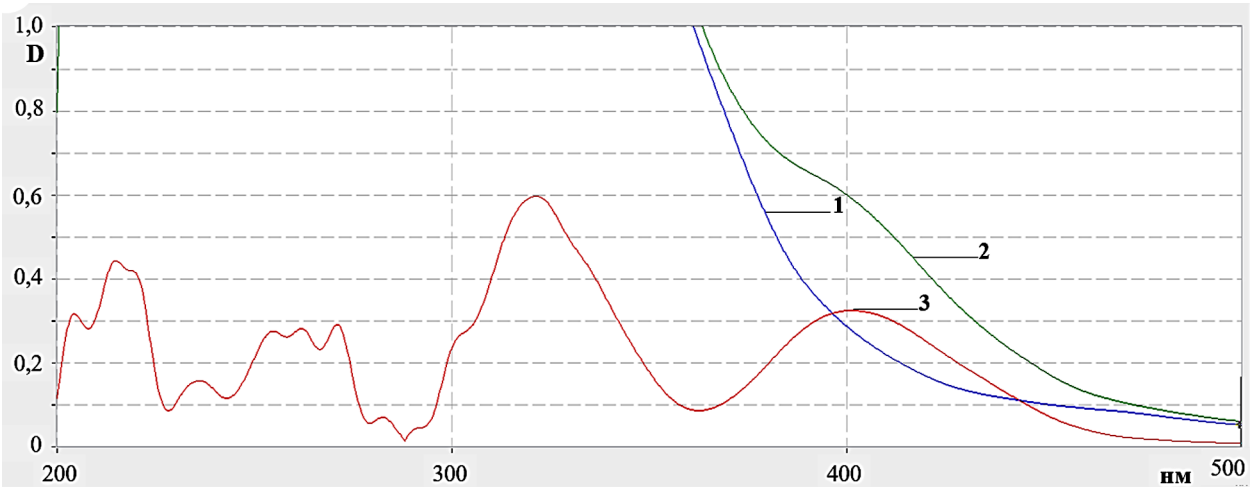

Figure 2 – Electronic spectra of solutions of aqueous-alcoholic extraction from Quercus robur L. buds
Note: 1 – extraction solution (direct spectrophotometry); 2 – extraction solution with addition of aluminum chloride; 3 – differential curve

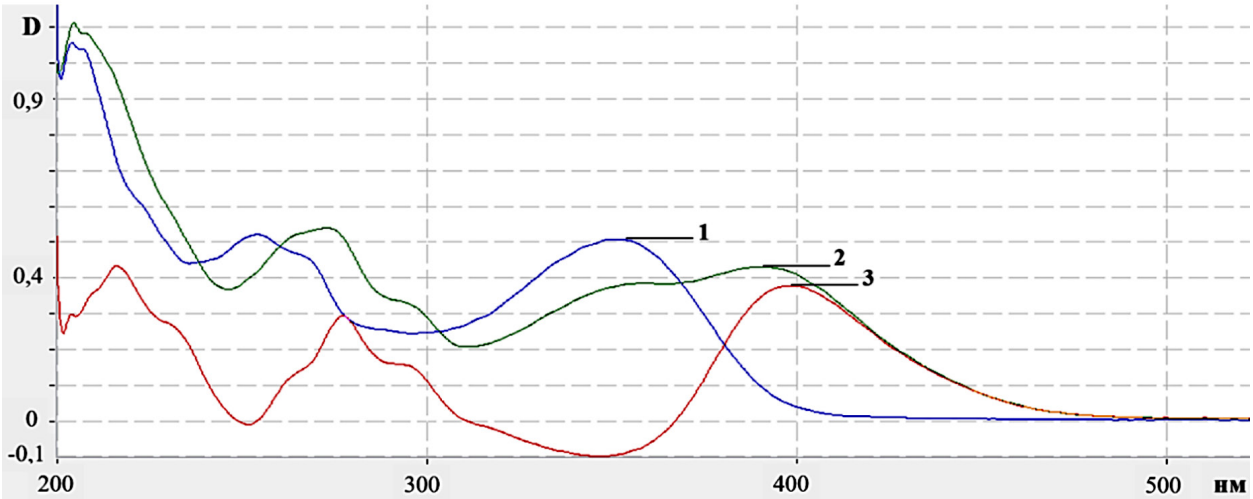

Figure 3 – Electronic spectra of aqueous-alcoholic solutions of cynaroside standard sample
Note: 1 – initial cynaroside solution (direct spectrophotometry); 2 – cynaroside solution with addition of aluminum chloride; 3 – differential curve of cynaroside (batochrome shift of short- and long-wave bands)

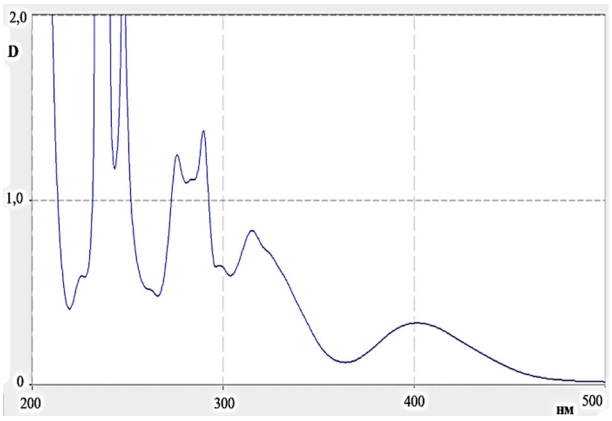

Figure 4 – Differential spectrum of aqueous-alcoholic extraction from Quercus robur L. buds

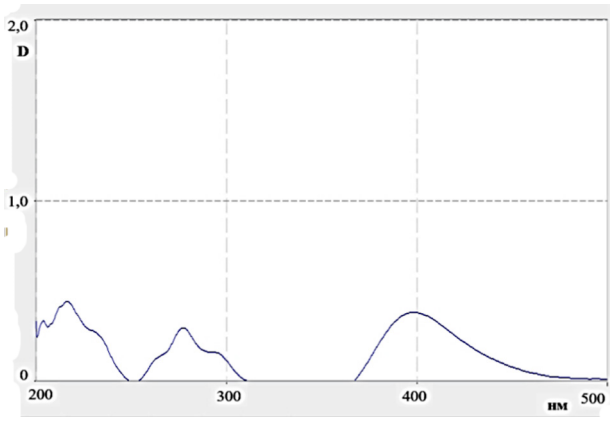

Figure 5 – Differential spectrum of cynaroside standard sample solution

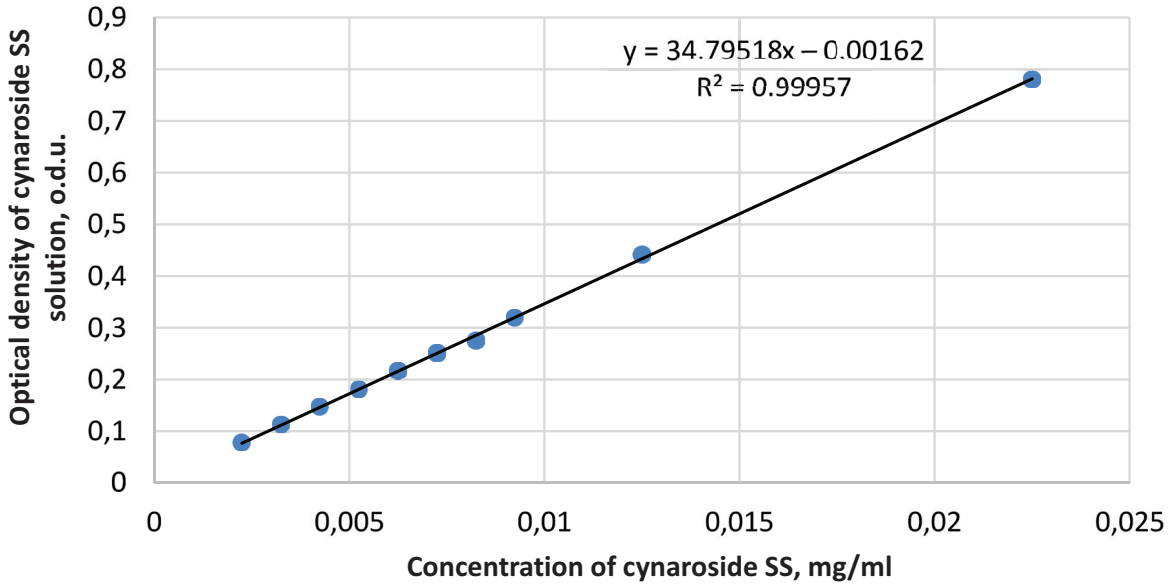

Figure 6 – Dependence of optical density values of cynaroside solutions with aluminum chloride on cynaroside concentration (differential version)

Table 1 – Optimal extraction rates of total flavonoids from *Quercus robur* L. buds at wavelength of 400 nm

No.	Extractor	"Raw materials: extractant" ratio	Extraction time, min	Degree of atomization, mm	Optical density value, D	Total flavonoids content per cyanaroside and absolutely dry raw materials, %
1	40% ethanol	1:30	60 min	2	0.3918	0.23±0.012
2	50% ethanol	1:30	60 min	2	0.4116	0.24±0.012
3	60% ethanol	1:30	60 min	2	0.4417	0.24±0.012
4	70% ethanol	1:30	60 min	2	0.4705	0.25±0.013
5	80% ethanol	1:30	60 min	2	0.4866	0.24±0.012
6	90% ethanol	1:30	60 min	2	0.4771	0.22±0.011
7	96% ethanol	1:30	60 min	2	0.4725	0.23±0.012
8	70% ethanol	1:30	30 min	2	0.4845	0.20±0.01
9	70% ethanol	1:30	45 min	2	0.5236	0.21±0.01
10	70% ethanol	1:30	60 min	2	0.4742	0.23±0.012

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No.	Extractor	"Raw materials: extractant" ratio	Extraction time, min	Degree of atomization, mm	Optical density value, D	Total flavonoids content per cyanaroside and absolutely dry raw materials, %
11	70% ethanol	1:30	90 min	2	0.383	0.23±0.012
12	70% ethanol	1:30	120 min	2	0.3883	0.26±0.013
13	70% ethanol	1:30	150 min	2	0.388	0.25±0.013
14	70% ethanol	1:20	120 min	2	0.3169	0.14±0.01
15	70% ethanol	1:30	120 min	2	0,6121	0.16±0.01
16	70% ethanol	1:50	120 min	2	0.4399	0.27±0.012
17	70% ethanol	1:100	120 min	2	0.6121	0.26±0.013
18	70% ethanol	1:50	120 min	1	0.5843	0.23±0.012
19	70% ethanol	1:50	120 min	2	0.6649	0.27±0.013
20	70% ethanol	1:50	120 min	3	0.6063	0.23±0.011

Table 2 – Input data for assessing methods linearity

No.	Concentration of cynaroside standard sample solution, mg/ml	The optical density value, o.d.u (average of three consecutive measurements)
1	0.00225	0.078411
2	0.00325	0.112547
3	0.00425	0.146935
4	0.00525	0.181541
5	0.00625	0.216048
6	0.00725	0.250947
7	0.00825	0.275401
8	0.00925	0.318974
9	0.0125	0.440864
10	0.0225	0.780564

Table 3 – Precision estimation results of quantitative determination methods of total flavonoids in *Quercus robur* L. buds (repeatability level)

Metrological characteristics	f	$\overline{X}$, %	S^2	SD	RSD	P, %	t (tab.)	$\Delta\overline{X}$, %	$\overline{\varepsilon}$, %
Values	9	0.24	0.00011738	0.011738	4.81%	95	2.262	±0.01	±3.44

Table 4 – Validation of laboratory precision of methods for determining total flavonoids in *Quercus robur* L. buds

Analyst 1	Analyst 2	Metrological characteristics	
X, %	X, %	Analyst 1	Analyst 2
0.24	0.26	$\overline{X}$ = 0.24	$\overline{X}$ = 0.25
0.24	0.25	S^2 = 0.000057	S^2 = 0.000080
0.23	0.26	SD = 0.00753	SD = 0.00894
0.25	0.24	RSD = 3.16%	RSD = 3.58%
0.23	0.24	$\overline{\varepsilon}$ = 3.63%	$\overline{\varepsilon}$ = 4.11%
0.24	0.25	$\overline{X} \pm \Delta\overline{X}$ = 0.24 ± 0.01	$\overline{X} \pm \Delta\overline{X}$ = 0.25 ± 0.01

Notes: $t_{calculated}$ = 2.44 < t (95%; 10); $F_{calculated}$ = 1.19 < F (95%; 5; 5), differences between the results obtained are random

Table 5 – Preparation scheme of aqueous-alcoholic extractions from *Quercus robur* L. buds with solutions addition of cynaroside standard sample

Initial cynaroside content, mg/ml aqueous-alcoholic extraction	Cyanaroside additive, mg/ml	Total calculated cynaroside content, mg/ml	Concentration level relative to nominal, %
2.30	1.84	4.14	80
2.30	2.30	4.60	100
2.30	2.76	5.06	120

Table 6 – Assessment results of correctness of quantitative determination method of total flavonoids in *Quercus robur* L. buds

Injected cyanaroside, mg/ml	Found, mg/ml	Openness, %	Characteristics calculated for opening value, %
0.84	0.80	95.24	$\overline{X}$ = 100.30% SD = 2.76% RSD = 2.75%
0.84	0.86	102.38	
0.84	0.83	98.81	
2.30	2.32	100.87	
2.30	2.26	98.26	
2.30	2.38	103.48	
2.76	2.81	101.81	
2.76	2.72	98.55	
2.76	2.85	103.26	

Table 7 – The content of total flavonoids in *Quercus robur* L. buds samples (in %) equivalent to cynaroside

No. n/a	Characteristics of raw material sample	Content of total flavonoids in absolutely dry raw materials (in %) calculated on cynaroside
1	Samara region, Pokhvistnevsky district, Pervomaysk village (March 2021)	0.27±0.01
2	Botanical Garden of Samara University, Samara (March 2021)	0.44±0.02
3	Natural forest park "Dubki", Samara (March 2021)	0.35±0.02

The results obtained testify to the satisfactory precision of the proposed quantitative determination methods of total flavonoids in *Quercus robur* L. buds equivalent to cynaroside at the levels of repeatability and in-laboratory precision.

It was found out that the average content of flavonoids in the studied sample of the raw materials was 0.24 ± 0.01% (the relative error of the determination was ±3.60%).

Thus, based on the experimental results validation, it can be concluded that this method is suitable for the quantitative estimation of total flavonoids equivalent to cynaroside.

Using this methods, three samples of *Quercus robur* L. buds, harvested at the same time (May-June 2021), were analyzed (Table 7). It was determined that the content of total flavonoids in the analyzed samples varies from 0.27%±0.01 to 0.44%±0.02 depending on its habitat (Table 7).

The presence of the flavonoid cynaroside in *Quercus robur* L. buds makes it possible to position them as a medicinal plant raw material. The medicines based on *Quercus robur* L. buds can be prescribed in diseases of chronic glomerulonephritis and pyelonephritis, complicated by a renal failure with hyperazotemia [25]. The indications for prescribing cynaroside may include hypertension, vasorenal hypertension complicated by nephrosclerosis and a chronic renal failure, as the flavonoid cynaroside alone has the above listed pharmacological effects [25]. The earlier studies on the research of the antimicrobial activity makes it possible to recommend *Quercus robur* L. buds as a raw material for the creation of antimicrobial agents [24].

The results obtained correlate with the data obtained for the buds of other plant species. If we take into account the fact that the determined total flavonoids in the buds of different species are converted to different substances, total flavonoids in *Aesculus hippocastanum* L. buds are equivalent to rhamnocitrin and varies from 1.24% to 2.31%. The content of total flavonoids in *Populus balsamifera* L. buds is equivalent to dihydroquercetin and rangers from 7.5% to 11.1%) [13–15].

Thus, the data obtained during the experiment suggest the feasibility of using the method of differential spectrophotometry for the quantitative determination of total flavonoids in *Quercus robur* L. buds. These results make it possible to recommend not less than 0.25% of total flavonoids for this type of raw material as a lower limit.

CONCLUSION

Thus, as a result of the study, the quantitative determination methods of total flavonoids in *Quercus robur* L. buds has been developed by differential spectrophotometry using a standard sample of cynaroside at the analytical wavelength of 400 nm. The content of total flavonoids has been determined for *Quercus robur* L. buds, which ranges from 0.27%±0.01 to 0.44%±0.02. The error of a single determination with a 95% confidence level is ±3.6%. The optimum values of the total flavonoids' extraction from *Quercus robur* L. buds, have been established. For *Quercus robur* L. buds, the total flavonoids content not less than 0.25%, can be recommended as the lower limit.

A validation assessment of the developed methods

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by the indicators of specificity, linearity, precision (a repeatability level), in-laboratory precision, correctness in accordance with SP (Russia), XIVth ed., has been carried out. Based on the results of the validation assessment of the experimental results, these methods can be suitable for the quantitative assessment of total flavonoids calculated on cynaroside.

This study has laid the foundation for the study of *Quercus robur* L. buds chemical composition, a quantitative assessment of total flavonoids of BAS in them by differential spectrophotometry. The results of the study can be used in the creation of herbal medicines based on *Quercus robur* L. buds and used in the treatment of kidney and dermatological diseases due to the content of total flavonoids in the raw material of biologically active substances and the substance of cynaroside alone.

The results obtained contribute to the development of the normative documentation for the promising species of the raw materials "*Quercus robur* L. buds" for the introduction to the State Pharmacopoeia (Russia).

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

Nikolay A. Ryabov – data collection, conducting the experiment, analysis and interpretation of the data obtained, preparation of the draft manuscript, literature analysis, writing the manuscript; Vitaly M. Ryzhov – study planning, participation in the development of study concept and design, collection of plant material for analysis; Vladimir A. Kurkin – final approval of the manuscript publication, processing of the obtained results, checking the critical intellectual content, statistical processing of the obtained results.

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SYNTHESIS AND ANTIOXIDANT ACTIVITY OF (*E*)-3-(3-(4-OXO-4*H*-CHROMEN-3-YL)ACRYLOYL) 2*H*-CHROMEN-2-ONE DERIVATIVES

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The aim is based on the results of the *in silico* prediction, to obtain and characterize a number of (*E*)-3-(3-(4-oxo-4*H*-chromen-3-yl)acryloyl)-2*H*-chromen-2-one derivatives, and also to study their antioxidant activity.

Materials and methods. The synthesis of the target compounds was carried out by condensation of substituted 3-formylchromones and 3-acetylcoumarins under the acid catalysis conditions. ^{1}H NMR spectra were recorded on the instruments of Bruker Avance-400 (400 MHz) and Bruker Avance-300 (300 MHz) in the solutions of $CDCl_3$ or DMSO-d_6. Mass spectra (ESI) were obtained on a Finnigan LCQ Advantage mass spectrometer (USA). The melting points of the compounds were determined on a PTP (M) instrument. Quantum-chemical calculations were carried out on the basis of a density functional theory using the Gaussian 09 program using the B3LYP/6-311G (d, p) method, as well as using the Way2Drug (PASS Online) online service. The antiradical activity of the compounds was studied by the DPPH test, and the chelating properties were assessed by the *o*-phenanthroline method.

Results. 15 derivatives of (*E*)-3-(3-(4-oxo-4*H*-chromen-3-yl)acryloyl)-2*H*-chromen-2-one have been obtained and characterized. The calculations based on the density functional theory showed that the highest occupied molecular orbital exhibiting electron-donating properties is localized on the propenone fragment, which confirms the likelihood of the manifestation of antiradical properties. According to the prediction of the probable spectrum of the biological activity, the obtained compounds are more likely to exhibit their direct antioxidant activity. According to the results of the *in vitro* study of the antioxidant activity, it was found out that compounds 1-15 are the most active in relation to the DPPH radical, which confirms the obtained prognostic data.

Conclusion. Thus, based on the *in silico* prediction data, 15 derivatives of (*E*)-3-(3-(4-oxo-4*H*-chromen-3-yl)acryloyl)-2*H*-chromen-2-one have been obtained and characterized, for which the method antioxidant activity has been studied *in vitro*. It was found out that compounds 1-15 exhibit the antiradical activity to a large extent.

Keywords: 3-formylchromone; 3-acetylcoumarin; chalcones; DFT calculations; antioxidant activity

Abbreviations: DFT – density functional theory; THF – tetrahydrofuran; DMF – dimethylformamide; HOMO – highest occupied molecular orbital; LUMO – lowest unoccupied molecular orbital; LPO – lipid peroxidation; TBA-AP – active products interacting with 2-thiobarbituric acid; ROS – reactive oxygen species.

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RESEARCH ARTICLE

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СИНТЕЗ И АНТИОКСИДАНТНАЯ АКТИВНОСТЬ ПРОИЗВОДНЫХ (*E*)-3-(3-(4-ОКСО-4*H*-ХРОМЕН-3-ИЛ) АКРИЛОИЛ)-2*H*-ХРОМЕН-2-ОНА

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Цель. На основе результатов прогноза *in silico* получить и охарактеризовать ряд производных (*E*)-3-(3-(4-оксо-4*H*-хромен-3-ил)акрилоил)-2*H*-хромен-2-она, а также изучить их антиоксидантную активность.
Материалы и методы. Синтез целевых соединений осуществляли конденсацией замещенных 3-формилхромонов и 3-ацетилкумаринов в условиях кислотного катализа. ЯМР^{1}Н спектры регистрировали на приборе Bruker Avance-400 (400 МГц) и Bruker Avance-300 (300 МГц) в растворах в дейтерированном хлороформе ($CDCl_3$) или дейтерированном диметилсульфоксиде (DMSO-d_6). Масс-спектры (ESI) были получены на масс-спектрометре Finnigan LCQ Advantage (США). Температуры плавления соединений определяли на приборе ПТП (М). Квантово-химические расчеты проводили на основе теории функционала плотности с помощью программы Gaussian 09 методом B3LYP/6-311G (d,p), а также с помощью онлайн-сервиса Way2Drug PASS Online. Антирадикальная активность соединений изучена методом DPPH-теста, а хелатирующие свойства оценены *o*-фенантролиновым методом.
Результаты. Получено и охарактеризовано 15 производных (*E*)-3-(3-(4-оксо-4*H*-хромен-3-ил)акрилоил)-2*H*-хромен-2-она. Расчеты на основе теории функционала плотности показали, что высшая занятая молекулярная орбиталь, проявляющая электронодонорные свойства, локализована на пропеноновом фрагменте, что подтверждает вероятность проявления антирадикальных свойств. По данным прогноза вероятного спектра биологической активности, полученные соединения с большей вероятностью могут проявлять прямую антиоксидантную активность. По результатам проведенного *in vitro* изучения антиоксидантной активности установлено, что соединения **1-15** проявляют наибольшую активность в отношение DPPH-радикала, что подтверждает полученные прогностические данные.
Заключение. Таким образом, на основании данных *in silico* прогноза получено и охарактеризовано 15 производных (*E*)-3-(3-(4-оксо-4*H*-хромен-3-ил)акрилоил)-2*H*-хромен-2-она, для которых методом *in vitro* изучена антиоксидантная активность. Установлено, что соединения 1-15 в значительной степени проявляют антирадикальную активность.
Ключевые слова: 3-формилхромон; 3-ацетилкумарин; халконы; DFT расчеты; антиоксидантная активность
Список сокращений: DFT – теория функционала плотности; ТГФ – тетрагидрофуран; ДМФА – диметилформамид; ВЗМО – высшая занятая молекулярная орбиталь; НСМО – низшая свободная молекулярная орбиталь; ПОЛ – перекисное окисление липидов; ТБК-АП – активные продукты, взаимодействующие с 2-тиобарбитуровой кислотой; АФК – активные формы кислорода.

INTRODUCTION

Currently, the relationship between the level of free radicals in the body and the development of a number of [1, 2], including malignant neoplasms [3], has been unambiguously established. These pathologies may be associated with the impaired DNA replication, as well as the normal functioning of membrane receptors, ion channels and membrane phospholipids [4].

Flavonoids are a wide class of natural polyphenolic compounds with a wide spectrum of a biological activity (including antioxidant) and a low toxicity [5–11]. Due to the antioxidant properties, the main types of the flavonoid activity are realized [12–15]. Flavonoids also include chalcones – compounds with an open pyran ring, in which two aromatic rings A and B which, in particular, exhibit the antimitotic activity, are linked by an α,β-unsaturated propenone fragment [16]. One of the possible mechanisms for the manifestation of the antiradical chalcones activity is the interaction of the vinylene group of the propenone fragment with reactive oxygen species. This mechanism occurs due to the transfer of electrons along the conjugation chain. From this point

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of view, a promising direction is the study of the effect of replacing one or both of the aromatic rings of chalcones with heterocyclic compounds.

In the article by Osipova et al. [17], chalcone analogs in which one of the rings was replaced by a 2*H*-chromen-2-one residue by the condensation of 3-acetylcoumarin and substituted benzaldehydes in butanol in the presence of acetic acid and piperidine, were synthesized. The obtained compounds showed a prolonged antioxidant activity on the systems of peroxidation of oleic acid and liver homogenate lipids.

THE AIM of the study is synthesis and an *in vitro* study of the antioxidant properties of chalcones analogs, in which one of the rings is replaced by a benz-γ-pyrone residue, and the second – by a benz-α-pyrone residue.

MATERIALS AND METHODS

Synthesis and determination of physical and chemical characteristics

The synthesis of the target compounds was carried out by condensation of substituted 3-formylchromones and 3-acetylcumarins under acid catalysis conditions. ^{1}H NMR spectra were recorded on a Bruker Avance-400 instrument (400 MHz) in the solutions of $CDCl_3$ or DMSO-d_6. Mass spectra at the atmospheric pressure with ionization by sputtering in an electric field (ESI) were obtained by full scanning of positive and negative ions on a dynamic tandem mass spectrometer Finnigan LCQ Advantage (USA). It was equipped with a mass analyzer with an ion trap MS Surveyor, an autosampler Surveyor, a generator nitrogen Schmidlin-Lab (Germany) and the information collection and the analysis system X Calibur (version 1.3, Finnigan) on a computer. The capillary temperature was 150°C, the field voltage between the needle and the counter electrode was 4.5 kV. The samples were injected into the ion source dissolved in acetonitrile using a syringe, through a 5 mL Reodyne injector at the carrier gas flow rate of 50 mL/min. The melting points of the compounds were determined in glass capillaries sealed from one end using a PTP (M) device.

General procedure for the preparation of *(E)*-3-(3-(4-oxo-4H-chromen-3-yl)acryloyl)-2H-chromen-2-one derivatives (1-15).

A mixture of 0.01 mol of the substituted 3-formylchromone and 0.01 mol of the corresponding 3-acetylcoumarin in 10 ml of AcOH was refluxed for 30 min in the presence of catalytic amounts of concentrated H_2SO_4. The precipitate obtained after cooling to room temperature, was filtered off and recrystallized from a THF-DMF mixture (7:3).

(*E*)-3-(3-(4-oxo-4*H*-chromen-3-yl)acryloyl)-2*H*-chromen-2-one (1)

Yield – 56%. MP 267–268°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.86 (s, 1H), 8.54 (s, 2H), 8.28 (d, J = 8.0 Hz, 1H), 7.85 (t, J = 8.0 Hz, 2H), 7.82–7.75 (m, 2H), 7.65 (d, J = 8.5 Hz, 1H), 7.59 (t, J = 7.6 Hz, 1H), 7.47 (t, J = 6.9 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 190.38, 162.11, 161.83, 161.68, 161.25, 160.82, 156.51, 155.04, 152.14, 138.76, 136.64, 136.27, 131.19, 127.54, 126.66, 126.42, 123.23, 119.41, 118.98, 117.23, 113.21, 110.38. Anal. Calcd (%) for $C_{21}H_{12}O_5$ (344.32): Calculated: C, 73.3; H, 3.5; O, 23.2. Found: C, 73.21; H, 3.53; O, 23.26. ESI-MS (m/z) 344 $[M + H]^+$

(*E*)-6-methyl-8-nitro-3-(3-(4-oxo-4*H*-chromen-3-yl) acryloyl)-2*H*-chromen-2-one (2)

Yield – 55%. MP 254–256°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.93 (s, 1H), 8.63 (s, 1H), 8.31 (d, J = 15.8 Hz, 1H), 8.24–8.20 (m, 1H), 8.16 – 8.11 (m, 1H), 8.07 (s, 1H), 7.88 – 7.82 (m, 1H), 7.71 (d, J = 8.4 Hz, 1H), 7.60 – 7.52 (m, 2H), 2.44 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 187.40, 175.20, 161.70, 155.01, 145.84, 144.74, 137.30, 135.55, 134.67, 129.33, 126.53, 126.26, 125.51, 123.56, 120.17, 118.38, 19.87. Anal. Calcd. (%) for $C_{22}H_{13}O_7$ (403.34): Calculated: C, 65.5; H, 3.2; N, 3.5; O, 27.8. Found: C, 65.63; H, 3.42; N, 3.39; O, 27.56. ESI-MS (m/z) 403 $[M + H]^+$

(*E*)-6-bromo-8-methyl-3-(3-(4-oxo-4*H*-chromen-3-yl)acryloyl)-2*H*-chromen-2-one (3)

Yield – 54%. MP 264–266°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.93 (s, 1H), 8.56 (s, 1H), 8.32 (d, J = 16.0 Hz, 1H), 8.14 (d, J = 8.0 Hz, 1H), 8.02 (s, 1H), 7.84 (dd, J = 16.8, 9.5 Hz, 2H), 7.71 (d, J = 7.8 Hz, 1H), 7.61–7.50 (m, 2H), 2.39 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 188.18, 175.64, 162.03, 158.30, 155.46, 152.38, 146.41, 137.40, 135.10, 130.25, 128.44, 126.69, 126.54, 125.96, 124.01, 120.28, 119.03, 118.88, 116.39, 15.13. Anal. Calcd. (%) for $C_{22}H_{13}BrO_5$ (437.24): Calculated: C, 60.4; H, 3; Br, 18.3; O, 18.3. Found: C, 60.29; H, 3.12; Br, 18.45, O, 18.14. ESI-MS (m/z) 437 $[M + H]^+$

(*E*)-6-chloro-3-(3-(4-oxo-4*H*-chromen-3-yl)acryloyl)-2*H*-chromen-2-one (4)

Yield – 59%. MP 231–233°C. ^{1}H NMR (400 MHz, DMSO-d_6) **δ** 8.95 (s, 1H), 8.60 (s, 1H), 8.35 (d, J = 15.8 Hz, 1H), 8.18–8.13 (m, 1H), 8.08 (d, J = 2.5 Hz, 1H), 7.89 – 7.83 (m, 1H), 7.80 – 7.71 (m, 2H), 7.60–7.52 (m, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 187.64, 175.20, 161.63, 157.90, 155.01, 153.11, 145.71, 136.93, 134.65, 133.55, 129.22, 128.55, 126.68, 126.39, 126.25, 125.50, 123.57, 119.77, 118.58, 118.42, 118.25. Anal. Calcd. (%) for $C_{21}H_{11}ClO_5$ (378.76): Calculated: C, 66.6; H, 2.9; Cl, 9.4; O, 21.1. Found: C, 66.57; H, 3.07; Cl, 9.52, O, 20.84. ESI-MS (m/z) 378 $[M + H]^+$

(*E*)-3-(3-(6-methyl-4-oxo-4*H*-chromen-3-yl)acryloyl)-2*H*-chromen-2-one (5)

Yield – 52%. MP 235–237°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.85 (s, 1H), 8.53 (t, J = 7.8 Hz, 2H), 8.19 (d, J = 2.1 Hz, 1H), 8.04–8.00 (m, 1H), 7.93 (d, J = 2.2 Hz, 1H), 7.79 (d, J = 15.5 Hz, 1H), 7.67 (dd, J = 8.7, 2.1 Hz, 1H), 7.55 (d, J = 8.7 Hz, 1H), 2.56 (s, 3H), 2.53 (s, 3H). Anal. Calcd. (%) for $C_{22}H_{14}O_5$ (358.34): Calculated: C, 73.7; H, 3.9; O, 22.3. Found: C, 73.67; H, 3.87; O, 22.46. ESI-MS (m/z) 358 $[M + H]^+$

(*E*)-6-methyl-3-(3-(6-methyl-4-oxo-4*H*-chromen-3-yl)acryloyl)-8-nitro-2*H*-chromen-2-one (6)

Yield – 54%. MP 248–251°C. ^{1}H NMR (400 MHz,

$CDCl_3$) δ 8.85 (s, 1H), 8.53 (t, J = 7.7 Hz, 2H), 8.19 (s, 1H), 8.02 (s, 1H), 7.93 (s, 1H), 7.79 (d, J = 15.5 Hz, 1H), 7.67 (d, J = 8.7 Hz, 1H), 7.55 (d, J = 8.6 Hz, 1H), 2.56 (s, 3H), 2.53 (s, 3H). Anal. Calcd. (%) for $C_{23}H_{15}O_7$ (417.37): Calculated: C, 66.2; H, 3.6; N, 3.4; O, 26.8. Found: C, 66.15; H, 3.57; N, 3.48; O, 26.8. ESI-MS (m/z) 417 $[M + H]^+$

(*E*)-6-bromo-8-methyl-3-(3-(6-methyl-4-oxo-4*H*-chromen-3-yl)acryloyl)-2*H*-chromen-2-one (7)

Yield – 57%. MP 256–258°C. ^{1}H NMR (400 MHz, $CDCl_3$) **δ** 8.68 (s, 1H), 8.60–8.43 (m, 2H), 8.04 (s, 1H), 7.81–7.59 (m, 4H), 7.51 (d, J = 8.3 Hz, 1H), 2.52 (s, 3H), 2.47 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.27, 161.84, 161.40, 160.96, 150.65, 139.80, 138.86, 137.98, 137.35, 130.36, 129.02, 125.23, 119.42, 118.42, 115.62, 112.79, 109.97, 20.55, 14.26. Anal. Calcd. (%) for $C_{23}H_{15}BrO_5$ (451.27): Calculated: C, 61.2; H, 3.4; Br,17.7; O, 17.7. Found: C, 61.15; H, 3.38; Br, 17.58; O, 17.89. ESI-MS (m/z) 451 $[M + H]^+$

(*E*)-6-chloro-3-(3-(6-methyl-4-oxo-4*H*-chromen-3-yl)acryloyl)-2*H*-chromen-2-one (8)

Yield – 54%. MP 236–238°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.72 (s, 1H), 8.51 (d, J = 19.6 Hz, 2H), 8.04 (s, 1H), 7.82–7.63 (m, 4H), 7.52 (d, J = 8.5 Hz, 1H), 7.40 (d, J = 8.9 Hz, 1H), 2.52 (s, 3H). Anal. Calcd. (%) for $C_{22}H_{13}ClO_5$ (392.79): Calculated: C, 67.3; H, 3.3; Cl, 9; O, 20.4. Found: C, 67.23; H, 3.35; Cl, 8.89; O, 20.53. ESI-MS (m/z) 392 $[M + H]^+$

(*E*)-3-(3-(6-methyl-8-nitro-4-oxo-4*H*-chromen-3-yl) acryloyl)-2*H*-chromen-2-one (9)

Yield – 56%. MP 261–263°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.83 (s, 1H), 8.55 (d, J = 14.7 Hz, 2H), 8.41 (s, 1H), 8.29 (s, 1H), 7.84 – 7.67 (m, 3H), 7.55–7.44 (m, 2H), 2.61 (s, 3H). ^{13}C ЯМР (101 MHz, $CDCl_3$) δ 189.55, 176.52, 161.56, 161.13, 160.70, 160.09, 155.07, 151.63, 138.58, 137.57, 136.55, 136.33, 132.42, 132.04, 127.97, 126.42, 124.98, 123.64, 120.27, 118.55, 117.21, 115.93, 113.10, 20.91. Anal. Calcd. (%) for $C_{22}H_{13}NO_7$ (403.34): Calculated: C, 65.5; H, 3.2; N, 3.5; O, 27.8. Found: C, 65.44; H, 3.27; N, 3.24; O, 28.05. ESI-MS (m/z) 403 $[M + H]^+$

(*E*)-6-methyl-3-(3-(6-methyl-8-nitro-4-oxo-4*H*-chromen-3-yl)acryloyl)-8-nitro-2*H*-chromen-2-one (10)

Yield – 56%. MP 213–216°C. ^{1}H NMR (400 MHz, CDCl3) δ 8.83 (s, 1H), 8.57 (s, 1H), 8.52 (s, 1H), 8.39 (s, 1H), 8.32 (s, 1H), 8.22 (s, 1H), 7.91 (s, 1H), 7.75 (d, J = 15.5 Hz, 1H), 2.62 (s, 3H), 2.57 (s, 3H). Anal. Calcd. (%) for $C_{23}H_{14}N_2O_9$ (462.37): Calculated: C, 59.7; H, 3.1; N, 6.1; O, 31.1. Found: C, 59.63; H, 3.12; N, 6.18; O, 31.07. ESI-MS (m/z) 462 $[M + H]^+$

(*E*)-6-bromo-8-methyl-3-(3-(6-methyl-8-nitro-4-oxo-4*H*-chromen-3-yl)acryloyl)-2*H*-chromen-2-one (11)

Yield – 49%. MP 275–276°C (decomp.). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.83 (s, 1H), 8.57 (s, 1H), 8.52 (s, 1H), 8.39 (s, 1H), 8.32 (s, 1H), 8.22 (s, 1H), 7.91 (s, 1H), 7.75 (d, J = 15.5 Hz, 1H), 2.62 (s, 3H), 2.57 (s, 3H). Anal. Calcd. (%) for $C_{23}H_{14}BrNO_7$ (496.26): Calculated: C, 55.7; H, 2.8; Br, 16.1; N, 2.8; O, 22.6. Found: C, 55.68; H, 2.77; Br, 16.21; N, 2.83; O, 22.51. ESI-MS (m/z) 496 $[M + H]^+$

(*E*)-6-chloro-3-(3-(6-methyl-8-nitro-4-oxo-4*H*-chromen-3-yl)acryloyl)-2*H*-chromen-2-one (12)

Yield – 47%. MP 283–284°C (decomp.). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.78 (s, 1H), 8.57 (s, 2H), 8.52 (s, 1H), 8.40 (s, 1H), 8.33 (s, 1H), 7.83 (s, 1H), 7.75 (d, J = 14.3 Hz, 2H), 7.44 (d, J = 8.9 Hz, 1H), 2.62 (s, 3H). Anal. Calcd. (%) for $C_{22}H_{12}ClNO_7$ (437.79): Calculated: C, 60.4; H, 2.8; Cl, 8.1; N, 3.2; O, 25.6. Found: C, 60.42; H, 2.74; Cl, 8.15; N, 3.13; O, 25.56. ESI-MS (m/z) 437 $[M + H]^+$

(*E*)-3-(3-(6-methyl-8-nitro-2-oxo-2*H*-chromen-3-yl)-3-oxoprop-1-en-1-yl)-4-oxo-4*H*- chromene-7-yl acetate (13)

Yield – 51%. MP 284–286°C (decomp.). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.78 (s, 1H), 8.62 (s, 1H), 8.28 (d, J = 15.8 Hz, 1H), 8.23 (s, 1H), 8.08 (s, 1H), 7.98 (d, J = 8.8 Hz, 1H), 7.54 (d, J = 15.7 Hz, 1H), 6.97 (d, J = 8.7 Hz, 1H), 6.90 (s, 1H), 2.45 (s, 3H), 1.91 (s, 3H). Anal. Calcd. (%) for $C_{24}H_{15}NO_9$ (461.38): Calculated: C, 62.5; H, 3.3; N, 3; O, 31.2. Found: C, 62.52; H, 2.93; N, 3.11; O, 31.44. ESI-MS (m/z) 461 $[M + H]^+$

(*E*)-3-(3-(6-bromo-8-methyl-2-oxo-2*H*-chromen-3-yl)-3-oxoprop-1-en-1-yl)-4-oxo-4*H*- chromene-7-yl acetate (14)

Yield – 52%. MP 243–246°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.79 (s, 1H), 8.55 (s, 1H), 8.28 (d, J = 15.8 Hz, 1H), 8.03 (s, 1H), 7.98 (d, J = 8.8 Hz, 1H), 7.82 (s, 1H), 7.52 (d, J = 15.7 Hz, 1H), 6.98 (d, J = 11.0 Hz, 1H), 6.90 (s, 1H), 2.39 (s, 3H). Anal. Calcd. (%) for $C_{24}H_{15}BrO_7$ (495.28): Calculated: C, 58.2; H, 3.1; Br, 16.1; O, 22.6. Found: C, 58.13; H, 3.12; Br, 16.18; O, 22.57. ESI-MS (m/z) 495 $[M + H]^+$

(*E*)-3-(3-(6-chloro-2-oxo-2*H*-chromen-3-yl)-3-oxoprop-1-en-1-yl)-4-oxo-4*H*-chromene-7-yl acetate (15)

Yield – 53%. MP 251–252°C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.80 (s, 1H), 8.58 (s, 1H), 8.30 (d, J = 15.7 Hz, 1H), 8.07 (d, J = 2.5 Hz, 1H), 7.98 (d, J = 8.8 Hz, 1H), 7.94 (s, 1H), 7.77 (dd, J = 8.9, 2.6 Hz, 1H), 7.55 (s, 1H), 7.51 (d, J = 6.6 Hz, 1H), 6.98 (dd, J = 8.8, 2.2 Hz, 1H), 6.91 (s, 1H), 2.88 (s, 3H). Anal. Calcd. (%) for $C_{23}H_{13}ClO_7$ (436.79): Calculated: C, 63.2; H, 3; Cl, 8.1; O, 25.6. Found: C, 63.15; H, 3.06; Cl, 7.96; O, 25.83. ESI-MS (m/z) 436 $[M + H]^+$

In vitro study of antioxidant activity

The DPPH free radical scavenging capacity was measured according to [18]. To 0.05 ml of a 0.4 mM DPPH (Sigma-Aldrich) methanol solution, 0.1 ml of a solution of the test compound in DMSO with concentrations of 1000 μg/ml; 500 μg/ml; 250 μg/ml; 125 μg/ml and 62.5 μg/ml was added. The mixture was incubated at room temperature for 30 min. Trolox (Sigma-Aldrich) in similar concentrations was used as a reference substance. After a 30-min incubation, a spectrophotometric detection at the wavelength of 518 nm against pure methanol was performed. A solution of DPPH in methanol was taken as a positive control (A_0). The percentage of the inhibition of the DPPH radical formation was calculated using the formula:

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$$\% \ inh. = \frac{A_x}{A_0} \times 100,$$

where: A_x – absorbance of the extract sample; A_0 – absorbance of the positive control sample.

IC_{50} was calculated based on the concentration-inhibitory ability relationship by the probit analysis.

The study of the chelating properties of compounds **1-15** was carried out by the *o*-phenanthroline method [19]. The incubation medium consisted of 1 ml of a 0.05% methanol solution of *o*-phenanthroline, 2 ml of iron (II) chloride (200 μM), and 2 ml of various concentrations of the test substances. The resulting mixture was incubated for 10 min. at room temperature. The absorbance of the samples was measured at 510 nm. The incubation medium without the addition of the studied substances served as a positive control. The percentage of inhibition was calculated by the formula:

$$\% \ inh. = \frac{B_x}{B_0} \times 100,$$

where: B_x – absorbance of the sample; B_0 – absorbance of the positive control sample.

When studying Fe^{2+}-ascorbate-induced lipid peroxidation, the incubation medium consisted of 100 mM Tris HCl buffer, pH 7.4; 0.5 mM ascorbate, 12 μM iron (II) sulfate and 100 μl of a rat brain homogenate. The reaction was carried out in a water bath at 37°C for 45 min. To determine the intensity of lipid peroxidation, 0.5 ml of the suspension was taken at time zero and after 60 minutes of incubation, then it was mixed in the cold with 1 ml of a 30% trichloroacetic acid solution. The resulting mixture was centrifuged at 3000 rpm for 15 minutes. 0.1 ml of a 5 M HCl solution and 1 ml of a 0.6% 2-thiobarbituric acid solution were added to the supernatant and heated in a water bath at 100°C for 15 minutes. The amount of TBA-AP was calculated using the molar extinction coefficient of malonic dialdehyde –$1{,}56\times10^5 M^{-1}\ sm^{-1}$ [20].

RESULTS AND DISCUSSION

(*E*)-3-(3-(4-oxo-4*H*-chromene-3-yl)acryloyl)-2*H*-chromene-2-one derivatives are obtained by condensation of 3-formylchromone with 3-acetylcoumarin in alcohol using organic bases – N,N-dimethylaminopyridine [21] or piperidine [22]. For this reaction, the catalytic properties of Lewis acids had been studied under various conditions [23, 24]. However, in the authors' opinion, this synthetic approach cannot be applied when 3-formylchromone is introduced into the interaction due to the high reactivity of the C (2) position: in the presence of even weak nucleophiles, the pyrone ring opens with the formation of substituted phenols.

(*E*)-3-(3-(4-oxo-4*H*-chromene-3-yl)acryloyl)-2*H*-chromene-2-one derivatives **1-15** were synthesized by refluxing of equimolar amounts of the corresponding substituted 3-formylchromones and 3-acetylcoumarins in glacial acetic acid in the presence of catalytic amounts of concentrated sulfuric acid (Scheme 1).

$R^1 = R^2 = R^3 = R^4 = R^5 = H$ (**1**); $R^1 = R^2 = R^3 = H$, $R^4 = NO_2$, $R^5 = Me$ (**2**); $R^1 = R^2 = R^3 = H$, $R^4 = Me$, $R^5 = Br$ (**3**); $R^1 = R^2 = R^3 = R^4 = H$, $R^5 = Cl$ (**4**); $R^1 = Me$; $R^2 = R^3 = R^4 = R^5 = H$ (**5**); $R^1 = R^5 = Me$, $R^2 = R^3 = H$, $R^4 = NO_2$ (**6**); $R^1 = R^4 = Me$, $R^2 = R^3 = H$, $R^5 = Br$ (**7**); $R^1 = Me$, $R^2 = R^3 = R^4 = H$, $R^5 = Cl$ (**8**); $R^1 = Me$; $R^3 = NO_2$, $R^2 = R^4 = R^5 = H$ (**9**); $R^1 = R^5 = Me$, $R^2 = H$, $R^3 = R^4 = NO_2$, (**10**); $R^1 = R^4 = Me$, $R^2 = H$, $R^3 = NO_2$, $R^5 = Br$ (**11**); $R^1 = Me$, $R^2 = R^4 = H$, $R^3 = NO_2$, $R^5 = Cl$ (**12**); $R^1 = R^3 = H$, $R^2 = OAc$, $R^4 = NO_2$, $R^5 = Me$ (**13**); $R^1 = R^3 = H$, $R^2 = OAc$, $R^4 = Me$, $R^5 = Br$ (**14**); $R^1 = R^3 = R^4 = H$, $R^2 = OAc$, $R^5 = Cl$ (**15**)

The information about some of the physicochemical parameters of compounds **1-15** is presented in Table 1.

At present, quantum-chemical calculation methods are actively used to predict the reactivity of compounds, one of the most promising of which is a density functional theory (DFT) calculation, which makes it possible to analyze the distribution of frontier molecular orbitals in a molecule. From the point of view of the search for new antioxidant agents, the use of this method is justified in view of the fact that the hydroxyl radical exhibiting electrophilic properties [25] will interact primarily with the highest occupied molecular orbital.

The distribution of the frontier molecular orbitals of compounds **1-15** was estimated on the basis of the structure analysis of the frontier orbitals. All necessary calculations were performed using the Gaussian 09 program based on the density functional theory (DFT) using the B3LYP / 6-311 G (d, p) basis set [26].

The parameters of the electron density distribution (the areas of the molecule where the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) are concentrated) for compound **1,** are shown in Fig. 1. For the calculations, water was chosen as a solvent, since all biological processes occur in the aquatic environment of the body.

As can be seen from Fig. 1, the highest occupied molecular orbital is mainly concentrated on the benzγ-pyrone fragment and the propenone fragment. Compounds **2-15** have similar frontier orbital distribution parameters.

In addition, *in silico*, calculated the probable spectrum of pharmacological activity using the PASS Online online service [27]. From the obtained data set, we selected only those types of activity that characterize the antioxidant properties of molecules. The values obtained are shown in Table 2.

As can be seen from the Table, derivatives **1-15** can, to a greater extent, exhibit a direct antioxidant activity. Compounds **13** and **14**, containing acetoxy groups in the structure, are most likely to manifest this type of activity (Pa> 0.5).

The study of the antiradical properties of compounds **1-15,** was carried out by the DPPH test. The data obtained are presented in Table 3.

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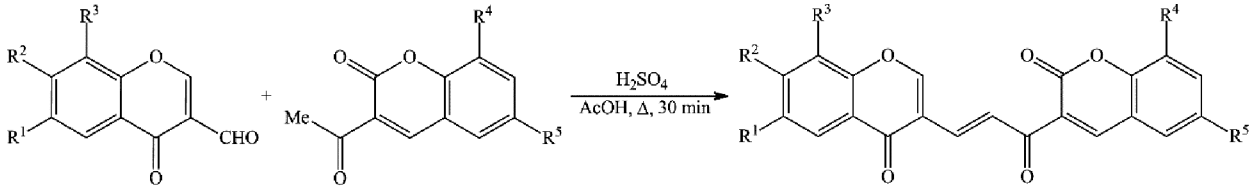
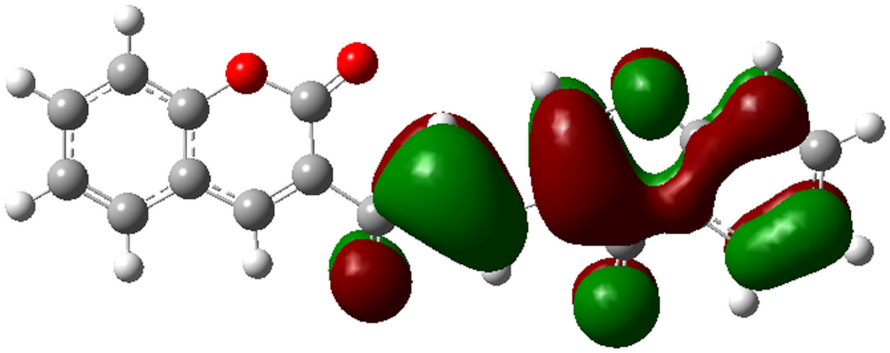


Scheme 1 – Synthesis of (*E*)-3-(3-(4-oxo-4H-chromene-3-yl)acryloyl)-2H-chromene-2-one derivatives



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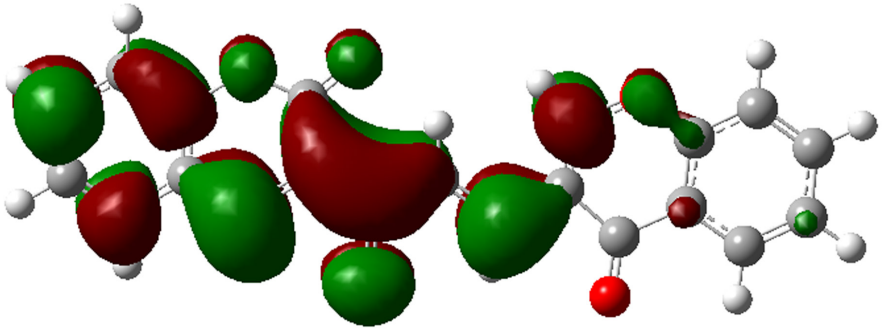

B

Figure 1 – Distribution of frontier orbitals in compound 1
Note: A – HOMO; B – LUMO

Table 1 – Physicochemical characteristics of *(E)*-3-(3-(4-oxo-4H-chromen-3-yl)acryloyl)-2H-chromen-2-one derivatives

Compound	Yield, %	MP (THF:DMF)	MW*	Molecular formula **
1	56	267–268 (lit. 270–272 [21])	344	$C_{21}H_{12}O_5$
2	55	257–259	403	$C_{22}H_{13}O_7$
3	54	258–260	437	$C_{22}H_{13}BrO_5$
4	59	231–233	378	$C_{21}H_{11}ClO_5$
5	52	235–237	358	$C_{22}H_{14}O_5$
6	54	248–251	417	$C_{23}H_{15}O_7$
7	57	256–258	451	$C_{23}H_{15}BrO_5$
8	54	236–238	392	$C_{22}H_{13}ClO_5$
9	56	261–263	403	$C_{22}H_{13}NO_7$
10	56	213–216	462	$C_{23}H_{14}N_2O_9$
11	49	275–276 (dif.)	496	$C_{23}H_{14}BrNO_7$
12	47	283–284 (dif.)	437	$C_{22}H_{12}ClNO_7$
13	51	284–286 (dif.)	461	$C_{24}H_{15}NO_9$
14	52	243–245	495	$C_{24}H_{15}BrO_7$
15	53	247–249	436	$C_{23}H_{13}ClO_7$

Note: * – according to the mass spectroscopy data (the obtained values correspond to the calculated ones); ** – the elemental analysis data are in agreement with the calculated values

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Table 2 – Prediction of the derivatives 1-15 antioxidant activity

Compound	Type of activity	
	Indirect antioxidant	Direct antioxidant
1	0.372	0.426
2	0.259	0.271
3	0.326	0.397
4	0.278	0.286
5	0.330	0.418
6	0.265	0.280
7	0.327	0.398
8	0.274	0.293
9	0.259	0.271
10	0.259	0.268
11	0.255	0.245
12	0.215	0.202
13	0.395	0.574
14	0.377	0.567
15	0.310	0.455

Table 3 – Antiradical activity of compounds 1-15

Compound	IC_{50}, mmol/ml	Compound	IC_{50}, mmol/ml	Compound	IC_{50}, mmol/ml
1	1.22±0.002	**6**	2.49±0.002	**11**	1.28±0.003
2	3.29±0.001	**7**	2.46±0.001	**12**	2.3±0.001
3	1.24±0.001	**8**	1.21±0.002	**13**	1.23±0.001
4	3.2±0.001	**9**	2.34±0.003	**14**	2.39±0.001
5	2.39±0.002	**10**	1.45±0.003	**15**	1.31±0.001
				Trolox	0.15±0.002

Table 4 – Chelating properties of compounds 1-15

Compound	IC_{50}, mmol/ml	Compound	IC_{50}, mmol/ml	Compound	IC_{50}, mmol/ml
1	2.9±0.161	**6**	3.03±0.183	**11**	3.05±0.069
2	2.11±0.224	**7**	3.15±0.089	**12**	3.36±0.128
3	2.47±0.235	**8**	3.55±0.26	**13**	3.64±0.185
4	2.86±0.13	**9**	2.73±0.168	**14**	3.16±0.13
5	3.58±0.202	**10**	3.26±0.098	**15**	4.98±0.097
				EDTA	1.52±0.014

Table 5 – IC_{50} values (mmol/ml) characterizing the ability of the studied compounds to affect Fe^{2+}-ascorbate-induced lipid peroxidation

Compound	IC_{50}, mmol/ml	Compound	IC_{50}, mmol/ml	Compound	IC_{50}, mmol/ml
1	9,88±0,197	**6**	7.16±0.145	**11**	9.6±0.058
2	8.73±0.183	**7**	7.76±0.137	**12**	7.34±0.158
3	9.84±0.07	**8**	7.32±0.199	**13**	7.03±0.091
4	8.27±0.047	**9**	9.8±0.125	**14**	8.19±0.122
5	8.8±0.091	**10**	9.78±0.101	**15**	9.42±0.073
				Trolox	2.3±0.003

Based on the data in Table 3, it can be assumed that compounds **1, 3, 11, 13** and **15** have the highest antiradical activity. Taking into account the peculiarity of the DPPH test, it can also be assumed that the studied substances have hydrogen-acceptor properties.

The data on the chelating properties of compounds **1-15** are presented in Table 4.

The most pronounced chelating properties in relation to Fe^{2+} are possessed by compounds **1-4**. This property is especially important in the processes of lipid peroxidation, induced by ions of divalent metals.

IC_{50} values (mmol/ml), characterizing the ability of the test compounds to affect Fe^{2+} -ascorbate-induced lipid peroxidation, are presented in Table 5.

Taking into account the high pathological role of Fe^{2+} – dependent oxidative processes in organs with a high metabolic activity, for example, the brain, the suppression of lipid peroxidation against the background of the studied substances, mainly **12** and **13**, can play a significant role in the survival of neurons and glial cells in case of brain damage of various etiologies.

An oxidative stress is an almost universal pathophysiological process that plays a role in the development and progression of a number of diseases, from atherosclerotic lesions of the vascular intima to a chronic obstructive pulmonary disease [28]. Modern concepts of the oxidative stress are based on two main postulates: damage to macromolecules is mediated by reactive oxygen species (ROS) or occurs because of the disruption of two-electron redox reactions of thiol chains. Herewith, the prevailing mechanism for the development of the oxidative stress is believed to be the oxidative modification of the cell structures under the influence of ROS in the presence of trace amounts of divalent metal ions, mainly Fe^{2+} known as lipid peroxidation (LPO). ROS are small molecules that have an unpaired electron and easily diffuse through the membrane lipid bilayer. ROS are very labile structures that spontaneously and without a significant energy expenditure, can undergo mutual transformations, which in most cases determines their pathogenetic significance in the development of diseases associated with an oxidative stress [29]. It has been established that ROS are more often of the intracellular origin and are generated in the processes of cellular respiration and metabolism. In the process of cellular respiration, during the sequential transfer of single electrons in the electron transport chain of mitochondria, intermediates with an odd number of electrons can “drop out” from the chain, forming ROS. At the same time, a large number of enzymes (xanthine oxidase, nitric oxide synthase, cytochrome, NADP-oxidase) produce ROS as a by-product of metabolism. It should be taken into consideration that ROS acquire cytotoxic properties only when the critical physiological concentration is exceeded or when antioxidant defense systems are dysfunctional [30].

A natural defense against ROS consists of ROS scavengers and antioxidant enzymes. Endogenous antioxidant defense enzymes are represented primarily by three superoxide dismutase isoenzymes, differing in the subcellular arrangement and catalyzing the dismutation of superoxide to oxygen and hydrogen peroxide. Catalase is also an important protective factor against ROS and degrades hydrogen peroxide to water. Thioredoxins, including several isoforms, make the reduction of oxidized proteins possible due to the thiol-disulfide exchange of cysteine. Glutathione peroxidases reduce lipid hydroperoxides to alcohols and hydrogen peroxide to water; Glutathione synthetase is responsible for the synthesis of the main cellular antioxidant glutathione and therefore plays an important role in the ROS detoxification. ROS scavengers are predominantly of the exogenous origin and are represented by active biomolecules such as tocopherol, ascorbic acid, carotenoids, uric acid, and polyphenols [31].

The main vector of the oxidative stress therapy is the use of substances with a direct antioxidant activity, both natural and synthetic. Thus, González et al., 2015 showed that propolis is an effective direct antioxidant [32]. Mai W, et al., 2020 showed that berberine, an isoquinoline’s alkaloid, significantly reduces the concentration of intracellular hydrogen peroxide due to the presence of radical-binding properties [33]. The most famous synthetic scavenger of ROS is N-acetylcysteine [34]. Direct antioxidant properties have also been established for some derivatives of chromone [35]. However, despite the presence of a fairly large number of compounds with the established antioxidant properties, expanding of the list of these substances, especially due to the targeted synthesis of effective antioxidants, is an urgent task of modern medicinal chemistry, as indicated by *Yang CS, et al., 2018.* [36]. In this regard, a study on the synthesis and on the antioxidant properties of (*E*)-3-(3-(4-oxo-4*H*-chromen-3-yl)acryloyl)-2*H*-chromen-2-one derivatives, was carried out. It showed that the analyzed compounds demonstrate a high level of a antiradical activity, which was confirmed by the data obtained during the DPPH test. It is also very important that the studied compounds have chelating properties with respect to Fe^{2+}, which can play a critical role in the termination of iron-induced lipid peroxidation and was confirmed in the course of this work. In addition, the toxicity analysis of related compounds suggests a low systemic toxicity of the analyzed compounds [37], which, together with a high antioxidant activity, makes a further study of these substances promising from the standpoint of drug development for a long-term systemic correction of the oxidative stress.

CONCLUSION

In this work, 15 derivatives of (*E*)-3-(3-(4-oxo-4*H*-chromen-3-yl)acryloyl)-2*H*-chromen-2-one have been synthesized and their structure has been confirmed by nuclear magnetic resonance, an elemental analysis , a

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mass spectrometry. According to *in silico* prediction data, the studied compounds are mainly characterized by a direct antioxidant activity. At the same time, the prediction data are generally consistent with the results of the *in vitro* studies of the antioxidant properties of the obtained compounds.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHORS' CONTRIBUTION

Stanislav S. Shatokhin – search and analysis of literature, quantum-chemical calculations, synthesis and establishment of the obtained compounds structure, text of the manuscript writing; Vladislav A. Tuskaev – synthesis and establishment of the obtained compounds structure; Svetlana Ch. Gagieva – synthesis and establishment of the obtained compounds structure; Dmitry I. Pozdnyakov – conducting pharmacological studies and the obtained data interpretation; Eduard T. Oganesyan – search and analysis of literature, the obtained results interpretation, text of the manuscript writing.

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TYPE 2 DIABETES MELLITUS'S DECOMPENSATED FORM: ON THE PROBLEM OF EFFECTIVE PHARMACOTHERAPY IN REAL CLINICAL PRACTICE

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The aim of this retrospective study was to analyze the pharmacotherapy regimens of the decompensated form of type 2 diabetes mellitus (DM2) and to evaluate its effectiveness, its compliance with clinical recommendations.
Materials and methods: A retrospective analysis of 54 medical cards of patients with decompensated DM2 was conducted. The 1st group (n=24; 44%) included the patients who had a decrease in glycated hemoglobin (HbA1c) by 50% or more in 3 months after hypoglycemic therapy; and the 2nd group (n=30; 56%) – the patients whose HbA1c level decreased by less than 50%.
Results. A HbA1c level was 10.4% in the 1st group and 13.2% in the 2nd group (p<0.001). However, the target levels of venous blood plasma glucose and HbA1c were not achieved in any of the patient groups. The total number of the drugs prescribed to the patients ranged from 4 (in 25% (n=6) and 10% (n=3) cases in the 1st and the 2nd groups, respectively) to 8 (in 12.5% (n=3) and 20% (n=6) cases in the 1st and the 2nd, groups, respectively). However, in a number of cases some violations of clinical recommendations were recorded: the prescription to the obese patients of insulin drugs, the administration of sulfonylureas derivatives to patients with a history of cardiovascular diseases of the atherosclerotic origin, but modern hypoglycemic drugs with proven benefits in reducing cardiovascular risks were rarely prescribed.
Conclusion. The tactics of pharmacotherapy in the patients with a decompensated form of DM2 does not fully comply with the approved clinical guidelines, which requires the effectiveness of treatment optimization of this medically and socially significant pathology.
Keywords: glycated hemoglobin; hypoglycemic drugs; insulin; polypragmasia; type 2 diabetes mellitus
Abbreviations: HbA1 – glycated hemoglobin; DM – diabetes mellitus; DM1 – type 1 diabetes mellitus; DM2 – type 2 diabetes mellitus; BMI – body mass index; iSGLT-2 – sodium glucose cotransporter inhibitor type 2; GLPra-1 – glucagon-like peptide-1 receptor agonist; iDPP-4 – dipeptidyl peptidase-4 inhibitor; BABs – beta-adrenoblocker; ACEi – angiotensin converting enzyme inhibitor; MRA – mineralocorticoid receptor antagonist; CCB – calcium channel blocker; HDL – high density lipoprotein; LDL – low density lipoprotein; p-value – level of static significance; OR – odds ratio; CI – confidence interval; Q1–Q3 – interquartile range; M – median; SD – standard deviation; QoL – quality of life.

ДЕКОМПЕНСИРОВАННАЯ ФОРМА САХАРНОГО ДИАБЕТА 2 ТИПА: К ВОПРОСУ ОБ ЭФФЕКТИВНОЙ ФАРМАКОТЕРАПИИ В РЕАЛЬНОЙ КЛИНИЧЕСКОЙ ПРАКТИКЕ

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RESEARCH ARTICLE

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Цель. Анализ схем фармакотерапии декомпенсированной формы сахарного диабета 2 типа (СД2) и оценка их соответствия клиническим рекомендациям.

Материалы и методы. Был выполнен фармакологический ретроспективный анализ 54 историй болезни пациентов с декомпенсированной формой СД2. В 1 группу (n=24; 44%) вошли пациенты, у которых по окончании 3-х месяцев гипогликемической терапии наблюдалось снижение уровня гликированного гемоглобина (HbA1) на 50% и более, а во 2 группу (n=30; 56%) – у которых уровень HbA1c снизился менее, чем на 50%.

Результаты. Уровень HbA1c в 1-й группе составил 10,4%, во 2-й группе 13,2% (p<0,001). Однако целевой уровень глюкозы плазмы венозной крови и HbA1c не были достигнуты ни в одной из групп пациентов. Общее количество назначаемых лекарственных средств составляло от 4 (в 25% (n=6) и 10% (n=3) случаев в 1 и 2 группах, соответственно) до 8 (в 12,5% (n=3) и 20% (n=6) случаев в 1 и 2 группах, соответственно), то есть полипрагмазия наблюдалась в абсолютном большинстве случаев. В ряде случаев были зафиксированы нарушения клинических рекомендаций: пациентам при наличии ожирения назначались препараты инсулина; при наличии в анамнезе сердечно-сосудистых заболеваний атеросклеротического генеза – производные сульфонилмочевины, но при этом редко назначались современные сахароснижающие лекарственные средства (ингибиторы натрий-глюкозного котранспортёра 2 типа, ингибиторы дипептидилпептидазы-4), обладающие доказанными преимуществами в отношении снижения сердечно-сосудистых рисков.

Заключение. Тактика лечения данной медико-социально значимой патологии в реальной клинической практике не в полной мере соответствует актуальным клиническим рекомендациям и требует дальнейшей оптимизации контроля эффективности.

Ключевые слова: гликированный гемоглобин; инсулин; полипрагмазия; сахарный диабет 2 типа; сахароснижающие лекарственные средства

Список сокращений: HbA1 – гликированный гемоглобин; СД – сахарный диабет; СД1 – сахарный диабет 1 типа; СД2 – сахарный диабет 2 типа; ЛС – лекарственное средство; ИМТ – индекс массы тела; иНГЛТ-2 – ингибитор натрий-глюкозного котранспортёра 2 типа; арГПП-1 – агонист рецепторов глюкагоноподобного пептида-1; иДПП-4 – ингибитор дипептидилпептидазы-4; БАБ – бета-адреноблокатор; иАПФ – ингибитор ангиотензинпревращающего фермента; АМР – антагонист минералокортикоидных рецепторов; БКК – блокатор кальциевых каналов; ЛПВП – липопротеины высокой плотности; ЛПНП – липопротеины низкой плотности; p – уровень статической значимости; ОШ – отношение шансов; ДИ – доверительный интервал; Q1–Q3 – интерквартильный размах; M – медиана; SD – стандартное отклонение.

INTRODUCTION

Diabetes mellitus (DM) is one of the most important medical and social problems of public health in the world, as it is a chronic, incurable disease, the therapeutic aspects of which require the patient to significantly change their lifestyle [1].

The total number of patients with DM in the Russian Federation (RF) as of January 2019 was 4 584 575 (3.12% of the population of the RF), including: type 1 diabetes mellitus (DM1) – 5.6% (256.2 thousand), type 2 diabetes mellitus (DM2) – 92.4% (4.24 million), other types of diabetes – 2% (89.9 thousand). Currently, the average prevalence of DM1 is 174.4 per 100 thousand population, DM2 – 2885.7 per 100 thousand, other types of DM – 61.2 per 100 thousand population[1]. Since 2000, the number of patients with DM in the RF has increased by 2.2 times: from 2.043 million to 4.58 million. As in many countries of the world, the RF continues to increase the prevalence of mainly DM2, with an annual increase of more than 250–300 thousand patients. During 2018, 10 805 new cases of DM1 and 298 628 of DM2 were identified [2]. However, these figures do not fully reflect the true scale of the non-communicable epidemic. The fact is that the register[2] records only officially registered the cases of the disease. At the same time, according to the national epidemiological study NATION [3], which included more than 26 thousand people in 63 subjects of the RF, the share of undiagnosed DM2 in the RF is 54% on average. Thus, the actual prevalence of DM2 with active screening for the level of glycated hemoglobin (HbA1c) is almost twice higher than officially registered, and can reach 8–9 million people [2].

A high medical and social significance of DM is due, among other things, to the high risk of associated micro- (nephropathy, retinopathy) and macroangiopathies (an ischemic heart disease, cerebrovascular diseases, and diseases of the arteries of the lower extremities). For example, DM is one of the leading risk factors for the development of acute cerebral circulatory disorders, leading to the so-called "vascular catastrophes" 3–4 times more often than in patients without carbohydrate metabolism disorders [4–6].

The level of glycated hemoglobin (HbA1c) is an integral indicator of glycemia, which serves as an indispensable diagnostic criterion in monitoring carbohydrate metabolism, evaluating the effectiveness of hypoglycemic therapy and predicting the course of diabetes, so its determination is currently mandatory [7, 8]. Thus, a 1% reduction in HbA1c in patients with DM2 reduces the risk of death by 21%, of an acute myocardial infarction – by 14%, and microvascular complications – by 37% [9, 10]. According to the World Health Orga-

[1] The Federal Register of Diabetes Mellitus of the Russian Federation. Available from: http://sd.diaregistry.ru/content/epidemiologiya.html
[2] Ibid.

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nization criteria, there are compensated diabetes (6.0–6.5% HbA1c), subcompensated diabetes (6.6–7.0% HbA1c) and decompensated diabetes (>7.0% HbA1c) [9].

Treatment of DM is one of the most expensive items of the health budget in many countries of the world. Thus, in 2017, the market volume of sugar-lowering drugs in the RF amounted to approximately 11612.5 million rubles. In the United States in 2012, 245 billion dollars were spent on the treatment of diabetes, in Italy in 2014 – about 20.3 billion euros [6, 11]. With effective therapy at an early stage of the disease, complications of the disease, disability and mortality are reduced. At the same time, there is an increase in costs at the initial stage, and then their reduction due to the prevention of hospitalizations associated with complications [12].

Patients with DM2, especially older age groups, often have concomitant chronic diseases, such as hypertension, dyslipidemia, a coronary heart disease, depressive disorders, a chronic kidney disease. They requires a simultaneous administration of several, usually more than 5–7 drugs; that exposes patients of this profile to a high risk of polypragmasia [13, 14].

From the standpoint of fundamental and clinical pharmacology, polypragmasia is the main cause of the undesirable side effects development in elderly and senile people [15, 16]. Polypragmasia bates the problems of drug interactions, reduces patients' adherence to antidiabetic therapy, and often causes suboptimal glycemic control. The presence of polypragmasia is also associated with a cascade of drug administrations, in which their side effects are misinterpreted as new pathological conditions, which can lead to the prescroption of new drugs. Polypharmacy has other negative health consequences, such as an increased risk of hospitalization, deterioration of a clinical status, poor quality of life (QoL) at patients, and significant economic consequences [13, 14].

THE AIM of this retrospective study was to analyze the pharmacotherapy regimens of the decompensated form of type 2 diabetes mellitus in settings of an endocrinological hospital, and to evaluate its effectiveness, as well as its compliance with clinical recommendations.

MATERIALS AND METHODS

The retrospective study was based on the analysis of medical cards of 54 patients with DM2 who were routinely hospitalized in a patient endocrinological facility in 2019. In the present study, only official documents (hospital history sheets) were studied, their analysis did not include direct identification of the patient's identity, therefore, the confidentiality of personal data was in no way violated. Thus, the planning and conduct of the study fully complied with the provisions on the ethical correctness of performing biomedical works[3] [17, 18].

The criteria for including patients in the study are: DM2 in the decompensation stage, the duration of the disease more than 10 years, a long-term and regular intake of hypoglycemic drugs. The criteria for excluding patients from the study are: DM1 and other disorders of carbohydrate metabolism, taking hypoglycemic drugs for less than 3 months, an inorganic and/or functional brain damage, a senile asthenia syndrome (according to the Fried criteria), a positive family history, thyroid diseases, liver diseases, abdominal cavity organs diseases, the age of patients up to 45 years.

A life history, modifiable, non-modifiable risk factors, biochemical parameters, therapeutic regimens and their modifications for the treatment of hyperglycemia and concomitant pathology were subjected to the pharmacological evaluation, including compliance with existing clinical recommendations, in order to choose the most optimal from the position of the attending physician and the patient. HbA1c was selected as a criterion for the therapy effectiveness.

Based on the assessment of the HbA1c level (the target levels ranged from 6.5% to 8%, the baseline – from 13% to 17.2%) in dynamics 3 months after hospitalization, two groups of patients were identified: the 1st group (n=24) included the patients who had a decrease in the HbA1c level by 50% or more, the 2nd group (n=30) – the patients whose HbA1c level decreased by less than 50%.

The patient groups were comparable in terms of gender, age, and the baseline clinical status ($p>0.05$). The general clinical characteristics of patients in the 1st and 2nd groups are presented in Tables 1 and 2.

The accumulation, correction, systematization of the initial information and visualization of the results were carried out in Microsoft Office Excel 2019 spreadsheets. The statistical analysis was performed using the IBM SPSS Statistics v. 26 program (IBM Corporation). The study materials were subjected to the statistical analysis using parametric and nonparametric analyses: Shapiro-Wilk test, Student t-test, Wilcoxon's rank sum test, F-test, Cramer's V, Spearman's rank correlation (the coefficient of the correlation was interpreted in accordance with the Cheddock scale), F-ratio test, Scheffe's test. The differences were considered statistically significant at $p<0.05$.

RESULTS AND DISCUSSION

First of all, the status of patients with the primary disease – DM2 in the stage of decompensation – at the

[3] The Federal Law "On the Fundamentals of Health Protection of Citizens in the Russian Federation" dated 21.11.2011 N 323-FZ. Russian

end of 3 months of hypoglycemic treatment was evaluated. It was discovered that the target level of venous blood plasma glucose and HbA1c had not been achieved in any of the patient groups. When comparing the average values of the HbA1c level using the Student t-test in the 1st and 2nd groups, statistically significant differences were found ($p<0.001$): the level of HbA1c (%) in the 1st group was 10.4% and in the 2nd group – 13.2%. By comparison of glucose values in the groups, statistically insignificant data were obtained ($p=0.264$): the level of venous blood plasma glucose was 8.5 mmol/L in the 1st group and 9.2 mmol/L in the 2nd group, respectively.

Subsequently, the details of the pharmacotherapeutic schemes of hypoglycemic therapy were analyzed. Table 3 shows the registered regimens and the frequency of their administration to the patients in 1st and 2nd groups.

Thus, 18 hypoglycemic therapy's regimens used were found. In both groups, the hypoglycemic drugs incompatible with each other were not prescribed. The recommendations regarding the prescription of a biguanide group representative – metformin, as the initiation of therapy in patients with DM2 and its use as the basis for further therapy in most patients which corresponds to both Russian and international recommendations, were followed [21, 22]. However, a detailed evaluation of these regimens (Table 4) revealed violations of current clinical guidelines in a number of cases [21].

Table 1 – General clinical characteristics (quantitative indicators) of patients in 1st and 2nd groups

Indicator	1st group (n=24)				2nd group (n=30)				p
	Men (n=10)		Women (n=14)		Men (n=14)		Women (n=16)		
	Me	Q1–Q3	Me	Q1–Q3	Me	Q1–Q3	Me	Q1–Q3	
Age	61.0	60.5–62.5	62	58.0–66.0	62.0	61.0–68.0	68.0	67.0–68.5	0.15
Glucose level in blood plasma before	12.8	10.9–14.0	12.5	12.0–13.6	16.0	14.0–16.0	15.0	14.0–16.6	0.10
Initial HbA1c level	17.0	16.2–17.1	13.7	13.6–15.3	16.6	13.2–16.7	14.4	13.9–14.8	0.40
Target HbA1c level (%)	7.0	6.8–7.3	7.0	6.8–7.5	7.0	7.0–7.5	7.0	6.8–7.5	0.80
BMI	M±SD	95% CI	M±SD	95% CI	M±SD	95% CI	M±SD	95% CI	p
	32.0±2.4	30.4–30.7	34.3±0.8	31.9–36.7	38.7±4.4	35.3–42.1	34.9±0.7	33.9–39.9	0.61

Note: 1st group (n=24) – patients with a decrease in HbA1c level by 50% or more; 2nd group (n=30) – patients with a decrease in HbA1c level by less than 50%; HbA1c – glycated hemoglobin; BMI – body mass index; Me – median; Q1–Q3 – interquartile range; SD – standard deviation; p – level of statistical significance (Shapiro-Wilk test).

Table 2 – General clinical characteristics (qualitative indicators) of patients in 1st and 2nd groups

Indicator	Patient groups								p*	V**	OR; 95% CI
	1st group(n=24)				2nd group (n=30)						
	n	%	n	%	n	%	n	%			
Gender	Male		Female		Male		Female		0.417	0.125	0.60; 0.20–1.8
	9	37.5	15	62.5	15	50	15	50			
Social status	Employed		Unemployed		Employed		Unemployed		0.692	0.178	–
	0	0	24	100	2	6.7	28	93.3			
Obesity	Present		Absent		Present		Absent		0.097	0.309	2.4; 1.6–3.4
	20	83.3	4	16.7	15	50	15	50			
Arterial hypertension	Present		Absent		Present		Absent		–	–	–
	24	100	0	0	30	100	0	0			
Coronary heart disease	Present		Absent		Present		Present		1.00	<0.001	1.0; 0.33–3.0
	12	50	12	50	15	50	15	50			
Hereditary predisposition to DM	Present		Absent		Present		Absent		0.558	0.098	0.67; 0.21–2.2
	18	75	6	25	21	70	9	30			
School of patients with DM	Attended		Did not attend		Attended		Did not attend		0.637	0.158	0.45; 0.07–2.7
	18	75	6	25	26	86.7	4	13.3			

Note: 1st group (n=24) – patients with a decrease in HbA1c level by 50% or more; 2nd group (n=30) – patients with a decrease in HbA1c level by less than 50%; n – absolute value; p-value – the level of static significance (statistically significant at $p<0.05$*; F-test); **V – Cramer's V-test; OR – odds ratio; 95% CI – 95% confidence interval (important when going beyond the border 1)

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Table 3 – Hypoglycemic therapy's regimens used in 1st and 2nd groups

	Hypoglycemic therapy's regimen	Number of patients receiving/not receiving treatment according to this regimen								p*	V**
		1st group (n=24)				2nd group (n=30)					
		Using		Not using		Using		Not using			
		n	%	n	%	n	%	n	%		
1	Insulin aspart – biphasic	0	0	24	100	3	10	27	90		
2	Insulin detemir + Insulin lispro	3	12.5	21	87.5	0	0	30	100		
3	Insulin-isophan [human biosynthetic] + Insulin soluble [human biosynthetic]	3	12.5	21	87.5	0	0	30	100		
4	Insulin detemir + Metformin	0	0	24	100	2	6.7	28	93.3		
5	Metformin + Glibenclamide	2	8.4	22	91.6	1	3.4	29	96.6		
6	Metformin + Gliclazide	0	0	24	100	7	23.4	23	76.6		
7	(Dapagliflozin + Metformin) + Glibenclamide	3	12.5	21	87.5	0	0	30	100		
8	(Dapagliflozin + Metformin) + Gosogliptine + Glibenclamide	1	4.2	23	95.8	0	0	30	100		
9	Insulin detemir + Insulin aspart + Metformin	1	4.2	23	95.8	3	10	27	90		
10	Insulin glargine + Insulin aspart + Metformin	1	4.2	23	95.8	0	0	30	100		
11	Insulin detemir + Metformin + (Antibodies to the C-terminal fragment of the β-subunit of the insulin receptor + antibodies to endothelial NO-synthase) affinity purified	0	0	24	100	3	10	27	90	<0.001	0.884
12	Insulin glargine + Metformin + Gosogliptine	3	12.5	21	87.5	1	3.4	29	96.6		
13	Insulin-isophan [human biosynthetic]+ Metformin + Glibenclamide	0	0	24	100	2	6.7	28	93.3		
14	Insulin-isophan [human biosynthetic] + Metformin + (Antibodies to the C-terminal fragment of the β-subunit of the insulin receptor + antibodies to endothelial NO-synthase) affinity purified	3	12.5	21	87.5	0	0	30	100		
15	Metformin + Glibenclamide + Alogliptin	3	12.5	21	87.5	0	0	30	100		
16	Insulin glargine + (Dapagliflozin + Metformin) + Metformin	1	4.2	23	95.8	3	10	27	90		
17	Insulin detemir + Metformin + Glibenclamide	0	0	24	100	3	10	27	90		
18	Metformin + Gosogliptine	0	0	24	100	2	6.7	28	93.3		

Note: 1st group (n=24) – patients with a decrease in HbA1c level by 50% or more; 2nd group (n=30) – patients with a decrease in HbA1c level by less than 50%; n – absolute value; p-value – the level of static significance (statistically significant at p<0.05*; Fischer's criterion); **V – Cramer's V-test

Table 4 – Groups of hypoglycemic drugs used in 1st and 2nd groups

Hypoglycemic drugs's groups	Number of patients with/no drugs as a component of therapy								p*	V**	OR; 95% CI
	1st group (n=24)				2nd group (n=30)						
	Presence		Absence		Presence		Absence				
	n	%	n	%	n	%	n	%			
Biguanides	14	58.4	10	66.7	26	86.7	4	13.3	0.028	0.321	0.215; 0.06–0.82
Insulin's drugs	19	79.2	5	20.8	19	63.4	11	36.6	0.243	0.172	2.2; 0.64–7.6
Sulfonylureas's drugs	15	62.5	9	37.5	17	56.7	13	43.3	0.783	0.059	0.78; 0.26–2.35
GLPra-1	3	12.5	21	87.5	1	3.3	29	96.7	0.312	0.174	4.14 0.4–42.66
iSGLT-2	0	0	24	100	3	10	27	90	0.245	0.217	0.59 0.41–0.69
iDPP-4	4	16.7	20	83.3	2	6.7	28	93.3	0.389	0.158	2.8; 0.47–16.8

Note: 1st group (n=24) – patients with a decrease in HbA1c level by 50% or more; 2nd group (n=30) – patients with a decrease in HbA1c level by less than 50%; n – absolute value; p-value – level of static significance (statistically significant at p<0.05*; Fischer's test); OR – odds ratio; 95% CI – 95% confidence interval (important when going beyond border 1); **V – Cramer's V-test; GLPra-1 – glucagon-like peptide receptor agonists 1; iSGLT-2 – sodium-glucose cotransporter inhibitor 2; iDPP-4 – inhibitors of dipeptidyl peptidase 4

Table 5 – Antihypertensive therapy's regimens used in 1st and 2nd groups

	Antihypertensive therapy's regimen	Number of patients receiving/not receiving treatment according to this regimen								p*	V**
		1st group (n=24)				2nd group (n=30)					
		Using		Not using		Using		Not using			
		n	%	n	%	n	%	n	%		
1	Bisoprolol + Indapamide + Perindopril	0	0	24	100	9	30	21	70	<0.001	0.942
2	Bisoprolol + Indapamide + Losartan	0	0	24	100	3	10	27	90		
3	Bisoprolol + Amlodipin + Perindopril	0	0	24	100	3	10	27	90		
4	Bisoprolol + Indapamide	0	0	24	100	3	10	27	90		
5	Perindopril + Indapamide	3	12.5	21	87.5	3	10	27	90		
6	Indapamide + Losartan	0	0	24	100	3	10	27	90		
7	Bisoprolol	0	0	24	100	3	10	27	90		
8	Bisoprolol + Indapamide + Amlodipine + Candesartan	0	0	24	100	3	10	27	90		
9	Bisoprolol + Moxonidine + Nifedipin	3	12.5	21	87.5	0	0	30	100		
10	Indapamide	3	12.5	21	87.5	0	0	30	100		
11	Bisoprolol + Moxonidine + Losartan + Spironolactone	3	12.5	21	87.5	0	0	30	100		
12	Metoprolol + Indapamide + Candesartan	3	12.5	21	87.5	0	0	30	100		
13	Amlodipine + Losartan	3	12.5	21	87.5	0	0	30	100		
14	Indapamide + Perindopril + Moxonidine + Bisoprolol	3	12.5	21	87.5	0	0	30	100		
15	Indapamide + Lisinopril + Amlodipine + Bisoprolol	3	12.5	21	87.5	0	0	30	100		

Note: 1st group (n=24) – patients with a decrease in HbA1c level by 50% or more; 2nd group (n=30) – patients with a decrease in HbA1c level by less than 50%; n – absolute value; p-value – level of static significance (statistically significant at p<0.05*; Fischer's criterion); **V – Cramer's V-test

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Table 6 – Groups of antihypertensive drugs used in 1st and 2nd groups

Antihypertensive drugs groups	Number of patients with drugs as a component of therapy								p*	V**	OR; 95% CI
	1st group (n=24)				2nd group (n=30)						
	Presence		Absence		Presence		Absence				
	n	%	n	%	n	%	n	%			
BABs	15	62.5	9	37.5	24	80	6	20	0.223	0.194	0.417; 0.12–1,4
ACEi	15	62.5	9	37.5	15	50	15	50	0.417	0.125	0.60; 0.2–1.8
Diuretics	18	75	6	35	24	80	6	20	0.748	0.06	0.75; 0.2–2.7
Sartans	9	37.5	15	62.5	9	30	21	70	0.577	0.079	1.4; 0.45–4.4
Statins	12	50	12	50	18	60	12	40	0.584	0.100	0.67; 0.23–1.9
MRAs	9	37.5	15	62.5	0	0	30	100	<0.001	0.50	0.34; 0.22–0.51
CCBs	9	37.5	15	62.5	6	20	24	80	0.223	0.194	2.4; 0.71–8.1

Note: 1st group (n=24) – patients with a decrease in HbA1c level by 50% or more; 2nd group (n=30) – patients with a decrease in HbA1c level by less than 50%; n – absolute value; p-value – level of static significance (statistically significant at p<0.05*; Fisher's test); OR – odds ratio; 95% CI – 95% confidence interval (important when going beyond the limit of 1); **V – Cramer's V-test; BABs – beta-adrenoblockers; ACEi – angiotensin converting enzyme inhibitors; MRAs – mineralocorticoid receptor antagonists; CCBs – calcium channel blockers

In the 1st group, 62.5% (n=15) of patients with a history of cardiovascular diseases of the atherosclerotic origin were prescribed drugs from the group of sulfonylurea derivatives. At the same time, there is some evidence that older representatives of sulfonylurea derivatives – glibenclamide, gliclazide, tolbutamide – violate the ischemic preconditioning, i.e., the process of adaptation of the myocardium to ischemia after a number of repeated episodes of transient ischemia of moderate severity. This may cause an increased risk of myocardial infarction and a worse prognosis after a myocardial infarction [19]. The administration of insulin's drugs to obese patients, which aggravates the course of this disease because insulin increases the expression of the Glut4 transporter and the activity of acetyl-CoA-carboxylase in adipocytes, as well as fatty acid synthase and lipoprotein lipase, which leads to rapid clearance from the circulation and deposition of glucose and lipids [19], also raises questions: out of 20 obese people, they were prescribed to 17 patients (85%). The fact that in a number of clinical trials in European countries (Germany, France, Spain) patients with an HbA1c level of more than 7% could not reach the target level of venous blood plasma glucose and HbA1c during a course of basal insulin therapy, was considered [21]. In addition, none of the 1st group patients received a sodium-glucose cotransporter inhibitor type 2 (iSGLT-2) and in a lower ratio compared to other drugs from the groups of glucagon-like peptide-1 receptor agonists (GLPra-1) (12.5% (n=3)) and dipeptidyl peptidase-4 inhibitors (iDPP-4) (16.7% (n=4)). That has proven benefits in patients with DM2 with associated cardiovascular diseases in terms of reducing cardiovascular and renal risks [19, 22]. In the process of a meta-analysis, it was found that, compared with the control group, the incidence of adverse cardiovascular events in the iSGLT-2 group (OR=0.86, 95% CI 0.80-0.93, p <0.0001), such as myocardial infarction (OR=0.86, 95% CI 0.79–0.94, p=0.001), as well as mortality from this pathology (OR=0.74, 95% CI 0.67-0.81, p<0.0001) was statistically lower [23]. As far as iDPP-4 group is concerned, in one of clinical stadies, the role of this group in the prevention of cardiovascular diseases was not so pronounced in comparison to iSGLT-2 [24].

In 2nd group, the number of patients receiving iSGLT-2, GLPra-1, and iDPP-4 was also insignificant: 10% (n=3), 3.3% (n=1), and 6.7% (n=2), respectively. The prescribed therapeutic regimen for the patients with concomitant risk-associated pathology also raises questions: 56.7% (n=17) of the patients with atherosclerotic cardiovascular pathology were prescribed sulfonylurea derivatives; 66.7% (n=10) of obese patients were prescribed insulin preparations. Thus, according to the results of the meta-analysis conducted in 2016 [25], it was found that metformin monotherapy was accompanied by a lower (≥2 years) mortality from complications of cardiovascular diseases compared with sulfonylurea monotherapy. The frequency of deaths from myocardial infarction was lower in the group where metformin alone was used (2 of 1454 participants (0.1%); the median follow-up was 4 years) than in the glibutide group (3 of 1441 participants (0.2%); the median follow-up was 3.3 years).

When assessing the contribution of a particular drugs group of achieving the target HbA1c level using the exact Fisher test and the Cramer's V, a statistically

significant level (p=0.028) with a relatively strong binding force was obtained for a representative of the biguanide group – metformin. In order to determine the role of this drug, a single-factor analysis (ANOVA) was performed, during which a statistically significant effect of metformin's usage (p=0.018) on the outcome of treatment in both groups was established. The contribution to the dispersion of metformin as a component of therapy was 10.3%.

When comparing the levels of venous blood plasma glucose and HbA1c with the number of prescribed hypoglycemic drugs, a statistically significant direct correlation of weak crowding was established and no correlation was found, respectively, on the Cheddock scale. Thus, the expediency of appointing more than 2 representatives of hypoglycemic drugs was absent.

The comorbidity of the patients presented in this study, also required an assessment of polypragmasia, which causes significant harm to human healths, leads to economic losses, and negatively affects the reputation of the doctor. In addition, a large number of prescribed drugs negatively affect the patient's compliance. The problem of polypragmasia is largely due to the lack of awareness of doctors about the drugs taken by the patient, which are prescribed by other specialists.

Arterial hypertension was considered as comorbid pathology present in 100% of patients in the 1st and 2nd groups. The particular pharmacotherapy regimens used and the groups of antihypertensive agents prescribed to patients, are shown in Tables 5 and 6, respectively.

When analyzing the pharmacotherapy of arterial hypertension, the following data were obtained. The patients from the 1st group received a selective beta-adrenoblocker (BAB) – bisoprolol in 62.5% (n=15) of cases. According to the literature data [26, 27] the usage of highly selective beta-blockers does not significantly change the metabolism of lipids (total cholesterol, HDL, LDL, triglycerides) in comparison with non-selective (BABs), which violate carbohydrate tolerance, increase insulin resistance, and have a hyperlipidemic effect. In 37.5% (n=9) of cases, 4 drugs were prescribed as a treatment for a high blood pressure and its complications. In 50% of cases (n=15), the 2nd group patients were prescribed 3 drugs for the treatment of a high blood pressure. In 30% (n=9) of cases, a two-component scheme was prescribed (BABs were not included in these schemes).

When comparing the levels of venous blood plasma glucose and HbA1c with the number of prescribed antihypertensive drugs, a negative correlation of weak crowding was established and no correlation was found, respectively, on the Cheddock scale.

When comparing the levels of venous blood plasma glucose and HbA1c with the number of prescribed hypoglycemic and hypotensive drugs, there was no correlation revealed and a negative correlation of weak crowding was established, respectively, according to the Cheddock scale.

The total number of drugs prescribed to patients of the 1st group (hypoglycemic drugs + antihypertensive drugs + statins) was: 4 drugs in 25% (n=6) of cases; 5 drugs 12.5% (n=3); 6 drugs 12.5% (n=3); 7 drugs 37.5% (n=9); 8 drugs 12.5% (n=3). The total number of the drugs prescribed to patients of the 2nd group (hypoglycemic drugs + antihypertensive drugs + statins) was: 4 drugs 10% (n=3); 5 drugs 30% (n=9); 6 drugs 20% (n=6); 7 drugs 20% (n=6); 8 drugs 20% (n=6). Thus, the phenomenon of polypragmasia was observed in the absolute majority of cases. At the same time, it should be noted once again that the target level of venous blood plasma glucose and HbA1c were are not achieved in any of the patient groups, so the existing polypragmasia was not justified from the point of view of the effectiveness of the pharmacotherapy.

However, it should be noted that this study, due to its retrospective nature, had some limitations, which must be taken into account when interpreting the results obtained.

CONCLUSION

According to the results obtained in the course of this retrospective analysis, we concluded that the tactics of pharmacotherapy in the patients with a type DM2 decompensated form, often does not fully comply with the approved clinical recommendations. In particular, patients are prescribed potentially non-recommended medications that significantly reduce the QoL and increase the risk of developing undesirable adverse reactions, and/or, conversely, the treatment regimen does not use potentially recommended medications necessary to improve the prognosis, reduce the risk of complications, and reduce the number of hospitalizations.

To solve the current situation, it can be necessary to consider the following theses.

1. For the treatment of DM2, the prescribed pharmacotherapy should be based on the current clinical recommendations.

2. To improve the prognosis of the DM2 course, to improve patient QoL, is possible only with a comprehensive approach, including, first, the prescription of adequate pathogenetic and personalized therapy, especially in the case of comorbid risk-associated pathologies's presence.

3. Each case of polypragmasia should be justified in the aspect of the "effectiveness-safety" ratio, and the choice of specific drugs for a joint use is based on considering the issues of their interaction from the point of view of fundamental pharmacology.

4. When following-up patients with DM2, it is extremely important to have a high professional level and a close cooperation of specialists of various profiles: endocrinologists, cardiologists, neurologists, nephrologists, ophthalmologists, and clinical pharmacologists.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHOR'S CONTRIBUTION

Andrey V. Safronenko, Elena V. Gantsgorn – working out the concept and study design, results interpretation and the final article edition; Ekaterina A. Sanina, Marina A. Khachumova – collection and primary processing of clinical materials, a draft article preparation; Stanislav O. Panenko – participation in the interpretation of the results obtained, translation of the article into English; Igor I. Kuznetsov – statistical processing of primary data, results interpretation; Anastasia A. Kivva, Viktoria I. Polyakova – participation in the literary references search and a draft article preparation.

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COST-MINIMIZATION ANALYSIS OF USING SHORT AND LONG-ACTING ERYTHROPOESIS-STIMULATING AGENTS FOR CORRECTION OF NEPHROGENIC ANEMIA AGAINST THE BACKGROUND OF SUBSTITUTION THERAPY

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Clinical trials conducted in various countries indicate that the use of epoetin alfa in patients with nephrogenic anemia in chronic kidney disease can increase the effectiveness of treatment, reduce the incidence of cardiovascular and infectious complications, and reduce mortality in patients with chronic kidney disease.

The aim of the article was to conduct a comparative clinical and economic assessment of the treatment costs of nephrogenic anemia in adult dialysis patients with recombinant human erythropoietins: epoetin alfa, darbepoetin and long-acting methoxy polyethylene glycol – epoetin beta.

Materials and methods. The study took into account direct medical costs of nephrogenic anemia pharmacotherapy on the basis of 1 year maintenance therapy according to the following scheme: epoetin alfa – 3 times per week, darbepoetin alfa – once per week, methoxy polyethylene glycol – epoetin beta – once per 2 or 4 weeks. A "costs minimization" analysis was performed for equivalent maintenance epoetins doses for intravenous and subcutaneous administrations. Epoetin alpha equivalents were calculated for an average patient weighing 75 kg by converting a weekly dose of short-acting epoetin (7500 IU) into equivalent doses using dose conversion factors.

Results. In the hypothetical cohort of patients under study, epoetin alfa, darbepoetin alfa, and methoxy polyethylene glycol – epoetin beta not differ in effectiveness in achieving target Hb values and in safety. With the equal effectiveness of the investigated drugs, in the studied patients, intravenous epoetin alfa can be less expensive drug therapy relative to the equivalent doses obtained by the calculation: darbepoetin by 14–24% and methoxy polyethylene glycol – epoetin beta by 4–30%. The change-over of patients to the subcutaneous administration makes it possible to decline a weekly dose of epoetin alfa by 20–30% by reducing the frequency of taking the drug to twice a week, and to reduce the cost of drug therapy by a third.

Conclusion. Intravenous and subcutaneous administrations of epoetin alfa 2500 IU may be a more economical drug therapy in comparison with the equivalent doses of darbepoetin and methoxy polyethylene glycol – epoetin beta.

Keywords: chronic kidney disease; anemia; erythropoiesis stimulating agent(s); epoetin alfa; economic assessment

Abbreviations: i.v. – intravenous; DA – darbepoetin; VEDs – vital and essential drugs; RRT – renal replacement therapy; CTs – clinical trials; MP – medicinal preparation; DF – dosage form; INN – international non-proprietary name; VAT – value added tax; s/c – subcutaneous; RCT – randomized controlled trial; r-HuEPO – recombinant human erythropoietin; ESA – erythropoiesis stimulating agent; CKD – chronic kidney disease; CKF – chronic kidney failure; EPO – epoetin.

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АНАЛИЗ МИНИМИЗАЦИИ ЗАТРАТ ПРИМЕНЕНИЯ СТИМУЛЯТОРОВ ЭРИТРОПОЭЗА КОРОТКОГО И ДЛИТЕЛЬНОГО ДЕЙСТВИЯ ДЛЯ КОРРЕКЦИИ НЕФРОГЕННОЙ АНЕМИИ НА ФОНЕ ЗАМЕСТИТЕЛЬНОЙ ТЕРАПИИ

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Клинические исследования, проведенные в различных странах, свидетельствуют о том, что применение эпоэтина альфа у пациентов с нефрогенной анемией при хронической болезни почек может повысить эффективность лечения, снизить число случаев сердечно-сосудистых и инфекционных осложнений, а также смертность у пациентов с хронической болезнью почек.

Цель. Провести сравнительную клинико-экономическую оценку затрат на терапию нефрогенной анемии у взрослых пациентов, находящихся на диализе, рекомбинантными человеческими эритропоэтинами: эпоэтином альфа, дарбэпоэтином и эпоэтином бета (метоксиполиэтиленгликоль) длительного действия.

Методы. В исследовании были учтены прямые медицинские затраты на фармакотерапию нефрогенной анемии из расчета 1 года поддерживающей терапии по схеме: эпоэтин альфа – 3 раза в неделю, дарбэпоэтин альфа – 1 раз в неделю, эпоэтин бета (метоксиполиэтиленгликоль) – 1 раз в 2 недели или 4 недели. Проведен анализ «минимизация затрат» для эквивалентных поддерживающих доз эпоэтинов для внутривенного и подкожного применения. Эквивалентные эпоэтину альфа дозы были рассчитаны для среднестатистического пациента с массой тела 75 кг, путем пересчета недельной дозы эпоэтинов короткого действия (7500 МЕ) в эквивалентные через коэффициенты конвертации доз.

Результаты. У исследуемой гипотетической когорты больных эпоэтин альфа, дарбэпоэтин альфа и эпоэтин бета (метоксиполиэтиленгликоль) не отличаются по эффективности в достижении целевых значений Hb и безопасности. При равной эффективности исследуемых препаратов, эпоэтин альфа внутривенно может являться у исследуемых больных менее затратной лекарственной терапией относительно эквивалентных доз, полученных расчетным путем: дарбэпоэтина на 14–24% и эпоэтина бета (метоксиполиэтиленгликоль) – на 4–30%. Перевод больных на подкожное введение позволяет снизить недельную дозу эпоэтина альфа на 20–30% за счет снижения частоты приема препарата до 2 раз в неделю, и сократить стоимость лекарственной терапии на треть.

Заключение. Эпоэтин альфа 2500 МЕ при внутривенном и подкожном введении может являться более экономичной лекарственной терапией в сравнении с эквивалентными дозами дарбэпоэтина и эпоэтина бета (метоксиполиэтиленгликоль).

Ключевые слова: хроническая болезнь почек; анемия; средства, стимулирующие эритропоэз; эпоэтин альфа; экономическая оценка

Список сокращений: в/в – внутривенный; ДА – дарбэпоэтин; ЖНВЛП – жизненно необходимые и важнейшие лекарственные препараты; ЗПТ – заместительная почечная терапия; КИ – клинические исследования; ЛП – лекарственный препарат; ЛФ – лекарственная форма; МНН – международное непатентованное наименование; НДС – налог на добавленную стоимость; п/к – подкожный; РКИ – рандомизированное контролируемое исследование; рчЭПО – рекомбинантный человеческий эритропоэтин; ССЭ – средства стимуляторы эритропоэза; ХБП – хроническая болезнь почек; ХПН – хроническая почечная недостаточность; ЭПО – эпоэтин

INTRODUCTION

Currently, there is an acute issue of the high prevalence of chronic kidney disease in the general population. It has been notified that on average, it can reach 13.4%, and in terms of coverage, it is comparable to arterial hypertension and diabetes mellitus. Chronic kidney disease (CKD) is often associated with nephrogenic anemia, which complicates the course of CKD and tends to worsen due to the CKD progresses. In modern clinical practice, anemia of the renal origin is a frequent complication and is observed with a decrease in creatinine clearance to 40–60 ml/min. It is widespread in all types of renal replacement therapy, but is especially pronounced in dialysis pa-

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tients (in the absence of treatment, the hemoglobin level below 10 g/dl can be observed in 90% of dialysis patients)[1,2]. Anemia is associated with a deterioration in patients' quality of life (QoL), an increase in cardiovascular complications and the frequency of CKD patients' hospitalizations[3]. In the pathogenesis of nephrogenic anemia, a key factor is a deficiency in the production of endogenous erythropoietin in the kidneys. In clinical practice, for the treatment of nephrogenic anemia, preparations of recombinant human erythropoietin (r-HuEPO) are widely used. Timely correction of anemia to the recommended targets can improve the effectiveness of treatment in general and reduce the incidence of cardiovascular and infectious complications in CKD[4] patients.

According to the national Russian Recommendations, the diagnosis of nephrogenic anemia is detected in CKD patients when the hemoglobin concentration drops below the average level by 2 standard deviations, taking into account the age and gender. Anemia in patients on renal replacement therapy (RRT) is considered as a decrease in hemoglobin lower than 11.5 g/dL in women and 13.5 g/dL in men, as well as lower than 12.0 g/dL in people over 70 years[5]. Currently, according to the majority of recommendations, the aim of treatment is to achieve a hemoglobin level of 10–12 g/dL both at the pre-dialysis stages of CKD, in dialysis patients and after kidney transplantation. International KDIGO 2012[6] guidelines and Russian Clinical Guidelines for CKD[7] prescribe starting treatment with r-HuEPO only when the hemoglobin level drops to 9–10 g/dL, while the upper limit for most patients is determined at 11.5 g/dL. Achieving higher Hb targets is not associated with significantly greater clinical effectiveness in terms of the risks of death or cardiovascular complications; on the contrary, it may be associated with a greater risk of adverse outcomes (increased blood pressure, vascular access thrombosis). Thus, the use of the lowest possible doses of erythropoiesis stimulating agents (ESAs) for the treatment of nephrogenic anemia in CKD patients makes it possible to avoid the risks of unfavorable vascular outcomes, as well as improve their quality of life (QoL) [1], eliminate anemic syndrome and reduce the frequency of blood transfusions in patients on hemodialysis[8].

It is generally accepted that the incidence of side effects such as seizures, headaches, increased need for heparin during hemodialysis, impaired dialyzer clearance, and hyperkalemia does not increase significantly with the use of ESA. One of the frequent and serious undesirable effects of r-HuEPO therapy is the occurrence or progression of arterial hypertension – up to 30% of patients may need to increase the doses of antihypertensive drugs. When treating patients with CKD complicated by diabetes mellitus, malignant neoplasms, strokes, a non-ischemic heart disease or a peripheral vascular disease, it is recommended to prescribe ESA with caution, the target hemoglobin range can be reduced to 10–11 g/dL.

In Russian databases, there is a number of published works on the pharmacoeconomic analysis of the use of short, intermediate and long-acting epoetins. The authors were most interested in the treatment of nephrogenic anemia in CKD patients undergoing substitution therapy in the system of domestic health care. Publication by R.I. Yagudina et al., 2009 [2], is devoted to assessing the effectiveness of EPO alpha (Eprex), EPO beta (Recormon) and DA (Aranesp) in dialysis and pre-dialysis patients. In the publication by M.V. Avksentieva, a comparative analysis of the costs of therapy with DA and EPO alfa (the original drug Eprex) was carried out [3]. The work by Krysanov I.S. et al., 2016 [4] is devoted to a comparative assessment of the use of EPO alfa (Eprex, Eralfon), DA (Aranesp) and long-acting EPO (Mircera) in dialysis patients in real clinical practice. These studies show high costs of managing such patients, but they were carried out in the period of 2009–2016, and not all studies reflect the investigated medicinal products, which determines the relevance of this study.

THE AIM of this study is to conduct a comparative cost-effectiveness analysis of short-, intermediate- and long-acting r-HuEPOs prescribed for the correction of nephrogenic anemia in adult patients with an end-stage chronic kidney disease requiring dialysis therapy.

MATERIALS AND METHODS

The objects of the study were:

1. Short-acting r-HuEPO – epoetin alfa (EPO alfa). Trade name: Eralfon®, release form: 2500 μg syringe, the holder of the Marketing authorization (LSR-006663/08) – CJSC PharmFirma Sotex, Russia;

2. Intermediate-acting rhEPO – darbepoetin alfa (DA). Trade name: Aranesp®, release forms: 20 μg, 30 μg syringe, the holder of the Marketing authorization (LSR-001710/07) – Amgen, the Netherlands. Trade name: Darbestim®, release forms: 20 μg, 30 μg, 40 μg syringe, the holder of the Marketing authorization (LP-005411) – CJSC "Biocad", Russia;

3. Long-acting r-HuEPO – methoxy polyethylene glycol – epoetin beta. Trade name: Mircera®, release forms: 50 μg, 75 μg, 100 μg syringe, the holder of the Marketing authorization (LSR-002182/08) – F. Hoffmann-La Roche Ltd., Switzerland.

The primary source of the data were clinical guidelines KDIGO 2012, updated Russian National Guidelines

[1] Clinical guidelines. Chronic kidney disease. 2019. 169 p. Russian
[2] Updated Russian national guidelines for the diagnosis and treatment of anemia in chronic kidney disease in the 2014 ed. Moscow, 2014. 34 p. Russian
[3] Clinical practical recommendations KDIGO on anemia in chronic kidney disease 2012. Nephrology and dialysis. 2013; 15(1): 14-53. Russian
[4] Updated Russian national guidelines for the diagnosis and treatment of anemia in chronic kidney disease in the 2014 edition.
[5] Updated Russian national guidelines for the diagnosis and treatment of anemia in chronic kidney disease in the 2014 ed.
[6] Clinical practical recommendations KDIGO on anemia in chronic kidney disease, 2012.
[7] Clinical guidelines. Chronic kidney disease. 2019.
[8] Ibid.

for the diagnosis and treatment of anemia in chronic kidney disease, Federal Clinical Guidelines for the diagnosis and treatment of anemia in chronic kidney disease, National Guidelines for CKD, as well as a retrospective, multicenter observational study (Choi P, 2013) [5].

The search and selection of literature data on the effectiveness and safety of r-HuEPO for the nephrogenic anemia treatment in adult dialysis patients was carried out in the available medical databases: PubMed database, Cochrane Library. The keywords were: epoetin [All Fields] AND CKD [All Fields] AND ("renal dialysis" [MeSH Terms] OR ("renal" [All Fields] AND "dialysis" [All Fields]) OR "renal dialysis" [All Fields] OR "dialysis" [All Fields] OR "dialysis" [MeSH Terms]). The search depth was 15 years – January 2006 – July 2020, the studies carried out in 2005 and earlier, were excluded from the further analysis.

In the selection of works, the preference was given to meta-analyzes, randomized controlled trials (RCTs), systematic reviews, then to clinical trials (CTs) without randomization. The following studies were excluded from the analysis: animal studies; the studies devoted to the research of the dosage form of the drug; CI 1–2 phases; research conducted in pediatric and/or geriatric practice; CTs in the cohort of pre-dialysis patients, CTs in the cohort of patients with concomitant diseases: cancer, diabetes, HIV infection, etc.

In the PubMed database, 140 publications were found, in the Cochrane Library database they were 4. For the pharmacoeconomic analysis, taking into account the inclusion and exclusion criteria, 12 publications were selected (Fig. 1). Six studies (4 meta-analyzes, 1 systematic review, 1 RCT) were used to assess the effectiveness and safety of therapy: the data from the World Health Organization report[9] and 5 peer-reviewed publications [6-10] the data from the pharmacoeconomic study [11] were adapted to the conditions of the domestic health care system and used in conducting a pharmacoeconomic study at the national level, 5 CTs [5, 12–15] served as a source of data for calculating equivalent doses of the studied drugs.

Russian and foreign Clinical guidelines recommend a 2-stage treatment of nephrogenic anemia. The first phase is the correction of anemia, the aim of which is to achieve a lower limit of the target hemoglobin level. In phase 1 of treatment, the starting r-HuEPO[10] doses are usually used. The second phase is maintenance therapy with the EPO doses, which are 20-30% lower than the starting ones[11]. Taking into account that the selection of doses and the duration of phase 1 are more dependent on the individual characteristics of a patient, the cost-effectiveness analysis was carried out for the maintenance phase of treatment at the rate of 1 year of continuous therapy. To determine the standard dosage regimens for EPO drugs at the stage of maintenance therapy (Table 1), the instructions for medications, international and National Clinical Guidelines[12] for the treatment of dialysis patients with anemia in the CKD[13], were analyzed.

[9] WHO EML 2016-2017 – Application for erythropoietin-stimulating agents (erythropoietin type blood factors). WHO EML 2016–2017 – Erythropoietin-stimulating agents December, 2016.
[10] Clinical guidelines. Chronic kidney disease. 2019.
[11] Updated Russian national guidelines for the diagnosis and treatment of anemia in chronic kidney disease in the 2014 ed.
[12] Clinical practical recommendations KDIGO on anemia in chronic kidney disease, 2012.
[13] Updated Russian national guidelines for the diagnosis and treatment of anemia in chronic kidney disease in the 2014 ed.

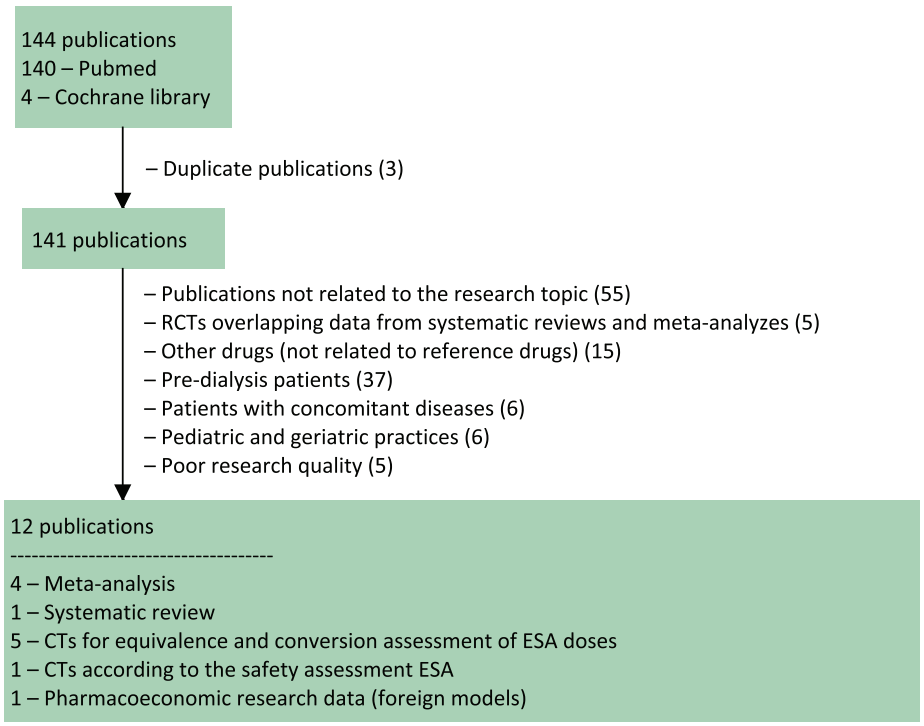

Figure 1 – Results of data selection for cost-effectiveness analysis

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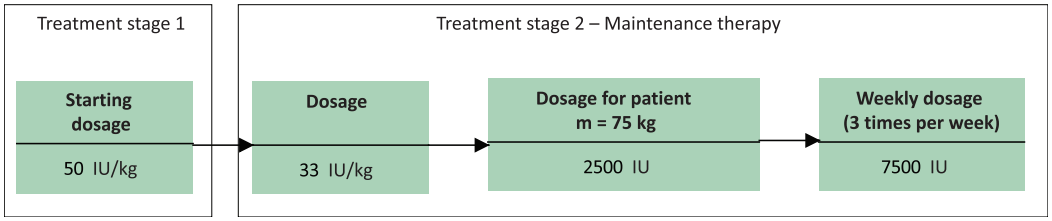

Figure 2 – Scheme of maintenance therapy with epoetin alfa

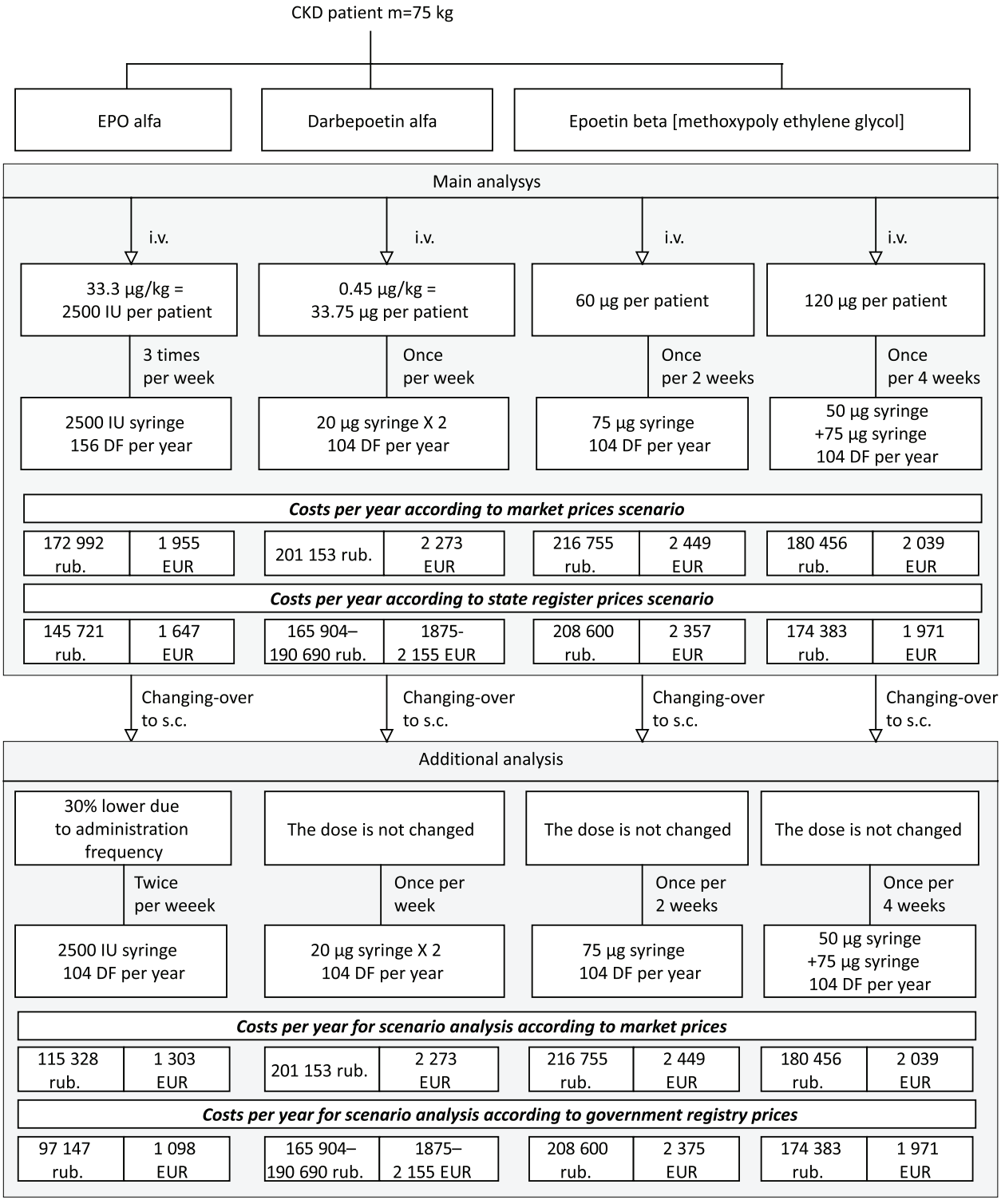

Figure 3 – Costs analysis for ESAs subcutaneous administration

Table 1 – Algorithms of maintenance drug therapy for nephrogenic anemia in target cohort of patients

International non-propriety name	Mode of administration	Maintenance therapy scheme
EPO alpha	i.v.	33.3 IU/kg – 3 times per week
	s.c.	reducing administration frequency to once-twice per week (weekly doses – 30% lower than in i.v. administration)
DA	i.v.	0.45 μg/kg – once per week
	s.c.	0.90 μg/kg – once per 2 weeks
Methoxy polyethylene glycol – epoetin beta	i.v. s.c.	When changing-over from other r-HuEPOs if the previous weekly dose was: <8000 IU per week EPO alpha <40 μg/week DA
		60 μg – once per 2 weeks
		120 μg – once per 4 weeks

Table 2 – Data of tender purchases of drugs – stimulants of erythropoiesis (01.01.2020-06.07.2020), top prices of the state drugs register

Trade name	INN	Dose	Qty. in pack	Price per unit, rub.	Price for 1 DF (syringe), rub.
Scenario 1 – Calculation at real pharmaceutical market prices					
Eralfon	EPO alpha	2500 IU	6	6 653.51	1 108.92
Aranesp	DA	20 μg	1	1 934.16	1 934.16
		30 μg	1	3 289.00	3 289.00
Mircera	EPO beta [methoxypoly ethylene glycol]	50 μg	1	5 544.48	5 544.48
		75 μg	1	8 336.74	8 336.74
		100 μg	1	10 222.74	10 222.74
Scenario 2 – Calculation at state register prices					
Eralfon	EPO alpha	2500ME	6	5 095.14	849.19 (934.11 with VAT)
Aranesp	DA	20 μg	1	1 666.87	1 666.87 (1 833.56 with VAT)
		30 μg	1	2 502.22	2 502.22 (2 752.44 with VAT)
Darbestim	DA	20 μg	1	1450.21	1450.21 (1 595.23 with VAT)
			4	5800.83	1450.21 (1 595.23 with VAT)
		30 μg	1	2176.51	2176.51 (2 394.16 with VAT)
			4	8706.02	2176.51 (2 394.16 with VAT)
		40 μg	Dosage (not included in VED list)		
Mircera	EPO beta [methoxypoly ethylene glycol]	50 μg	1	4 900.93	4 900.93 (5 391.02 with VAT)
		75 μg	1	7 293.72	7 293.72 (8 023.09 with VAT)
		100 μg	1	9 293.47	9 293.47 (10 222.82 with VAT)

Note: average price for a pack in pharmaceutical market – median for 1–2 qtrs., 2020; * 1 pack price is indicated in brackets at state register price including VAT

Table 3 – Equivalent doses of EPO

Initial data	Converting coefficient	Data source	Source characteristics	Equivalent dosage per week.	Equivalent DF (amp., syringe)
Clinical trials					
Short-acting EPO → Darbepoetin alfa					
<8000 IU per week	---	Sulowicz W et al, 2007 [15]	Controlled, open-label, randomized CT with parallel groups, phase 3, assessment of EPO effectiveness and safety in CKD patients, n = 572	<40 μg	20 μg * 2 DF per week 30 μg per week
7500 IU per week	200:1	Kuwahara M et al, 2015 [13]	Prospective CT, evaluation of EPO effectiveness in CKD patients over 144 weeks long, n = 297	37.5 μg per week	20 μg * 2 DF per week
7500 IU per week	206:1	Fuller DS et al, 2018 [17]	Data analysis of prospective cohort CT "DOPPS", phase 5 data for 9 countries*, 164 medical organizations, n = 3 281, CKD dialysis patients	36.4 μg per week	20 μg * 2 DF per week

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Initial data	Converting coefficient	Data source	Source characteristics	Equivalent dosage per week.	Equivalent DF (amp., syringe)
7500 IU per week	222:1	Raymond CB et al, 2008 [14]	Retrospective analysis of real practice (Canada) in Manitoba Renal Program framework, n = 295, peritoneal dialysis	33.8 μg per week	20 μg * 2 DF per week
7500 IU per week	244:1	Raymond CB et al, 2008 [14]	Retrospective analysis of real practice (Canada) in Manitoba Renal Program framework, n = 1086, hemodialysis	30.7 μg per week	30 μg per week *(assumption)*
Short-acting EPO → Methoxy polyethylene glycol – epoetin beta					
7500 IU per week	255:1	Fuller DS et al, 2018 [17]	Data analysis of prospective cohort CT "DOPPS", phase 5 data for 9 countries *, 164 medical organizations, n = 3 281, CKD dialysis patients	59 μg per 2 weeks 118 μg per 4 weeks	75 μg * 2 DF per month 75+50 μg per month
Darbepoetin alfa → Methoxy polyethylene glycol – epoetin beta					
24.1 μg per week	1:1.17	Choi P et al, 2013 [5]	Retrospective multicenter research, study and determination of equivalence coefficient of weighted average EPO doses in CKD dialysis patients, n = 302	28.6 μg per week 57.2 μg per 2 weeks 114.4 μg per 4 weeks	75 μg * 2 DF per month 75+50 μg per month
30.7 μg per week	1:0.93	Kuwahara M et al, 2015 [13]	Prospective CT, evaluation of EPO effectiveness in CKD patients over 144 weeks long, n = 297	28.6 μg per week 57.2 μg per 2 weeks 114.4 μg per 4 weeks	75 μg * 2 DF per month 75+50 μg per month
<40 μg	–	Sulowicz W et al, 2007 [15]	Controlled, open-label, randomized CT with parallel groups, phase 3, evaluation of EPO effectiveness and safety in CKD patients, n = 572	60 μg per 2 weeks 120 μg per month	75 μg * 2 DF per month 75+50 μg per month
Methoxy polyethylene glycol – epoetin beta → Darbepoetin alfa					
118 μg per 4 weeks	0.89: 1	Fuller DS et al, 2018 [17]	Data analysis of prospective cohort CT "DOPPS", phase 5 data for 9 countries*, 164 medical organizations, CKD dialysis patients, n = 3 281	>26.3 μg per week	30 μg
27.4 mg per week	1.06:1	Donck J et al, 2014 [12]	Multicenter, observational CT, search for equivalent EPO doses in real practice in CKD hemodialysis patients, n = 1027	25.6 μg per week	30 μg
International and Russian recommendations					
Short-acting EPO → Darbepoetin alfa					
7500 IU per week	200:1	KDIGO 2012[14]	KDIGO Clinical Practice Guidelines for Anemia in Chronic Kidney Disease 2012	37.5 μg per week	20 μg * 2 DF per week
7500 IU per week	200:1	Russian Recommendations 2014[15]	Updated Russian national guidelines for anemia diagnosis and treatment of chronic kidney disease, revised in 2014	37.5 μg per week	20 μg * 2 DF per week
7500 IU per week	200:1	Instructions on drugs	Official instructions on medicinal products approved by the Ministry of Health (grls.rosminzdrav.ru)	37.5 μg per week	20 μg * 2 DF per week
7500 IU per week	240:1	Federal Recommendations 2014[16]	Federal clinical guidelines for anemia diagnosis and treatment in chronic kidney failure, 2014	31.25 μg per week	20 μg *2 DF per week 30 μg per week *(assumption)*
Short-acting EPO → Methoxy polyethylene glycol – epoetin beta					
<8000 IU per week	---	Instructions on drugs	Official instructions on medicinal products approved by the Ministry of Health (grls.rosminzdrav.ru)	60 μg per 2 weeks 120 μg per month	75 μg *2 DF per month 75+50 μg per month
Darbepoetin alfa → Methoxy polyethylene glycol – epoetin beta					
<40 μg	---	Instructions on drugs	Official instructions on medicinal products approved by the Ministry of Health (grls.rosminzdrav.ru)	60 μg per 2 weeks 120 μg per month	75 μg * 2 DF per month 75+50 μg per month

Note: * countries included in the analysis: Belgium, France, Germany, Italy, Russia, Spain, Sweden, Turkey, United Kingdom

[14] Clinical practical recommendations KDIGO on anemia in chronic kidney disease 2012.
[15] Updated Russian national guidelines for the diagnosis and treatment of anemia in chronic kidney disease in the 2014 ed.
[16] Ibid.

Table 4 – Number of annual i.v. doses of r-HuEPO for maintenance therapy – main analysis

INN	Average dosage per m = 75 kg	Frequency of administration	Equivalent DF	Qty of doses	Qty of DFs
EPO alpha	2500 IU	3 times per week	2500 IU	156	156
DA	37.5 µg	once per week	20+20 µg	52	104
Methoxy polyethylene glycol – epoetin beta	60 µg	once per 2 weeks	75 µg	26	26
	120 µg	once per 4 weeks	75+50 µg	13	26

Table 5 – Analysis of EPO cost minimization in i.v. administration

Medicinal product	INN	Qty of DF per year	Cost of 1 DF, rub./euro	Costs per year, rub. (euro)	Differences in changing-over to EPO alpha	
					rub. / euro	%
Scenario 1 – Calculation at real pharmaceutical market prices						
Eralfon 2500 IU	Epoetin alfa	156	1 108.92 / 12.53 €	172 992 / 1 955 €	---	---
Aranesp 20 µg	Darbepoetin alfa	104	1 934.16 / 21.85 €	201 153 / 2 273 €	– 28 161 / – 318 €	–14
Mircera 75 µg	Epoetin beta [methoxypoly ethylene glycol]	26	8 336.74 / 94.20 €	216 755 / 2 449 €	– 43 763 / – 494 €	–20
Mircera 50 µg + 75 µg		13 13	5 544.48 / 62.65 € 8 336.74 / 94.20 €	180 456 / 2 039 €	– 7 464 / -84 €	–4
Scenario 1 – Calculation at the prices of the state register						
Eralfon 2500 IU	Epoetin alfa	156	934.11 / 10.55 €	145 721 /1 647 €	---	---
Aranesp 20 µg	Darbepoetin alfa	104	1 833.56 / 20.72 €	190 690 /2 155 €	– 44 969 / -508 €	–24
Darbestim 20 µg	Darbepoetin alfa	104	1 595.23 / 18.03 €	165 904 /1 875 €	– 20 183 / – 228 €	–12
Mircera 75 µg	Methoxy polyethylene glycol – epoetin beta	26	8 023.09 / 90.66 €	208 600 /2 357 €	– 62 879 / –710 €	–30
Mircera 50 µg + 75 µg		13 13	5 391.02 /60.92 € 8 023.09 /90.66 €	174 383 / 1 971 €	– 28 662 / – 324 €	–16

According to the recommendations of KDIGO 2012, in a hemodialysis patient, the most effective frequency of short-acting ESA – EPO alfa administration is 3 times per week. Among prolonged-release drugs, DA is usually started with a dose of 0.45 µg/kg body weight once per week subcutaneously (s. c.) or intravenously (i. v.) for dialysis patients. In the maintenance phase of treatment, DA continues to be administered once a week, or the administration is changed-over to once per two weeks, increasing the initial dose twice, the most optimal dosing regimen for methoxy polyethylene glycol – epoetin beta is the administration of the drug once per 4 weeks. In patients not receiving dialysis treatment or receiving treatment with peritoneal dialysis, the subcutaneous administration is preferable, and in hemodialysis patients it is intravenous. It is important to notify that for short-acting ESA, the effectiveness of the subcutaneous administration in hemodialysis patients is higher than that of the intravenous administration, which makes it possible to reduce the weekly dose of the injected drug by 30%, while for long-term ESA, the effectiveness of subcutaneous and intravenous administrations is equivalent.

The study time horizon was 1 year of continuous erythropoietins therapy. Since modern approaches to the anemia treatment often involve the beginning of anemia correction at the pre-dialysis stage of CKD treatment, when calculating the costs for long-acting epoetin beta, the study included dialysis patients and the ones who had been treated with EPO preparations in the volume equivalent to the average maintenance dose of EPO alfa or DA (<8000 IU per week and <40 µg per week, respectively) 1, 3.

The costs were calculated according to two scenarios: according to the data of tender purchases of the investigated drugs in 2020 (01.01.2020-06.07.2020), and according to the maximum selling prices of the manu-

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facturer (including value added tax (VAT)), since all the investigated dosage forms are included in the current list of "Vital and Essential Drugs" (VED).

According to the instructions on the medicinal product, pre-filled syringes are used for only one injection, and therefore, when calculating the costs, it was taken into account that if it was necessary to administer a part of the dose to one patient, the remainder of the unclaimed medicinal product in the syringe was disposed of and not used. In this regard, for the tpharmacoeconomic analysis, the price for 1 dosage form (DF) (syringe) was calculated (Table 2). The average price per unit in the pharmaceutical market was determined as the median among all prices of completed tenders at the time of the study (the main analysis is Scenario 1) or as the price of the state register + value added tax (VAT – 10%) (the main analysis is Scenario 2).

For darbepoetin with the trade name Darbestim, which is a reproduced drug, there were no sales data for the analyzed period (01.01.2020-06.07.2020). From 02.09.2020 the drug was included in the VED list; therefore, it was calculated at the state register prices. When converting the costs in euros, the average annual rate of the euro in 2021 was used – 1 euro = 88.5 rubles.

RESULTS

The meta-analysis by Amato, 2017 [6] of 30 studies results covered the sample of 7843 CKD patients, 21 studies of which included hemodialysis or peritoneal dialysis patients. The results showed the equal effectiveness and safety of epoetin alfa, DA and methoxy polyethylene glycol – epoetin beta. However, DA was statistically significantly superior to EPO alfa in reducing the number of transfusions. The meta-analysis by Saglimbene, 2017 [9] included 27 publications (n = 5410): 7 – pre-dialysis patients, 19 – dialysis patients, 1 – a patient requiring kidney transplantation. It was found out that methoxy polyethylene glycol – epoetin beta in various dosages significantly little differs in terms of safety and effectiveness profiles in comparison with EPO alfa and DA. The meta-analysis by Palmer, 2014 [7] included 21 publications (n = 8328). A comparative analysis of DA and placebo was carried out in 1 publication; a comparison of DA and epoetin alfa – in 16; DA and methoxy polyethylene glycol – epoetin beta were compared in 4 publications; DA in different dosage modes – in 3 works; the analysis of various methods of DA administration (i.v. or s.c.) – in 4 publications. It was revealed that DA reduced the number of transfusions in CKD patients at stages 3–5, but it did not affect the mortality and patients' QoL, and its overall effectiveness was comparable to its analogs (EPO alpha, methoxy polyethylene glycol – epoetin beta). Another meta-analysis by Palmer, 2014 [8], which included 56 publications and 15596 patients, also found out no differences in the effectiveness and safety of EPO alfa versus DA and methoxy polyethylene glycol – epoetin beta. A systematic review by Wilhelm-Leen, 2014 [16] also showed that DA is equivalent to epoetin alfa in terms of effectiveness and safety. A multicenter randomized controlled open-label study carried out by Locatelli, 2019 [10], investigated the safety of methoxy polyethylene glycol – epoetin beta in comparison with EPO alpha, EPO beta and DA in 2818 CKD patients (dialysis and predialysis patients) with a history of anemia. The researchers found out that in the target cohort of patients, taking methoxy polyethylene glycol – epoetin beta once a month is no worse than taking analog drugs in terms of adverse cardiovascular complications or mortality from all causes. Thus, based on the analyzed data of meta-analyzes, a systematic review and RCTs, a conclusion was made about the comparable effectiveness in achieving the target values of Hb and the safety of EPO alpha relative to DA and methoxy polyethylene glycol – epoetin beta.

At the next stage of the study, the dosage of EPO alfa for the maintenance therapy stage according to the instructions on the drug was calculated. Thus, for maintenance therapy, it is recommended to reduce the starting dose of 50 IU / kg by 1.5 times (Fig. 2).

A number of studies [5, 12–15, 17] devoted to the calculation of the coefficients for converting EPO doses into equivalent ones, have been analyzed. The results obtained (Table 3) made it possible to substantiate the choice of equivalent doses of EPO preparations of various generations for an average patient with a body weight of 75 kg.

It should be notified that when converting short-acting EPO to DA, the dose conversion factors varied from 200: 1 to 244: 1. When converting short-acting EPO doses to methoxy polyethylene glycol – epoetin beta, the conversion factor was 255: 1, or it was indicated that a weekly dose of epoetin alpha less than 8000 μg is equivalent to 60 μg/once per 2 weeks or 120 μg/once per 4 weeks. When choosing equivalent doses, the emphasis was made on the coefficients recommended by international and Russian clinical recommendations and guidelines, as well as the conversion rates used in real clinical practice [17]. One of the limitations of the model is also the fact that in real clinical practice the maintenance doses that CKD dialysis patients with anemia can receive, vary significantly and are more dependent on the individual characteristics of patients.

Thus, the equivalent ESA doses for the main clinical and economic study were determined and scientifically substantiated:

• Epoetin alfa – 2500 IU * 3 times per week (the intravenous administration, according to the instructions and clinical guidelines);

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• Darbepoetin alfa – 20 + 20 μg (30.7–40 μg) * once per week (the dosage of 30 μg/week is included in the sensitivity analysis);

• Epoetin methoxy polyethylene glycol – epoetin beta – 75 μg (57.2–60 μg) * once per 2 weeks or 75 + 50 μg (114.4–120 μg) * once per 4 weeks. At the same time, in a number of studies it is notified that the dose of long-acting EPO in dialysis patients can vary from 100–200 μg per 4 weeks (100 μg (n = 585) [18], 119 μg (n = 63) [19], 100-200 μg (n = 60) [20], 115 μg (n = 184) [21], 153 μg (n = 3281) [17]). In the present study, the calculations were performed for patients receiving high doses of long-acting EPO equivalent to the doses according to clinical guidelines.

Table 4 shows the results of calculating the number of intravenous ESA injections per year per 1 CKD patient at the end-stage (with an average body weight of 75 kg).

Thus, on average, the frequency of injections of short-acting epoetins is 156 per year, darbepoetin alfa – 52 injections per year, methoxy polyethylene glycol – epoetin beta – 13 injections or 26 injections per year for once per 4 weeks and once per 2 weeks, respectively.

The results of the main cost-effectiveness analysis of cost minimization for drugs used i. v., are presented in Table 5.

It was revealed that when calculated at tender prices in the public procurement system (according to the data of 2020), the costs for treating anemia with epoetin alfa 2500 IU will be 172,992 rubles (€ 1,955) per patient per year. In comparison with DA, the use of epoetin alfa will reduce costs by 28,161 rubles (318 €) per year per patient (14%). Compared to methoxy polyethylene glycol – epoetin beta, epoetin alfa can save from 4 to 20%.

When calculated at the state register prices, the differences in costs will be more significant – compared to DA, the costs will be lower by a quarter (–24%), compared to methoxy polyethylene glycol – epoetin beta, and the differences in costs varied from –16% to –30%.

It should be notified that in dialysis patients, short-acting EPO can also be used subcutaneously, which makes it possible to reduce the administered dose and, accordingly, the cost of drugs by 20-30%. Fig. 3 below shows the results of the cost minimization analysis of the study drugs in the comparative aspect when administered s / c.

Since to achieve a similar effect, in s.c. EPO alfa administration a patient needs the dose up to 30% lower than in i.v. administration this makes it possible to use a lower frequency of the drug administration (twice per week). In its turn, it makes it possible to reduce the cost of pharmacotherapy by a third – from 172,992 rubles up to 115, 328 rubles (from 1,955 € to 1,303 €), or from 145,721 rubles up to 97 147 rubles (from € 1,647 to € 1,098) for market prices and state register prices, respectively. Prolonged action ESAs can also be used s/c, however, no adjustment of the administered dose is made.

Thus, when changing-over from i.v. to s. c. administration of the studied ESAs, the savings in choosing epoetin alfa for the correction of nephrogenic anemia can reach 50% (36–47% and 44–53% for market prices and state register prices, respectively).

DISCUSSION

According to the Russian clinical guidelines on the treatment of CKD, meta-analyses [6-9], clinical [10] and retrospective studies [17], it can be stated with a high evidence level that no significant differences in the effectiveness of different ESAs for the correction of anemia have been revealed. No significant differences have been either shown between original and biosimilar epoetins [6, 22, 23]. At the same time, there are cases of an increase in the partial erythrocytes aplasia in the use of some locally produced epoetins (in Asia and Latin America). These kinds of drug production should be accompanied by strict protocols for the approval of biosimilar drugs by the regulatory authorities of some countries, including the RF[17]. The use of CKD makes it possible to achieve the target hemoglobin values (100–120 mg/ml) with maintenance therapy for 6 months or more in more than 80% of patients [24].

This research made it possible to update the previously obtained data from a similar study by Krysanov, 2019 [25]. The previous study included a marketing analysis of prices for 3 quarters of 2018 (01.01.2018-31.09.2018). In this study, the prices for medicines were updated according to the tenders carried out in 2020 (01.01.2020-06.07.2020). In connection with the mandatory re-registration of the manufacturers' maximum selling prices for the drugs included in the VED list, an additional analysis of the treatment costs was carried out and calculated on the basis of the state register prices.

The updated results also indicate that epoetin alfa (Eralfon®) has an economic advantage relative to darbepoetin alfa (Aranesp®) and methoxy polyethylene glycol – epoetin beta (Mircera®) in both the intravenous and subcutaneous administrations of the investigated drugs when used in equivalent maintenance doses in adult patients who have previously received EPO therapy, with equal effectiveness and safety. It should be notified that the intravenous administration is preferable for dialysis patients; however, changing-over to the subcutaneous administration of the drug can also be considered for such a cohort of patients. For short-acting ESAs, the effectiveness of the subcutaneous administration is higher than intravenous, which makes it possible to reduce

[17] Clinical guidelines. Chronic kidney disease. 2019.

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the frequency of the drugs administration in this group (twice per week), reduce the dose and the cost of drug therapy in relation to long-term ESAs by 30%. Herewith, the effectiveness in the subcutaneous and intravenous administrations appears to be equal at the investigated dosage frequencies[18]. It should be notified that in real clinical practice, the subcutaneous method of administration could hardly be suitable to all patients due to its high painfulness or other clinical features.

The sensitivity analysis of the data obtained in this study, to a change in the dosing regimen of darbepoetin alfa showed that with a decrease in the weekly dose of DA with 2 DF* 20 μg per week up to 30 μg (1 DF* 30 μg) ([8, 10]), the per year costs for therapy with epoetin alfa will be insignificantly higher than with darbepoetin. In comparison with INN Aranesp, the per year cost of therapy with epoetin alfa is 1% higher (172 992 rubles and 171,028 rubles, or 1,955 € and 1,933 €), or 2% (145,721 rubles and 143,127 rubles, or 1,647 € and 1,617 €) when calculated at market prices and the prices of the state register, respectively. In comparison with INN Darbestim 30 μg once a week, the cost of therapy with epoetin alfa per year is 17% higher (145,721 rubles and 124,496 rubles, or 1,647 € and 1,407 €) when calculated at the prices of the state register, i.e., the results of calculations with respect to reproduced DA are sensitive to changes in the dosage regimen. Thus, for a cohort of patients treated with DA in the weekly dose of 30 μg, the differences in the costs of the annual treatment in comparison with the original DA (1–2%) can be considered insignificant, and in comparison, with the reproduced drug – as significant (17%).

The sensitivity analysis of the data obtained to changing-over the dosage regimen of long-acting EPO from the 120 μg scheme once per 4 weeks (13 DFs per year) to 120 μg once a month (12 DFs per year) showed that the differences in costs varied within +4% to –9%. The costs depend on the method of price calculation – at real market prices or at VED prices, respectively. It should be notified that the calculations carried out, have limitations and reflect the costs in a comparative aspect in the patients administrated with high doses of Mircera (more than 100 μg per month). According to the conversion factors based on the international and Russian clinical guidelines, high doses of Mircera (more than 100 μg per month) are equivalent to the dosage regimen of epoetin alfa 2500 IU per patient * 3 times per week (7500 IU per week). A large-scale study conducted in 9 countries of the world, including the Russian Federation, shows that in real clinical practice, the average prescribed long-acting EPOs are close to high doses [17].

It should be notified that in the study by Krysanov et al., 2016 [4], the use of short, intermediate and long-acting epoetins in hemodialysis patients with nephrogenic anemia, were also studied. The patients were administrated with different dosages of drugs depending on the target hemoglobin value. So, to achieve the index of 9 ± 1 g/dL, on average, the patients are administrated with epoetin alfa (biosimilar) 2,558 IU 3 times per week (7,674 IU – a weekly dose), DA 25 μg per week and long-acting EPO 161 μg per month. Under the assumption of neglecting the differences in the calculated values of single doses up to 6%, the above-listed doses will be equivalent: epoetin alfa syringe 2500 IU * 3 times per week – neglecting an error of 2%, darbepoetin syringe 30 μg * once per week – provided that a 30 μg syringe is used to achieve a single dose of 25 μg and methoxy polyethylene glycol – epoetin beta 100 + 50 μg * once per 4 weeks – neglecting an error of 6%. It should be notified that in the present study, EPO alpha 2,500 IU was comparable in price to DA 30 μg (the differences in costs did not exceed 2%) and more economical in comparison with methoxy polyethylene glycol – epoetin beta 75 + 50 μg. Thus, a conclusion can be made about comparable costs versus DA and the economic advantage versus long-acting EPO beta 100 + 50 μg, since the preparation with a dosage of 100 μg has a higher cost per pack in comparison with the release form of a lower dosage.

The obtained comparison results for the intravenous administration in the pair of EPO alpha and EPO beta long-acting* once per 2 weeks are insensitive to the price increases for EPO alpha (Eralfon®) up to +20%. The comparison results in the pair of EPO alfa and DA 20 μg per week are insensitive to the price increase up to + 15%; EPO alpha remains an economically more profitable alternative. At the same time, in the pair of EPO alpha and methoxy polyethylene glycol – epoetin beta * once per 4 weeks, the results are sensitive up to 5% and the price increases for EPO alpha. The comparison results for the subcutaneous administration are insensitive to price increases for EPO alpha (it will be more economical even if prices rise by 50%).

The conclusions reached are relevant when calculating the cost per patient with an average body weight of 75 kg, and assuming that the number of dosage forms per injection is rounded to integers, the calculations may differ for other body weights.

CONCLUSION

Thus, with equal effectiveness and safety of the studied drugs, EPO alfa 2,500 IU 3 times per week administrated intravenously and subcutaneously, is a more economical drug technology in relation to darbepoetin 40 μg once per week and methoxy polyethylene glycol epoetin beta with the scheme both 60 μg twice per month and 120 μg once per month.

[18] Clinical practical recommendations KDIGO on anemia in chronic kidney disease, 2012.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

Ivan S. Krysanov – development of research design, article editing and final approval; Viktoria Yu. Ermakova – article editing, planning and developing the study design; Larisa B. Vaskova – article editing, planning and developing the study design; Marina V. Tiapkina – collecting material, data processing, writing and editing the article.

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RESEARCH ARTICLE

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ANTIHISTAMINES: RESEARCH AND ANALYSIS OF THE REGIONAL RETAIL MARKET

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The aim. Focused on optimizing the assortment portfolio of pharmacy organizations and improving the process of drug supply to end-consumers, the aim of the study was to analyze the regional pharmaceutical market for antihistamines.
Materials and methods. In the study, the following methods were used: a content analysis of regulatory documents; a documentary observation method of the volume of antihistamines sales; a sociological survey method. The objects of the study were accounting registers in 32 pharmacy organizations for 2020, as well as the sociological survey results of 174 respondents – consumers of antihistamines.
Results. The classification analysis of 38 international non-proprietary trade names of antihistamines, represented by 187 names of drugs, revealed the prevalence of the first-generation drugs (63%). On the Russian pharmaceutical market, there were also 55% of foreign production drugs. At the regional level, there are 66 types of drugs in circulation, 50% of which are second-generation ones. The cost analysis showed rather a wide rage of the pricing proposal and the economic availability of antihistamines for patients. The sociological survey revealed the fact that 46% of the consumers were ready to pay for the necessary drugs in the price range "over 100 and up to 500 rubles" (over $ 1.38 and up to $ 6.88) for one conventional package. A medical-demographic profile of the antihistamines consumer at the regional level has been made up, and guidelines for pharmaceutical specialists on managing the assortment portfolio of pharmaceutical organizations have been developed.
Conclusion. As a result of the study, the following facts have been established: the seasonal peaks in the antihistamines consumption; a gradual renewal of the pharmacies assortment portfolio due to the increased consumption of the second and third generation antihistamines. The medical and demographic profile of the consumer should be taken into consideration when planning a drug provision for the patients with allergic pathologies, and it is connected with the growth in pharmacies profits due to the sale of drugs in the range from 100 to 500 rubles (from $ 1.38 to $ 6.88). The methodical recommendations have been brought to the attention of the management of regional pharmacy organizations.
Keywords: antihistamines; pharmaceutical market; pharmacy organizations; assortment portfolio; end-consumers
Abbreviations: AHMPs – antihistamine medicinal preparations; MP – medicinal preparation; INN – international non-proprietary name; CMW – Caucasian Mineral Waters; VEM – vital and essential medicines.

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ИССЛЕДОВАНИЕ И АНАЛИЗ РЕГИОНАЛЬНОГО РОЗНИЧНОГО РЫНКА АНТИГИСТАМИННЫХ ЛЕКАРСТВЕННЫХ ПРЕПАРАТОВ

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Цель. Анализ регионального фармацевтического рынка антигистаминных препаратов, направленный на оптимизацию ассортиментного портфеля аптечных организаций и совершенствование процесса лекарственного обеспечения конечных потребителей.

Материалы и методы. В исследовании использовались: контент-анализ нормативных документов; метод документального наблюдения за объемами реализации антигистаминных препаратов; метод социологического опроса. Объектом исследования служили регистры бухгалтерского учета в 32-х аптечных организациях за 2020 г., а также результаты социологического опроса 174 респондентов – потребителей антигистаминных лекарственных препаратов.

Результаты. Классификационный анализ 38 международных непатентованных наименований антигистаминных лекарственных средств, представленных 187 наименованиями препаратов, выявил превалирование на российском фармацевтическом рынке препаратов первого поколения (63%), зарубежного производства (55%). На региональном уровне в обращении находится 66 наименований лекарственных препаратов, из которых 50% составляют препараты второго поколения. Анализ стоимости показал достаточную широту ценового предложения и экономическую доступность антигистаминных препаратов для пациентов. Социологический опрос выявил, что 46% потребителей готовы платить за необходимые препараты в ценовом диапазоне «свыше 100 и до 500 рублей» (свыше 1,38$ и до 6,88$) за одну условную упаковку. Сформирован медико-демографический портрет потребителя антигистаминных лекарственных препаратов на региональном уровне и разработаны методические рекомендации для фармацевтических работников по управлению ассортиментным портфелем аптечных организаций.

Заключение. В результате исследования установлены сезонные пики потребления антигистаминных препаратов; постепенное обновление ассортиментного портфеля аптек за счет роста потребления антигистаминных препаратов второго и третьего поколений; рост прибыли аптек идет за счет продаж препаратов в диапазоне от 100 и до 500 рублей (от 1,38$ и до 6,88$); медико-демографический портрет потребителя следует использовать при планировании лекарственного обеспечения пациентов с аллергическими патологиями. Методические рекомендации доведены до руководства региональных аптечных организаций.

Ключевые слова: антигистаминные лекарственные препараты; фармацевтический рынок; аптечные организации; ассортиментный портфель; конечные потребители

Список сокращений: АГЛП – антигистаминные лекарственные препараты; ЛП – лекарственный препарат; МНН – международное непатентованное наименование; ЖНВЛП – жизненно необходимые и важнейшие лекарственные препараты; КМВ – Кавказские Минеральные Воды.

INTRODUCTION

Currently, pathologies of an allergic nature represent a serious medical and social problem due to the constantly growing list of allergens, impending environmental problems, global climatic changes, migration flows of large masses of people, the emergence of new features in the nature and course of allergic reactions, and others [1]. According to the White Book on Allergy of the World Allergy Organization (WAO)[1], among the entire population of the planet, up to 40% of people are susceptible to at least one sensitizing agent, and 10 to 30% of them from suffer from allergic irritation of the upper respiratory tract (including rhinitis), up to 10% – from allergic reactions to drugs. Urticaria, in the form of the eruption (rashes on the body), significantly reduces

[1] WAO White Book on Allergy 2013. Available from: https://www.worldallergy.org/UserFiles/file/WhiteBook2-2013-v8.pdf

the quality of life, and occurs at least once during a lifetime in 20% of people [2–4].

According to "The All-Russian Public Opinion Research"[2], about 26% of Russians suffer from allergic diseases, the etiology of which is a contact with natural plant allergens (pollen or plant fluff). Almost all of them use special drugs, and one in five is monitored by a doctor. A great number of factors affecting the prevalence of various types of allergic diseases in adults and children are known to Russian medical science and practices. The combination of these factors leads to a permanent increase in morbidity, increased disability and mortality, as well as significant economic costs for the treatment and prevention of allergies [5].

The main group of pharmacotherapeutic agents used for the prevention and treatment of allergic diseases is AHMPs, the range of which is represented on the Russian pharmaceutical market by a wide range of trade names. In such a situation, medical specialists do not always correctly prescribe necessary AHMPs, thereby causing unreasonable polypragmasy, which contributes to the deterioration of the patients' healths [6]. Allergic pathologies prevent patients from leading an active social mode of life, as well as negatively affect their working capacity and life in general, leading to constant ailments of a chronic or seasonal nature [7, 8]. On the other hand, the current situation on the pharmaceutical market also complicates the work of pharmaceutical specialists in the formation of an optimal pharmacy assortment, which should positively affect the amount of income of a pharmacy organization. The optimal range of drugs in pharmaceutical retail facilities is one of the main components of the drug supply system for the population and, which is important, it plays a decisive role in ensuring the competitiveness of pharmacy organizations under the market conditions [9–11]. At the same time, this assortment is not frozen or constant; it must be continuously changed and improved to maintain the socially necessary level of drug consumption by the population [12, 13]. Improving the mechanisms for organizing a drug provision should be considered as elements of the pharmaceutical safety system in our country and the safety of pharmaceutical services provided by pharmacy organizations to the population [14, 15].

Focused on optimizing the assortment portfolio of pharmacy organizations and improving the process of drug supply to end-consumers, **the aim** of the study was to analyze the regional pharmaceutical market for antihistamines.

Research objectives:

1. Explore the basic AHMPs assortment in the circulation on the Russian pharmaceutical market.

2. Analyze the actual assortment to carry out research on the AHMPs consumer market at the level of regional pharmacy organizations.

3. Substantiate the medico-pharmaceutical profile of the regional AHMPs consumer.

4. Formulate scientifically grounded proposals for optimizing the assortment portfolio of pharmacy organizations according to the AHMPs nomenclature at the regional level.

MATERIALS AND METHODS

Research objects

The object of the study was the data on the presence and movement of AHMPs in pharmaceutical retail entities for 2020, as well as the results of a sociological study of AHMPs consumers, carried out in pharmacy organizations.

The experimental part of the work was carried out on the basis of 32 pharmacies serving about 140 thousand people living in the cities of Pyatigorsk, Yessentuki and Kislovodsk in the CMW region, located on the territory of the Stavropol Territory – a subject of the Russian Federation.

Research methods

During the study, the following methods have been used: the content analysis of the research papers on the AHMPs pharmaceutical, as well as regulatory legal documents governing the civil circulation of AHMPs in the Russian Federation. Various electronic sources of information were used as an empirical base, including Russian scientific electronic libraries: eLIBRARY.RU; CyberLeninka; National Electronic Library (NEB); State Public Scientific and Technical Library (SPSL); Russian State Library (RSL). Besides, archives of scientific works of the Higher Attestation Commission of the Ministry of Science and Higher Education of the Russian Federation; international scientometric databases SCOPUS, Web of Science, Chemical abstract, Pubmed and others, have also have been used. The search was carried out by screening the texts by selected keywords (antihistamines, allergic diseases, pharmacy organizations, assortment portfolio, end-consumers), followed by studying the content of the selected works for compliance with the research topic.

The content analysis of regulatory legal documents was carried out using the "Consultant Plus" legal reference system, which provides the relevant texts of the required documents. Regarding the studied AHMPs nomenclature, the juridical documents regulating the circulation of medicines in the Russian Federation, i.e. "The vital and essential medicines" (VEM) and "The minimum range of drugs necessary for the provision of medical care" (further, minimum range of drugs), approved by the Order of the Russian Federation Government dated October 12, 2019 No. 2406-r for 2021, were analyzed[3]. In addition, the State Register of Medicines, posted on

[2] TASS. Available from: https://tass.ru/obschestvo/6448616

[3] On the approval of the list of vital and essential medicines for 2020, as well as the lists of medicines for medical use and the minimum range of medicines required for the provision of medical care: Order of the Government of the Russian Federation of 12.10.2019, No. 2406-r (as amended. and additional, entered into force on 01.01.2021). Available from: http://www.consultant.ru/cons/cgi/online.cgi. Russian

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the official website of the Ministry of Health of the Russian Federation, was used to analyze the Russian pharmaceutical market[4].

The method of documentary observation was used to study the volumes of AHMPs retail sales to end-consumers. In value terms, the required data were selected from the accounting registers of pharmaceutical organizations, which had been used as an experimental data base for the study.

The classification analysis of medicinal preparations used in the pharmacotherapy of allergic pathologies was used according to the standard technique; the attitude of the studied drugs to different generations, the countries in which their industrial production and medicinal preparations are localized, were identified as the main classification signs of AHMPs.

A sociological survey of visitors to pharmacy organizations was used to study the regional AHMPs consumer market. For this, a special questionnaire had been developed (Table 1), which contained the questions related to socio-demographic and medico-pharmaceutical data.

A sociological study was carried out during May-September 2020 on the basis of pharmacy organizations selected as an experimental base. The selection of respondents was conducted by a continuous sampling method, when the specialists of the "first department" suggested that all end-users who purchased AHMPs should go through a voluntary anonymous questionnaire, the results of which formed the basis of the experimental part of the study. The survey involved 174 respondents.

RESULTS AND DISCUSSION

The carried out content analysis of the scientific publications made it possible to establish that the features of the AHMPs pharmaceutical market had been studied in detached regions of Russia [16-18]. A fairly large body of research on the characteristics of the drug therapy for allergic diseases is regularly carried out in the Russian Federation [19-21] and abroad [22-24]. Over the past period, the range of AHMPs has been significantly updated and expanded.

Herewith, new methodological approaches to the formation of the assortment portfolio of pharmacy organizations were not developed. In addition, each region of Russia has its own distinctive features in terms of the diseases spread, the effective demand of the population and the quality of pharmaceutical consulting. On the regional pharmaceutical market of resort cities of the CMW region of the Stavropol Territory, no full-fledged studies devoted to improving the management of the assortment policy at the level of pharmacy organizations, using the example of AHMPs, have been carried out. That determines the scientific relevance and practical significance of the present work.

[4] State Register of Medicines / Official website of the Ministry of Health of Russia 2021. Available from: https://grls.rosminzdrav.ru/Default.aspx. Russian

The content analysis of the State Register of Medicines revealed 38 INNs of medicines registered in the Russian Federation and used in the prevention and treatment of allergic diseases. They are represented by 187 trade names of medicinal preparations from various manufacturing organizations, dosages and dosage forms. The list of antihistamines registered in the Russian Federation is presented by three generations (Table 2).

Among the 38 registered INNs, the share of antihistamine drugs belonging to the first generation is 63%; to the second – 32%; to the third – 5% of the total number of the trade names.

In the course of the classification analysis on the basis of the localization of the organization-manufacturer of medicines, it was established that foreign-made drugs prevail among AHMPs (55%), the drugs produced at Russian pharmaceutical enterprises accounted for 45% of the total number of INNs. The main volumes of AHMPs supplies to the pharmaceutical market are accounted for 47 Russian drug manufacturers. The number of importers of AHMPs to the Russian Federation tends to decrease; at the time of the study, the supply of AHMPs to the Russian pharmaceutical market was carried out by drug manufacturing organizations from 19 countries. India has been holding the leading position for many years, while its share is about 22% of the total import AHMPs supplies. The leading Western European drug manufacturers come next: Switzerland – 11%, Hungary – 10% and Germany – 5%. In addition to these countries, the supply of AHMPs to Russia is carried out by Slovenia, Bulgaria, Israel and other countries.

On the basis of the dosage form release, it was determined that the most demanded AHMPs are in the form of a variety of tableted dosage forms. Their share is about 48% of the total number of dosage forms. As before, dosage forms in the form of solutions for injections are still relevant for use in the clinic of emergency and urgent medical care, their share is about 18%. Recently, AHMPs have been gaining popularity in the form of such liquid dosage forms as suspensions, solutions and syrups for internal use (16%), and dosed aerosols (11%). In addition, the popularity of antiallergic drugs produced in the form of nasal and eye drops, sprays and ointments does not decrease, their share is 7%.

Further on, according to the studied AHMPs nomenclature, the judicial documents regulating the circulation of medicines in the Russian Federation were analyzed, i.e.: VEM and minimum range of drugs, established for 2021 (Table 3).

As evidenced by the data in Table 3, VEM and minimum range of drugs include only 4 and 2 INNs, respectively, of the entire range of the antihistamines, in various dosage forms. They are represented by 22 medicinal preparations, 2 of which are the third generation medicinal preparations, 18 are the second generation and 2 are the first generation.

Table 1 – Questionnaire of patient with allergic pathology

Question formulation	Respondents' answer options
1. Sociodemographic data	
1.1. Specify your gender	– male – female
1.2. Specify your age (number of full years)	– up to 20 years old inclusive – from 21 to 30 – from 31 to 40 – from 41 to 50 – from 51 and older
1.3. Where do you live?	– up in town – in rural areas
1.4. What is your education?	– secondary general – vocational secondary – higher
2. Medical-pharmaceutical data	
2.1. How long have you been suffering from an allergic disease?	– less than 2 years – from 2 to 5 years – more than 5 years
2.2. How often do you seek medical help for an allergic disease?	– "I never apply" – "I apply only in urgent cases" – "I always apply"
2.3. What clinical manifestations of allergies do you encounter?	– rhinorrhea – lacrimation – skin rashes – skin itching – other
2.4. 2.4. What sources of information about antihistamines do you use?	– medical and pharmaceutical specialists – relatives and acquaintances – internet – medical literature – mass media
2.5. What influences your choice when buying the drug you need?	– recommendations of medical and pharmaceutical specialists – recommendations of friends – personal experience – the price of the drug
2.6. Which of the characteristics of the drug has a prevailing influence on you when choosing it?	– manufacturing organization – efficiency – mode of application – no side effects – the price of the medicinal preparation
2.7. What price for the required antihistamine are you willing to pay for one conventional package?	– up to 100 rubles (up to $ 1.38)[5] – over 100 to 500 rubles (over $ 1.38 to $ 6.88) – over 500 rubles (over $ 6.88)

Table 2 – Antihistamines on the Russian pharmaceutical market

Antihistamines accordingt o INNs		
First generation	Second generation	Third generation
1. Alimemazine	1. Azelastine	1. Fexofenadine
2. Antazoline	2. Akrivastin	2. Cetirizine
3. Bromodiphenhydramine	3. Астемизол	
4. Brompheniramine	4. Бамипин Bamipin	
5. Hydroxyzine	5. Dimetindene	
6. Dexchlorpheniramine	6. Desloratadine	
7. Dimenhydrinate	7. Cromoglycic acid	
8. Diphenhydramine	8. Mizolastine	

[5] At the rate of the Central Bank of Russia as of 01.07.2021 – 72.72 rubles. for 1 US dollar.

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Antihistamines accordingt o INNs		
First generation	Second generation	Third generation
9. Doxylamine	9. Oxatomide	
10. Quifenadine	10. Terfenadine	
11. Xylomethazolin hydrochloride	11. Ebastin	
12. Mebhydrolin	12. Epinastine	
13. Meclizine		
14. Mepiramine		
15. Oxomemazine		
16. Pyrilamine		
17. Promethazine		
18. Sevifenadine		
19. Trimeprazine		
20. Pheniramine		
21. Chloropyramine		
22. Chloropheniramine		
23. Cyclizine		
24. Cyproheptadine		

Table 3 – Antihistamines in VEM and minimum range of drugs

ATC-code	Group name according to ATC classification	INN	Dosage form
1. Vital and Essential Medicines			
R06AA	alkylamine esters	diphenhydramine	solution for intravenous and intramuscular administration; pills
R06AC	substituted ethylenediamines	chloropyramine	solution for intravenous and intramuscular administration; pills
R06AE	piperazine derivatives	cetirizine	drops for oral administration; syrup; film-coated tablets
R06AX	other antihistamines of systemic action	loratadine	syrup; oral suspension; pills
2. Minimum range of medicines necessary for provision of medical care			
R06AC	substituted ethylenediamines	chloropyramine	solution for intravenous and intramuscular administration; pills
R06AX	other antihistamines of systemic action	loratadine	syrup; oral suspension; pills

Table 4 – Antihistamine sales volumes in 2020

Month	Sales volume per month		Share of total annual sales volume, %	Sales quarter volume		Share of total annual sales volume, %
	rub.	$		rub.	$	
January	827 228,59	11 375,53	7,8	2 357 875,38	32 424,03	22.33
February	721 475,12	9 921,28	6,8			
March	809 171,67	11 127,22	7,7			
April	910 097,56	12 515,09	8,6	2 994 956,98	41 184,78	28.36
May	1 042 776,36	14 339,61	9,9			
June	1 042 083,06	14 330,08	9,9			
July	874 039,77	12 019,25	8,3	2 949 921,83	40 565,48	27.94
August	1 166 084,11	16 035,26	11,1			
September	909 797,95	12 510,97	8,6			
October	774 654,34	10 652,56	7,3	2 256 337,87	31 027,75	21.37
November	725 095,54	9 971,06	6,8			
December	756 587,99	10 404,13	7,2			
Total	10 559 092,06	145 202,04	100,0	10 559092,06	145 202,04	100.0

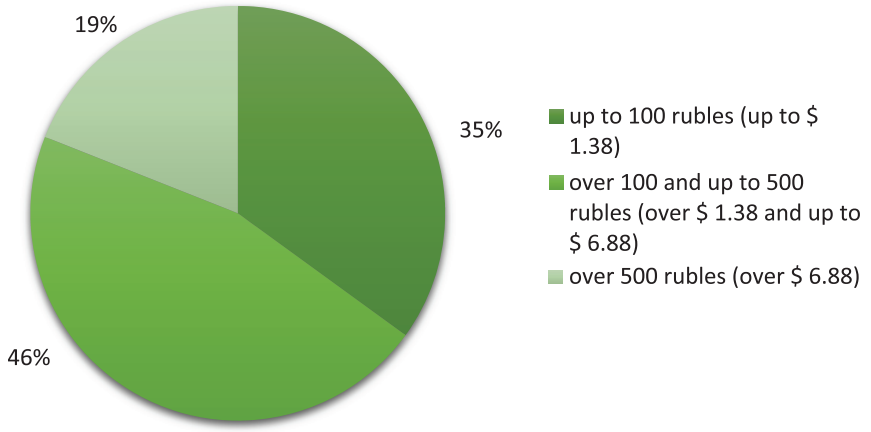

Figure 1 – Distribution of respondents' survey results about price preferences, %

Thus, it is possible to say that there is a necessary and sufficient range of AHMPs in circulation on the Russian pharmaceutical market, which ensures the provision of a full-fledged pharmacotherapeutic assistance to the patients suffering from allergic pathologies.

Further on, on the basis of 32 pharmacy organizations, the actual assortment of AHMPs was investigated. It includes 66 names of drugs in various dosages and dosage forms that are in circulation on the regional pharmaceutical market. It was established that pharmaceutical organizations have the AHMPs included in the VEM, which ensures the state regulation of the maximum selling prices for these drugs. In addition, the institutional requirements for the availability of AHMPs in the distribution network included in the minimum range of drugs, are fully met. As a result of the analysis, it was notified that the stocks of regional pharmaceutical retail organizations fully ensure the economic and physical accessibility of drugs for the prevention and treatment of allergic diseases to the general population.

The classification analysis of the regional pharmaceutical market of AHMPs in terms of their creation time, state registration and being in civil circulation, determined their belonging to three generations of anti-allergic drugs.

18 drugs of the 66 studied, belong to the first generation (27.5% of the total), 33 (50%) to the second and 15 (27.5%) to the third one. The overwhelming presence of second-generation AHMPs in pharmaceutical retail subjects indicates a clear reorientation of end-users to relatively new drugs that have undeniable pharmacotherapeutic advantages of new ones in comparison with the first-generation drugs.

Using the method of documentary observation of the availability and movement of material and monetary funds in the accounting registers, the sales volumes of AHMPs in 32 surveyed pharmacy organizations for 2020 by months and quarters have been determined (Table 4).

The data of Table 4 clearly demonstrate the growth in the sales of AHMPs depending on the seasons and periods of "pollen" plants blossom-time characteristic of the CMW region [25, 26]. In connection with the beginning of blossom- and pollination time of plants, the "peak" of AHMPs sales was registered in the spring-summer period (from April to September).

When analyzing the cost range of the AHMPs presented on the regional pharmaceutical market, it was found out that it was in the range of 30.92 rubles. ($ 0.43)[6] for one package (mebhydrolin 50 mg per pill, No. 10 per package) up to 787.67 rubles. ($ 10.83) per package (desloratadine 5 mg per tablet, No. 30 per package). The data obtained made it possible to distribute all AHMPs into three price subgroups: I – costing up to 100 rubles (up to $ 1.38); II – over 100 and up to 500 rubles (over $ 1.38 and up to $ 6.88); III – over 500 rubles (over $ 6.88) for one conventional package.

The first price subgroup (I) included 23 trade names of drugs, which accounted for 34.8% of the sold range of drugs in this group. The drug mebhydrolin presented in 14 dosages and dosage forms, prevails here. The price of mebhydrolin varies from 35.23 rubles ($ 0.48) up to 71 rubles ($ 0.98) per package, depending on the manufacturing organization and dosage. Patients' adherence to the purchase of mebhydrolin 100 mg in pills, No. 10 per package, should be notified; more than 2900 packages of mebhydrolin were sold during the study period.

In addition to Mebhydrolin, this subgroup includes such frequently sold drugs as xylometazoline in the form of a 0.1% nasal spray, 10 ml in a vial; cromoglycic acid in the form of 2% eye drops, 10 ml in a vial. For a complete satisfaction of patients' preferences, it is necessary to constantly have these drugs in the assortment of pharmacy organizations. As a rule, the affordable price of AHMPs for a patient is the propulsion source of a spontaneous and quick purchase, which has a positive effect on the pharmacy organization's revenue.

The second price subgroup (II) included 37 names of drugs (56.06% of the total number of trade names). The most popular drugs were: chloropyramine tablets, No. 20 per pack; dimetindene, 30 (50) g per tube; cro-

[6] At the rate of the Central Bank of Russia as of 01.07.2021 – 72.72 rubles. for 1 US dollar.

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moglycic acid 2%, 15 ml, aerosol. In this price subgroup, AHMPs are presented in a wide variety of dosage forms: sprays, eye drops, tablets, gels, emulsion creams, which creates preferable conditions for the consumer when choosing the most convenient dosage form at an acceptable cost.

The third price subgroup (III) included 6 drugs (9.09% of the total number of trade names). This group includes the third generation AHMPs (fexofenadine and cetirizine), an important difference of which is the absence of such a side effect as sedation.

The conducted cost analysis of the regional AHMPs pharmaceutical market showed a sufficient range of the price offer, which, against the background of the current economic availability, fully meets the needs of the patients suffering from allergic pathologies.

The data obtained after generalizing and analyzing the questionnaires of the respondents indicate that women prevail among the users of AHMPs (62%). Most of all, AHMPs are purchased by the consumers from the third age category (from 31 to 40 years old) – 56%, followed by the representatives of the second age category (from 21 to 30 years old) – 25%, 15% is accounted for the fourth age category (from 41 to 50 years old). Among the surveyed respondents, the smallest number of consumers entered the first (under 20 years old) and fifth (over 51 years old) age categories, their shares amounted to 1% and 3%, respectively.

Urban residents make up 58% of the number of respondents. Among the respondents, persons with higher education accounted for 33%, vocational secondary – 41%, secondary general – 26%.

The results of the respondents' survey concerning medical-pharmaceutical issues showed that most often, the allergic disease is chronic – it lasts more than 5 years (69%), 22% of respondents suffer from allergies from 2 to 5 years, and only 9% of the respondents notify their allergic manifestations for less than 2 years.

In terms of the frequency of applying for medical care, the respondents' answers were distributed as follows: "I never apply" – 84%; "I apply only in urgent cases" – 10%; "I always apply" – 6%.

Among the respondents, the following clinical manifestations of allergy prevailed: rhinorrhea – 28%; lacrimation – 31%; skin rashes – 16%; skin itching – 14%; other – 11%.

The information sources about AHMPs, used by their consumers, were distributed in the following sequence: relatives and friends – 30%; medical and pharmaceutical workers – 27%; internet – 15%; mass media – 18%; medical literature – 10%.

On buying the necessary medicinal preparation, the influence on its choice is exerted by: recommendations of medical and pharmaceutical specialists – 22%; recommendations of friends – 12%; personal experience – 35%; the price of the drug – 31%.

The results of the survey showed that for consumers, the most significant characteristics of AHMPs that affect their choice are: the price – 37%, the efficiency – 34%, the absence of side effects – 22% and a manufacturing organization – 7%.

The willingness of consumers to pay for the appropriate AHMPs showed that they give a price preference to the drugs in the price range "over 100 and up to 500 rubles" (over $ 1.38 and up to $ 6.88) for one conventional package (Fig. 1).

The generalized data of the sociological survey made it possible to form a medical and demographic profile of the AHMPs end-consumer at the regional level. Therefore, the average AHMPs end-consumer is a person predominantly of a female sex (62%), belonging to the age group from 31 to 40 years old (56%), living up in town (58%), and having a vocational secondary education (41%). Such people suffer from an allergic disease for more than 5 years (69%); practically do not apply for medical help (84%), with the most common clinical manifestation of allergy in the form of lacrimation (31%). They usually take information about AHMPs from communication with relatives and friends (30%), relying on the choice AHMPs, mainly based on personal experience (35%), take into account the price (37%) when choosing a drug. In addition, they are ready to pay for the necessary drug in the price range "over 100 and up to 500 rubles" (over $ 1.38 and up to 6, $ 88) for one conventional package (46%).

CONCLUSION

Based on the research conducted, methodological recommendations in the form of an information letter for pharmaceutical workers involved in the formation of an assortment portfolio of pharmacy organizations, have been prepared. They can be presented in the form of basic theses:

– pronounced seasonal peaks in the AHMPs consumption associated with the periods of "pollen" plants blossom-time in the CMW region, have been established, and these require the creation of a current stock of drugs to meet the increasing volumes of their consumption;

– the analysis of the federal and regional market supply for the nomenclature of the drugs aimed at the prevention and treatment of allergic diseases, indicates the dynamics of the first AHMPs generation displacement from the assortment portfolio of pharmaceutical organizations, and that makes it possible to focus purchases on the second and third drugs generations with their active promotion on the pharmaceutical market using various marketing technologies (merchandising, advertising on the Internet and in the media, etc.);

– a study of the AHMPs sales volumes revealed that the bulk of the pharmacy organizations profits comes from the drugs sale of the second price subgroup – "over 100 and up to 500 rubles" (over $ 1.38 and up to $ 6.88) for one conventional package. The medicines of this par-

ticular price subgroup are ready to be purchased by a potential end-user. This situation can serve as an economic benchmark for pharmaceutical specialists who manage the assortment portfolio of a pharmacy organization;

– the formed medical and demographic profile of the average consumer makes possible predicting the AHMPs consumption at the regional level and planning the distribution of funds for their purchases in order to maintain the socially necessary level of the drug consumption at the regional level.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

Viktoria V. Prokopenko – data collecting, conducting an experiment, analyzing and interpreting the data obtained, preparing a manuscript draft, literature analysis; Taisiya I. Kabakova – study planning, participation in the concept development and study design; Maxim V. Chernikov – final approval of the manuscript publication, processing of the results obtained, verification of critical intellectual content; Andrey B. Goryachev – literature search and analysis, writing and execution of the final manuscript version, tracking the article publication; Svetlana A. Mikhailova – description and analysis of the results obtained, manuscript writing and its final publication approval; Olga I. Knysh – manuscript writing, a critical research analysis.

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RESEARCH ARTICLE

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ACTUAL PROBLEMS OF PROFESSIONAL AND PERSONAL DEVELOPMENT QUALIFIED PERSONS RESPONSIBLE FOR QUALITY OF MEDICINAL PRODUCTS FOR HUMAN USE

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The aim of the study is to investigate topical problems of the professional and personal development of qualified persons responsible for quality of medicinal products for human use.
Materials and methods. In the period from April 6 to May 10, 2020, an online survey of leading employees in the field of quality assurance of Russian manufacturers was conducted. 176 people took part in the survey; the return of questionnaires was about 17.9%.
Results. From the standpoint of D. Super's theory of professional development, the largest number of respondents was at the maintenance stage, holding their achieved positions (53.2%). All respondents, regardless of age, were motivated for professional development. Most often qualified persons had chemical engineering (27.3%) and pharmaceutical education (22.2%). Most of them had a working experience in 1–2 divisions of the enterprise, and combined the functions of qualified persons with managerial positions (74.5% and 71.9%, respectively). The majority of the qualified persons (86.4%) indicated the sufficiency of the available knowledge and the lack of knowledge on certain issues. Knowledge and skills in the quality risk management, specific GMP issues and statistical methods (59.0%, 49.2 and 44.2%, respectively); communication and interpersonal skills and, in particular, stress management, emotion management and the art of negotiation (49.4%, 41.3% and 40.9%, respectively), were most popular. About 36% of respondents notified the need for the digital economy competencies, while only 5.1% notified the presence of an electronic batch production record at the enterprise. Finally, only half of the respondents (50.5%) had a formal training plan for qualified persons.
Conclusion. This pilot study revealed the need for the revision of the Exemplary Additional Professional Training Program for Qualified Persons and the professional standard, the urgent need for the regulatory body to develop a scheme and principles for the continuous professional development of qualified persons, and showed the direction of further research in this area.
Keywords: qualified person; pharmaceutical company; professional development; medicines; additional vocational training
Abbreviations: GMP – Good Manufacturing Practice; RSC – Royal society of chemistry; CPD – Continuing professional development; CE – continuous education; EAPTP QPs – Exemplary Additonal Professional Training Program for Qualified Persons; Ps – Pharmaceuticals; QCD – Quality Control Division; QAD – Quality Assurance Division; QP – qualified person; PQS – Pharmaceutical Quality System.

АКТУАЛЬНЫЕ ПРОБЛЕМЫ ПРОФЕССИОНАЛЬНО-ЛИЧНОСТНОГО РАЗВИТИЯ УПОЛНОМОЧЕННЫХ ЛИЦ ПО КАЧЕСТВУ ПРОИЗВОДИТЕЛЕЙ ЛЕКАРСТВЕННЫХ СРЕДСТВ ДЛЯ МЕДИЦИНСКОГО ПРИМЕНЕНИЯ

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Цель. Изучение актуальных проблем профессионально-личностного развития уполномоченных лиц по качеству производителей лекарственных средств для медицинского применения.
Материалы и методы. В период с 6 апреля по 10 мая 2020 года был проведен онлайн-опрос ведущих сотрудников в области обеспечения качества российских производителей. Участие в опросе приняло 176 человек, возврат анкет составил около 17,9%.
Результаты. С позиций теории о профессиональном развитии Д. Сьюпера наибольшее количество респондентов находилось на этапе поддержания, сохранения достигнутых позиций (53,2%). Все респонденты вне зависимости от возраста мотивированы на профессиональное развитие. Наиболее часто уполномоченных лиц имели химико-технологическое (27,3%) и фармацевтическое образование (22,2%). Большинство имели опыт работы в 1–2 подразделениях предприятия, а также совмещали функции уполномоченных лиц с руководящими позициями (74,5% и 71,9%, соответственно). Большинство уполномоченных лиц (86,4%) указали достаточность имеющихся знаний и нехватку знаний по отдельным вопросам. Наиболее востребованными оказались знания и умения по управлению рисками для качества, специфические вопросы GMP и статистические методы (59,0%, 49,2% и 44,2%, соответственно); коммуникативные и межличностные умения и, в частности, управление стрессом, управление эмоциями и искусство переговоров (49,4%, 41,3% и 40,9%, соответственно). Около 36% респондентов отметили потребности в компетенциях цифровой экономики, и при этом только 5,1% отметили наличие на предприятии электронного досье на серию. И наконец, всего лишь у половины респондентов (50,5%) имелся формальный план обучения уполномоченных лиц.
Заключение. Данное пилотное исследование выявило необходимость пересмотра примерной дополнительной профессиональной программы повышения квалификации уполномоченных лиц и профессионального стандарта, острую необходимость разработки регуляторным органом схемы и принципов непрерывного профессионального развития уполномоченных лиц, а также показало направление дальнейших исследований в этой области.
Ключевые слова: уполномоченное лицо по качеству; фармацевтическое предприятие; профессиональное развитие; лекарственные средства; дополнительное профессиональное обучение
Список сокращений: GMP – Надлежащая производственная практика; RSC – Королевское химическое общество Великобритании; CPD – Непрерывное профессиональное развитие; CE – непрерывное образование; ДПП УЛ – Примерная дополнительная профессиональная программа повышения квалификации уполномоченных лиц; ЛС – Лекарственные средства; ОКК – Отдел контроля качества; ООК – Отдел обеспечения качества; УЛ – Уполномоченное лицо по качеству; ФСК – Фармацевтическая система качества.

INTRODUCTION

The personnel professional development, especially that of key employees, is considered the most important element of the enterprise management and one of the conditions for the success of their activities [1-9]. Depending on the theoretical approaches to the study of this process, different definitions are used. From the point of view of psychologists, “professional development is a change in the psyche in the process of mastering and performing vocational, educational, labor and professional activities” [1]. From the standpoint of acmeology, this is “the process of actualizing the potential of an individual and achieving the highest forms of professionalism” [7]. Many researchers emphasize the idea that the basis of professional development is self-development as “the process of transforming one’s own life into an object of practical transformation in connection with the requirements of a professional activity, leading to creative self-realization in the profession” [10], self-directed learning and self-esteem [11, 12].

From the standpoint of sociology, professional development can be described as a process of socialization of an individual with the meaning of their organization activities, the need for respect and consideration of opinions when making decisions [13]. The Royal Society of Chemistry of Great Britain (RSC) defines continuous professional development of a chemist as the responsibility of individuals to systematically maintain, improve and expand knowledge and skills to ensure professional competence throughout their working life (career)[1]. The Irish Pharmaceutical Society, in its Professional Development Models report, emphasizes that continuing professional development (CPD) is a self-directed process that allows professionals to develop and deepen a wide range of knowledge, skills and motivations consistent with their current and future work activities. The need to separate continuing professional development (CPD) and continuous education (CE) is also highlighted. Under CE, it is suggested to consider structured educational experience (planned training) and practical activities in the postgraduate period in order to improve and expand knowledge, skills and competencies. As a self-governing process, CDP involves the specialists’ determination of their educational and other needs, an assessment of the achievement of current goals and objectives of their development. CE is one of the components of professional development[2].

[1] The Royal Society of Chemistry – Continuing Professional Development. Available from: https://www.rsc.org/cpd/
[2] Pharmaceutical Society of Ireland Review of International CPD Models. Final report. – PSI, Dublin Ireland, 2010. Available from: ttps://www.thepsi.ie/Libraries/Education/PSI_International_Review_of_CPD_Models.sflb.ashx.

The research of the professional development problems is aimed at finding approaches and methods to improve the management of an organization, in particular, the management of personnel and their professional training, both internal (corporate) and external; psychological and personal aspects, a staff motivation. The problems of national schemes for attestation (accreditation) of specialists in regulated professions (medical and pharmaceutical specialists, aviation specialists, teachers and lecturers, etc.) and their effectiveness for ensuring the life and safety of the population are also investigated.

For a drug manufacturer, professional development of the personnel, in particular, a qualified person (QP), as well for other regulated professions, this is also a prerequisite for carrying out production activities. Thus, in the Rules of Good Manufacturing Practice (GMP) which are in force in the Russian Federation and in the Eurasian Economic Union (EAEU) states parties, Appendix 16 explicitly states: "Qualified persons must maintain their qualifications up to date in the light of scientific and technological progress and take into account changes in the management system quality related to products, the compliance of which with the established requirements is confirmed by an qualified person". In EU GMP, there are similar requirements[3]. Therefore, professional development issues are not only the responsibility of an qualified person, but should be also included in the scope of the pharmaceutical quality system of the enterprise. The considered professional group in our country is characterized by a structural development, when the most competent employee is selected to perform these functions [5].

Requirements for the qualified person's qualifications have been established by regulatory legal acts, since this person is personally responsible for the release of a series of medicinal products into civilian circulation and is often forced to make up difficult decisions in the conditions of uncertainty. Thus, the legislation determines that "the following specialists are allowed for certification: the ones who have at least 3 years of experience in the field of production, or quality assurance, or quality control of medicines and completed higher education in one of the following areas – chemical, chemical-technological, chemical pharmaceutical, biological, biotechnological, microbiological, pharmaceutical, medical". Qualified persons must also undergo training in 12 chemical, biomedical and pharmaceutical disciplines, or when receiving higher education, or as a part of additional professional training[4]. Similar requirements are established in all the countries of the Eurasian Economic Union[5] and are available in all the countries of the European Union[6]. The main labor actions of qualified persons when confirming the compliance of each batch of a medicinal product and releasing the batch into civil circulation, are indicated in GMP[7]. The description of the labor functions, knowledge and skills of the qualified person can be found in the professional standard "Expert on manufacturing pharmacy in the field of Pharmaceutical Quality Assurance". It was approved by the Order of the Ministry of Labor of Russia dated 05.22.2017 No.429n (Labor function B / 05.7 Evaluation of the batch production record of a medicinal product with registration of a decision on release into circulation)[8]. There is an exemplary additional professional training program of advanced vocational training for qualified persons, approved by the Ministry of Health of Russia[9]. Thus, the state participates in the formation of the personnel potential of the pharmaceutical industry, although, as in other industries, according to experts, it is not very effective [14].

[3] [1] Order of the Ministry of Industry and Trade of Russia of June 14, 2013 No. 916 (as amended on December 18, 2015) "On the approval of the Good Manufacturing Practice Rules" (Registered at the Russian Ministry of Justice on September 10, 2013 No. 29938). [2] Rules of Good Manufacturing Practice of the Eurasian Economic Union, approved by the Decision of the Council of the Eurasian Economic Commission dated 03.11.2016 No. 77. [3] EudraLex – Volume 4 – Good Manufacturing Practice (GMP) guidelines.

[4] [1]. Order of the Ministry of Health of the Russian Federation "On Approval of the List of Documents Submitted by a Certified Qualified Person of a Manufacturer of Medicines of a Member State of the Eurasian Economic Union, Stages of the Procedure and Procedure for Making Decisions on Certification of Qualified Persons of Manufacturers of Medicinal Products for Medical Use in accordance funds approved by the decision of the Council of the Eurasian Economic Commission of November 3, 2016 No. 73 "On the procedure for certification of qualified persons of drug manufacturers." No. 73 "On the Procedure for Attestation of Qualified Persons of Medicinal Products Manufacturers. [3] Federal Law "On the Circulation of Medicines" dated 12.04.2010 No. 61-FZ.

[5] Agreement on uniform principles and rules for the circulation of medicines within the Eurasian Economic Union dated December 23, 2014.

[6] Directive 2001/83/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to medicinal products for human use.

[7] [1] Order of the Ministry of Industry and Trade of Russia of June 14, 2013 No. 916 (as amended on December 18, 2015) "On the approval of the Good Manufacturing Practice Rules" (Registered at the Russian Ministry of Justice on September 10, 2013 No. 29938). [2] Rules of Good Manufacturing Practice of the Eurasian Economic Union, approved by the Decision of the Council of the Eurasian Economic Commission dated 03.11.2016 No. 77. [3] EudraLex – Volume 4 – Good Manufacturing Practice (GMP) guidelines.

[8] Order of the Ministry of Labor of Russia dated May 22, 2017 No. 429n "On the approval of the professional standard" Specialist in industrial pharmacy in the field of quality assurance of medicines "(Registered with the Ministry of Justice of Russia on July 20, 2017 No. 47480).

[9] Order of the Ministry of Health of Russia dated January 22, 2014 No. 37-n "On the approval of exemplary additional professional pharmaceutical education programs" (Appendix No. 2) (Registered with the Ministry of Justice of Russia on April 18, 2014 No. 3203).

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Despite rather a long period (almost 8 years) of the formation of the professional group in Russia considered in this article, and the importance of the qualified persons' professional development for the health of the population, no publications on the professional development of qualified persons of Russian drug manufacturers and related issues, had been found. Therefore, **THE AIM** of the described pilot study was to investigate the current situation in this area.

MATERIALS AND METHODS

The study of relevant problems of qualified persons' professional and personal development was carried out by a questionnaire method. A broad educational need was investigated both in the professional knowledge and skills described above, which belong to the category of "hard-skills", and universal competences (over-professional skills, skills of the 21st century, etc., soft-skills) that qualified persons should have as highly qualified specialists [15–17]. In this study, the structure of universal competencies given in the "Target Model of Competencies 2025", was used[10].

The questionnaire included 42 questions related to various aspects of this professional group's work in our country, as well as problems of professional development, socio-demographic and professional factors influencing it. Depending on the position from which professional development is considered, various factors affecting it are distinguished. They are external (regulatory requirements and recommendations), internal (work with personnel in the organization, financial and procedural opportunities, etc.). Very importantan factors are personal (professional potential, motivation, personal goal-setting, etc.); socio-demographic (gender, age, social status, education) and psychophysiological (psychophysiological potential, goal commitment, a sense of mastery, interpersonal interaction); social and professional (the content of the profession, ways of performing professional tasks, professional experience) and socio-economic (the level of wages, the demand for certain professional knowledge and skills, "professional success", etc.) [2–4, 8, 18–20].

When developing the questionnaire, the provisions of regulatory legal acts related to the qualified persons' professional development, were taken into account[11]. 27 people with knowledge of qualified persons' work took part in checking the readability and clarity of the questionnaire.

The survey was conducted online by Sechenov University in cooperation with the National Chamber of Pharmacy from April 10 to April 30, 2020. The questionnaires were sent by email to potential respondents (982 people, 48 constituent entities of the Russian Federation, more than 300 enterprises). 176 people took part in the survey; respectively, the return of questionnaires was about 17.9%. All respondents are acting qualified persons or have performed functions of qualified persons in the past (96% and 4%, respectively). Most of the respondents are female (86.2%), have been working at pharmaceutical enterprises for more than 10 years (72.6%).

RESULTS AND DISCUSSION

The resulting sample includes employees of various sizes enterprises, producing various dosage forms (Fig. 1).

According to the modified theory of D. Super, the respondents are at different stages of professional development [3, 5, 21]:

1) at the stage of stabilization, consolidation and promotion – 26.6%;

2) at the stage of maintaining, keeping the achieved positions – 53.2%;

3) at the stage of declining professional and social activities – 20.2%.

It should be notified that no unwillingness to study and develop professionally in the age groups in the range of 40-60 years, which should have been expected from the literature, have been revealed [2, 3]. On the contrary, all respondents, regardless of age, are motivated for professional development.

The distribution of qualified persons by vocational education is shown in Fig. 2. The most common are chemical engineering (27.3%) and pharmaceutical education (22.2%).

Slightly more than a third of survey participants (37.5%) have work experience in only one department: quality control department (QC), quality assurance department (QA) or a production unit; 36.9% have experience in two divisions (the combinations of the aforesaid plus a regulatory division), the rest have experience in three or more divisions; 71.6% of the respondents are heads of enterprise structural divisions or occupy even higher administrative positions. These data make possible to conclude that a combination of horizontal (a change in the professional and functional activity areas) and vertical (advancement in the organizational and managerial hierarchy) directions, is characteristic for the professional and official development of qualified persons [5].

When asked about the sufficiency of knowledge

[10] Butenko V, Polunin K, Kotov I, Sycheva E, et al. Russia 2025: from personnel to talents. The Boston Consulting Group, 2017: 70 p. Russian
[11] The Royal Society of Chemistry – Continuing Professional Development. Available from: https://www.rsc.org/cpd/

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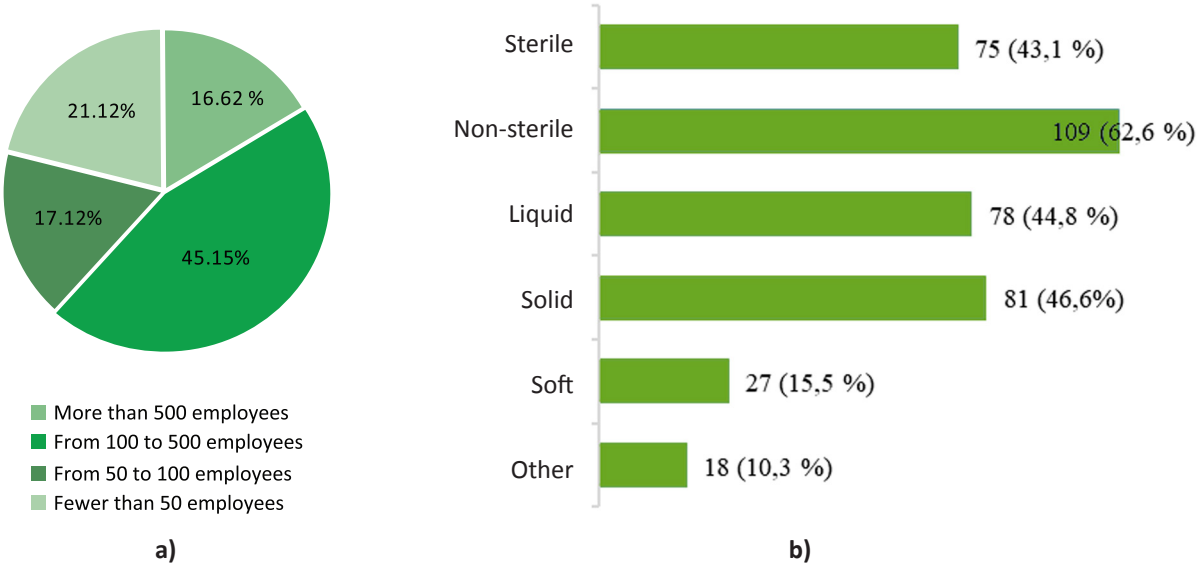

Figure 1 – General characteristics of qualified persons who took part in the survey
Note: a) distribution of respondents by enterprise size; b) dosage forms produced by the enterprises where the respondents work

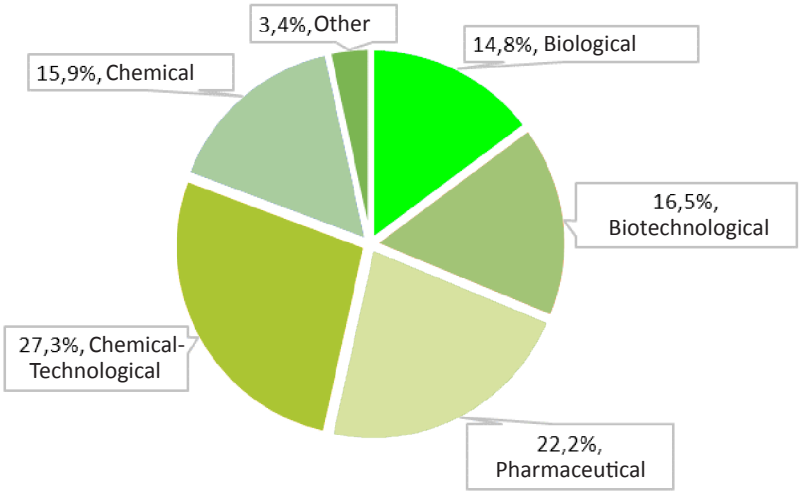

Figure2 – Distribution of respondents by vocational education

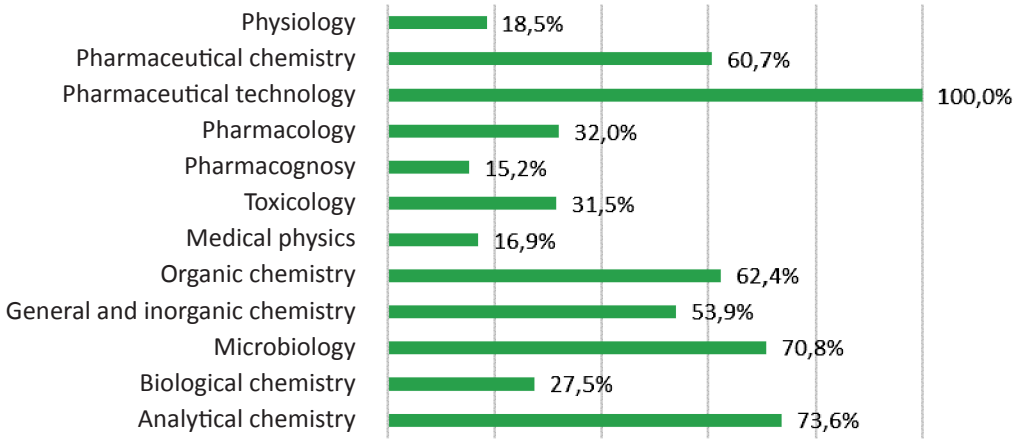

Figure 3 – Use of knowledge of compulsory subjects in qualified persons' practical activities

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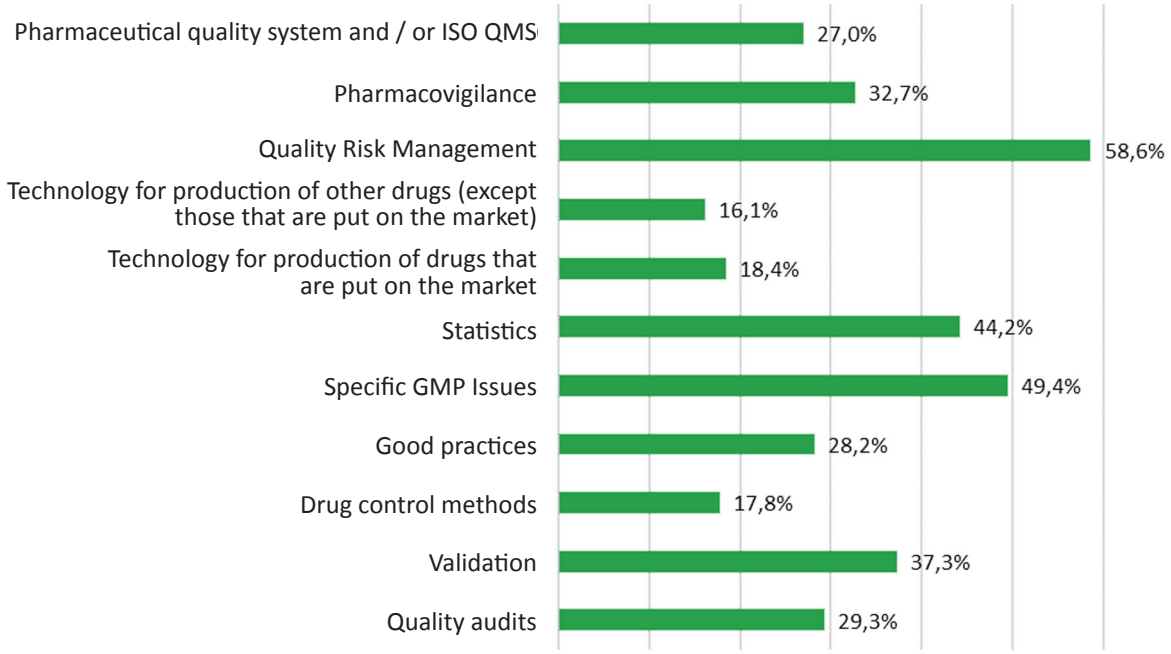


Figure 4 – Qualified persons' need for training in various professional competencies (hard skills)

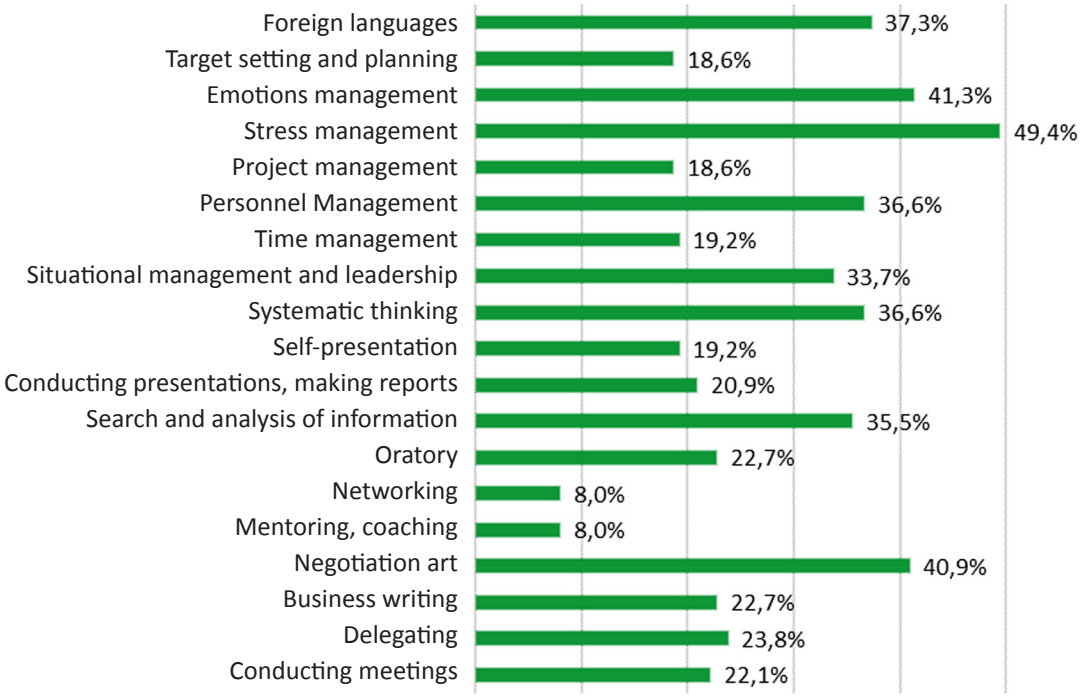
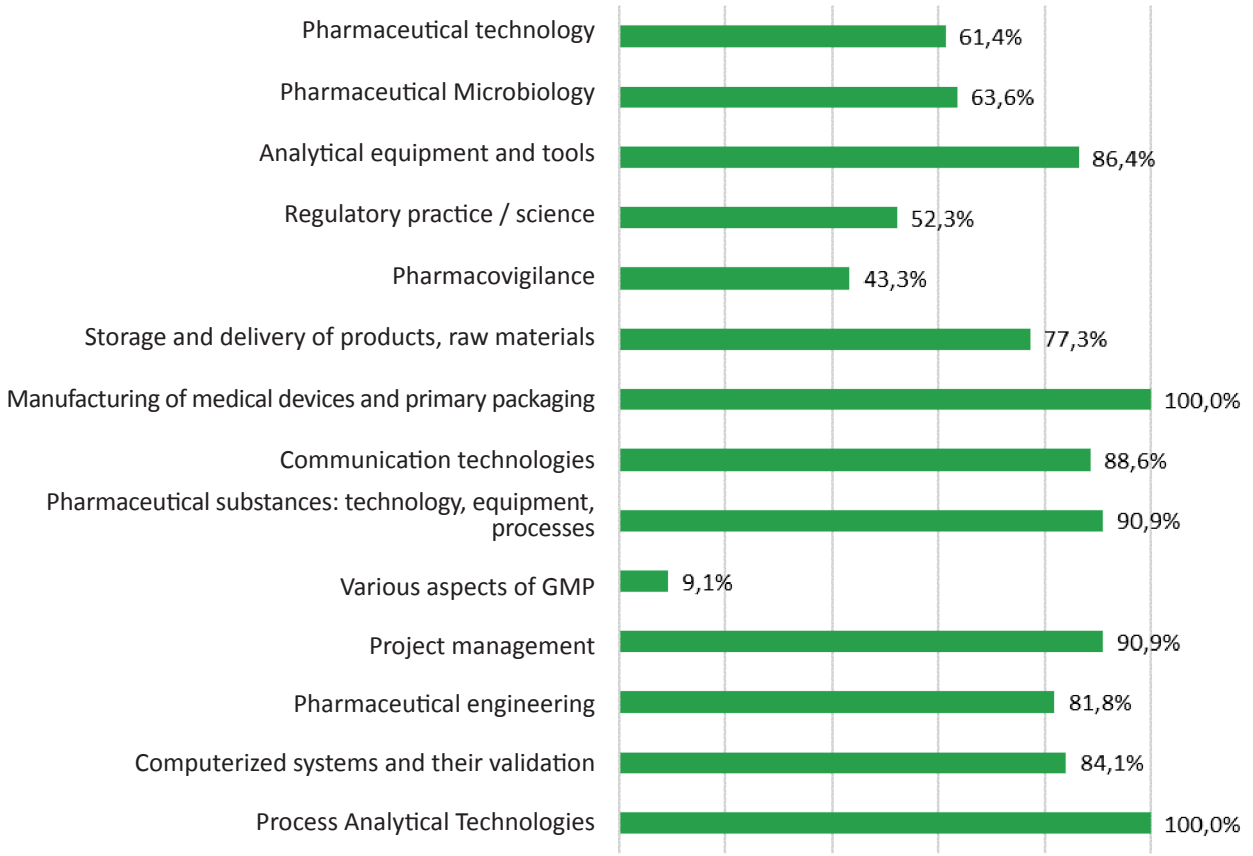


Figure 5 – Qualified persons' need for training in various universal competencies (soft skills)



Figurer 6 – Topics not presented in qualified persons' external and internal training programmes in 2018–2021
Note: according to reports on professional activities in Term 1, 2021

to perform the functions of qualified persons, only 9% of the respondents answered unequivocally in the affirmative. This group included people with different kinds of education, but mainly with biotechnological and chemical-technological ones (50.0% and 31.2%, respectively), at different stages of professional development, but with an equally long experience of work at pharmaceutical enterprises (100% – more than 10 years). Even fewer respondents (4.5%) indicated that their education is insufficient to perform the discussed functions. This group was also dominated by persons with chemical-technological education (62.5%), which is explained by a great number of qualified persons with this kind of education in the obtained sample, as well as by a great number of profiles (directions) of educational programs in chemical technology, many of which do not include study of industrial pharmacy problems. Only 14.6 percent of the respondents indicated that their knowledge is sufficient to perform the functions of qualified persons, but at the same time, they still notified the presence of an educational need for both professional and universal competencies. The data obtained practically coincide with the data of the survey carried out in 2012: 71.2% of employers and 100% of the surveyed specialists in the pharmaceutical industry noted the need to acquire new knowledge in order to fulfill their official duties [22]. In general, these data show, on the one hand, the effectiveness of the regulatory requirement for qualified persons' mandatory professional development once every 5 years[12] (their knowledge is updated and there are no significant gaps in the current knowledge). On the other hand, the obtained data show the need to optimize the content of the above-mentioned additional professional programs and, indirectly, the fact that enterprises do not allocate sufficient resources for the professional development of qualified persons.

The data on the use of knowledge on the disciplines compulsory for studying by qualified persons in professional practice, are shown in Fig. 3. Noteworthy is rather a small number of qualified persons using knowledge of pharmacology (32.0%); the largest percentage of them are persons with pharmaceutical education (40.4%). These results can be explained, first, by the time period when the survey was conducted (2020): the requirements for the compulsory study of pharmacology and other disciplines described above, by qualified persons, although established in 2016, came into effect on the territory of our country only in 2021. Second, these results can be explained by the absence of this and other biomedical disciplines in engineering and natural science educational programs.

To answer the question about the required profes-

[12] Resolution of the Government of the Russian Federation of 06.07.2012 No. 686 "On approval of the Regulation on licensing the production of medicines".

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sional (hard skills) and universal (soft skills) competencies, the respondents were offered an open list of knowledge and skills with the ability to indicate additional competencies that had not been included in the questionnaire. The data obtained are shown in Fig. 4 and 5.

The most popular were knowledge and skills in quality risk management (58.6%), which is explained by their almost widespread use in GMP-regulated organizations. It should be notified that these results coincide with the data of the authers' 2020 survey on the industry practice of quality risk management in the Russian pharmaceutical industry, in which 59.0% of the respondents emphasized the lack of specialists with competence in quality risk management [23]. Quite a lot of respondents also notified the need to study specific GMP issues (the narrow issues highlighted in the annexes of the rules and various guidelines) and statistical methods (49.2% and 44.2%, respectively). The authors were unable to determine any significant correlations in the identified educational needs with socio-demographic and professional factors. This need was not influenced by qualified persons' vocational education, age and work experience in the pharmaceutical industry, the nature of products and the size of the enterprise, the number of qualified persons at the enterprise, the position held and work experience in various divisions of the enterprise (QCD, QAD, etc.). The least popular were knowledge and skills in the field of drug production technology and pharmacopoeial analysis (18.4 and 17.8%, respectively). In the group that indicated the presence of such an educational need, there was a fairly large number of respondents with education in the field of natural sciences (38.0%), which, in the authors' opinion, is explained by the absence of pharmaceutical technology and pharmacopoeial analysis in educational programs in biology and chemistry. The authors did not find any correlation with other socio-demographic and professional factors. The identified educational needs of qualified persons with the Exemplary Additonal Professional Training Program for Qualified Persons[13] (EAPTP QPs) – manufacturers of medicines for medical use, were compared. The modules "Pharmaceutical development and production of dosage forms", "Pharmaceutical analysis and quality control of medicines", "Development and production of pharmaceutical substances" turned out to be the least demanded or the most studied out of 10 modules of the program (less than 20% of respondents). Taking into account the current requirements for the compulsory mastering of 12 biomedical and pharmaceutical disciplines, the EAPTP QPs modules related to these issues should be shortened or eliminated. At the same time, the expansion and deepening of the content of such modules as "Quality Management System of a Pharmaceutical Enterprise", "Statistical Methods Used in a Pharmaceutical Enterprise" are definitely required. It is also necessary to include a module on the basics of pharmacovigilance in EAPTP QPs, and to highlight the issues of validation in an independent module at a pharmaceutical enterprise. Since the survey was conducted among the acting Qualified Persons, it may be necessary to have two Exemplary Additonal Professional Training Programs of Qualified Persons at different levels (beginners' and advanced).

The most demanded professional knowledge and skills identified during the questionnaire, are also indicated in the list of knowledge and skills necessary to perform labor function B / 05.7. It is entitled as "Evaluation of the batch production record of a medicinal product with registration of a decision on release into circulation)[14]: a pharmaceutical quality system, quality audits, quality risk management, methods of statistical quality management, mathematical statistics used in assessing the results of tests and validation performed, principles of validation of technological processes and analytical methods, qualifications of premises and equipment, engineering systems. At the same time, only 26.1% of respondents carefully studied this professional standard, and other 38.6% have just looked it through.

Thus, the identified educational needs of qualified persons in terms of professional knowledge and skills indicate the shortcomings of the Exemplary Additonal Professional Training Program for Qualified Persons, the need for an even closer connection between the EAPTP QPs and the corresponding professional standard, as well as the insufficient skills of qualified persons and drug manufacturers to assess their educational and other needs, and plan professional development using professional standards. It can be also argued that the impact of professional standards on the content of advanced professional education, professional programs, including the ones for qualified persons, is not the same as expected by the regulatory body in the field of education[15]. According to the regulation on federal state control (supervision)[16], the work of the organizations carrying out activities in the field of advanced professional education (APE), is not a subject of a federal state control (supervision) and is carried out by the constituent entities of the Russian Federation within the framework of licensing control. Herewith, that does not include the

[13] Order of the Ministry of Health of Russia dated January 22, 2014 No. 37-n "On the approval of exemplary additional professional pharmaceutical education programs" (Appendix No. 2) (Registered with the Ministry of Justice of Russia on April 18, 2014 No. 3203).

[14] Order of the Ministry of Health of Russia dated January 22, 2014 No. 37-n "On the approval of exemplary additional professional pharmaceutical education programs" (Appendix No. 2) (Registered with the Ministry of Justice of Russia on April 18, 2014 No. 3203).

[15] Order of the Ministry of Labor of Russia dated May 22, 2017 No. 429n "On the approval of the professional standard" Specialist in industrial pharmacy in the field of quality assurance of medicines" (Registered with the Ministry of Justice of Russia on July 20, 2017 No. 47480).

[16] [1] Letter of the Ministry of Education and Science of Russia dated 09.10.2003 No. 06-737 "On additional professional education", [2] Methodological recommendations for the development of basic professional educational programs and additional professional programs, taking into account the relevant professional standards, approved by the Minister of Education and Science D.V. Livanov 01/22/2015 No. DL-1 / 05vn.

assessment of the content of APE programs. Therefore, the assessment of educational programs is solely the responsibility of qualified persons and a pharmaceutical enterprise (for example, on compliance with GMP, professional standards and the Exemplary Additonal Professional Training Program for Qualified Persons, identified educational needs of qualified persons). Unfortunately, there was no significant improvement in the situation with APE in the field of industrial pharmacy in comparison with the authors' assessment carried out in 2012 [24].

In general, the most demanded universal competencies among qualified persons were communicative and interpersonal skills from the group of socio-behavioral skills, and, in particular, stress management and emotions management (49.4% and 41.3%, respectively). These data can be explained by qualified persons' working conditions, including relations with other structural divisions of the enterprise: almost two or three respondents from this group (67.3%) reported the presence of conflict situations in the performance of their professional functions (in the entire sample obtained there are less than 58% of them). This, in our opinion, explains the demand for the art of negotiations (40.9%). It should be notified that these skills are also specified in the discussed professional standard, but are absent from the Exemplary Additonal Professional Training Program for Qualified Persons.

The average demand (about 20–22% of respondents' answers) for managerial competencies (conducting meetings, delegating, business writing, etc.) practically coincided with the number of respondents combining the functions of qualified persons with the functions of the top administrative head (29.2%). Although, in general, the number of respondents in managerial positions (the head of a department, laboratory or production site and higher positions) was 71.5%, which can be explained by both the presence of a hidden educational need of qualified persons and the absence of the employer's need to develop managerial skills among middle managers. Moreover, there is no correlation between the educational need for managerial competencies and the size of a pharmaceutical company.

It was also impossible to identify socio-demographic and professional factors that determined a small number of respondents who indicated the need for training in mentoring (coaching) and networking (8% each). Most of the qualified persons who took part in the survey are, probably, not involved in the system of internal personnel training, or are not sufficiently informed about the composition of these competencies, and the lack of the need for networking training is associated with a high degree of closeness of Russian enterprises, the lack of qualified persons' culture of collaboration in the domestic pharmaceutical industry. On the other hand, the data obtained correlate with the results of the carried out survey of employers and pharmaceutical industry professionals in 2012 [22]. Then the competences in teaching and educational activities, including mentoring, were listed among the most popular (6 points out of 10 possible), and, perhaps, their demand by employers led to the following result: for example, such training at the majority of enterprises is included in corporate educational programs. Rather a small number of respondents (35.5%), who indicated the presence of educational needs for the search and analysis of information, was also unexpected. On the one hand, it can indicate a fairly high level of digital literacy among qualified persons (in 2012, this competence was among the most demanded – 7.2 points out of 10 possible). On the other hand, it can be caused by the absence of a real need of employers for these competencies of their employees due to the low speed of digital transformation of the Russian pharmaceutical industry. For example, only 5.1% of respondents indicated that the evaluation of the batch production record is electronically maintained at the enterprise. There is also no state attention to the formation of digital economy competencies among graduates of educational programs in chemical technology, pharmacy, biology, medicine: within the framework of the Federal Target Program "Personnel for the Digital Economy" it is believed that only graduates of chemistry and biotechnology programs develop two or more such competencies[17]. The authors believe that more substantive and in-depth research is required to unambiguously assess the situation and understand the factors influencing the development of the competencies of the Federal Target Program in digital economy.

A practice analysis of planning qualified persons' professional development at Russian pharmaceutical enterprises showed that only half of the respondents (50.5%) have a formal training plan for qualified persons', while only 34% of them undergo formal internal certification, which indicates employers' lack of attention to the problems of qualified persons' professional development. To analyze the contents of plans, topics, forms and modes of teaching qualified persons, 60 reports on professional activities reviewed by the Expert Group of the Sechenov University Attestation Commission for the certification of qualified person – manufacturers of medicines for medical use of the Ministry of Health of Russia (hereinafter the "Expert Group"), were studied. All the reports were submitted to the Expert Group in the first half of 2021 and, according to the new template, included training data for the reporting period (i.e., for the last 3 years)[18]. In half of the reports, there was no mention of the past internal training (53.3%), which was confirmed by the data of the carried out survey. In 72.9%

[17] Decree of the Government of the Russian Federation of June 25, 2021 No. 997 "On approval of the Regulation on federal state control (supervision) in the field of education."

[18] Order of the Ministry of Economic Development of Russia dated January 24, 2020 No. 41 "On Approval of Methods for Calculating Indicators of the Federal Project" Personnel for the Digital Economy "of the National Program" Digital Economy of the Russian Federation " and more core competencies of digital competencies).

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of reports, internal training related to the implementation of the EAEU GMP Rules at the enterprise. Of the 32 people who indicated the presence of internal training, only half (16 people) indicated that they underwent it annually. All the qualified persons who applied for certification in the Expert Group (hereinafter referred to as "applicants") underwent external training, and 80% – in addition to the advanced training program developed by the Exemplary Additonal Professional Training Program for Qualified Persons, studied at educational webinars on certain issues of GMP Rules, registration of medicines in the EAEU, validation and qualifications at a pharmaceutical enterprise. About one third of the applicants underwent external training not more frequently than 5 years, 20% – from 3 to 5 years, and the rest – almost every year. The data on the topics of internal and external training are shown in Fig. 6. Most often, Supplementary Programmes for Qualified Persons were trained in various aspects of the GMP Rules, more than half of these Programmes studied pharmacovigilance (56.7%, almost equally in internal and external training), regulatory practice / science (47.7%). There was practically no training in process analytical technologies, the production of medical devices and primary packaging, the technology for the production of pharmaceutical substances, project management, and others (Fig. 6). No correlation either with the size of the pharmaceutical company and the types of products manufactured, or with socio-demographic and professional factors, has been found. In general, according to the reports on the professional activity of the qualified persons, the results of the analysis of training coincided with the trends identified during the survey.

Currently, EAEU GMP Appendix 16[19] aimed at harmonizing it with the similar GMPEC text, is being revised. In the current EU version of this annex, there is a requirement that qualified persons should prove their continuous learning in relation to the type of product, technological processes, technical innovations and GMP changes (the term "continuing" is used in the EAEU draft). The volume of continuing education required, and the type of training acceptable by the regulator, and the type of training evidence, have not been clearly defined. At the same time, in other documents related to qualified persons, for example, in the UK, recommendations on how to ensure the fulfillment of the requirements under consideration, can be found.

Professional development activities are a condition for qualified persons' annual renewal of the membership in a trade union (a prerequisite for qualified persons' certification in the UK). The members of the society send a short report on professional activities to the secretariat. They reflect the maintenance of 12 professional competencies defined by this society, and 5 types of educational activities determined by the Science Council of Great Britain[20] and are randomly checked (the qualified persons' certification was examined in detail in this country [24]). This approach was also used in the template for the annual report of qualified persons' RSC on continuing professional development: the Supplementary Programme is recommended to correlate the following types of professional development with professional activities and performance of office duties, and attach the relevant evidence:

– on-the-job training (performing the functions of a staff internship / student internship manager, developing training proposals, writing reports);

– professional activities (participation in a professional society, mentoring);

– formal training (writing scientific and popular scientific articles / documents, additional professional training);

– self-study (reading magazines, reviewing books and articles);

– other (intellectual volunteering, social activities).

The following examples are indicated as evidence in the template: certificates and testimonies, training materials, reports, a list of studied publications, reviews.

The UK Qualified Persons' Code of Practice has an entire professional development[21] section that details the GMP and professional society requirements discussed above. Additionally, recommendations are given on the procedure for fulfilling the GMP requirements on preliminary training in case of significant changes in qualified persons' labor functions. For example, these can be: changing / expanding the range of medicinal forms released into circulation); when moving to a new place of work and when returning to the activities of qualified persons' after a break: the presence of a training plan approved by the management, which indicates the identified gaps in knowledge and skills and the required training with a time schedule.

As the foregoing example shows, the choice of form(s) and matter(s) of professional development falls on qualified persons themselves. On the other hand, the presence of qualified persons' professional development is a GMP requirement, and, accordingly, is included in the complex of actions of a pharmaceutical company to comply with all established requirements. Therefore, in the authors' opinion, the presence of clear criteria for assessing the adequacy of professional development, established by the regulatory body in the field of assessing the compliance of an enterprise with GMP requirements, by analogy with the regulation and organization in our country and

[19] Orlov VA. An overview of the main innovations in the new version of Appendix No. 16 to GMP rules – "Requirements for confirmation by an qualified person of the conformity of a series of products for the purpose of its release". Available from: https://gilsinp.ru/?wpfb_dl=369

[20] The Science Council Continuing Professional Development. Standards for Registrants. Available from: https://sciencecouncil.org/web/wp-content/uploads/2021/01/Updated-CPD-Standards-for-Registrants.pdf

[21] Qualified Person involved in the manufacture of pharmaceuticals. QP Code of Practice. Available from:https://www.rsc.org/globalassets/09-careers/personal-professional-development/practising-scientists/qpr/qp-code-of-practice-2018.pdf

in the world of continuous medical and pharmaceutical education [25, 26], would have had a significant positive impact on the current situation.

This pilot study did not set the task of identifying the industry practice of organizing internal personnel training, including the methods used in it, which are widely considered in the literature, and assessing their effectiveness [3,4, 12, 27–29]. The study did not investigate the main barriers that hinder qualified persons' professional development, such as lack of time and heavy workload, lack of technical or financial possibilities or doubts about the effectiveness of this process, misunderstanding of the continuous professional development concept [30, 31]. All these problems require a further study.

CONCLUSION

Professional development is the responsibility of qualified persons themselves, as well as the responsibility of the pharmaceutical company. The guidelines for planning professional development are the requirements of the legislation of the country in which qualified persons operate, and other requirements on which their admission to professional activities depends. The professional and official development of qualified persons in our country is characterized by a combination of horizontal (change in the professional and functional areas of activities) and vertical (advancement in the organizational and managerial hierarchy) directions.

The analysis of the open training needs identified in the course of the study, showed that a revision of the Exemplary Additonal Professional Training Program approved by the Ministry of Health of Russia in 2014, is required to improve qualified persons' qualifications of medicines for medical use manufacturers, an update of the professional standard in order to take into account the 2025 (or similar) competency model, and also the competencies of the digital economy.

The identified problems indicate the urgent need for the regulatory authorities to develop schemes and principles for the professional development qualified persons. These will ensure the compliance of individuals and enterprises with the new requirements for this professional group, which are planned to be introduced into Appendix 16 of the Rules of Good Manufacturing Practice of the EAEU, including criteria for CPD of qualified persons, forms and mechanisms for confirming and obtaining the required evidence.

All the foregoing indicates that the problems of qualified persons' professional development are very relevant and require further research, including those identified in this article.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

Zhanna I. Aladysheva – idea, research design development, consultation at all research stages, article writing; Natalya V. Pyatigorskaya – research planning; consultation on the conduct of all study stages, article writing; Vasily V. Belyaev – literature analysis, article writing, consultation on research planning and data processing; Natalya S. Nikolenko – data processing, bibliography formalization; Ekaterina I. Nesterkina – consultation on the conduct of separate study stages; Sofya A. Loseva – data processing, bibliography formalization.

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