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CURRENT STATE OF RESEARCH IN THE FIELD OF CHEMICAL COMPOSITION AND PHARMACOLOGICAL EFFECTS OF *ZEA MAYDIS STYLI CUM STIGMATIS*

E.B. Nikiforova, N.M. Bat, N.A. Davitavyan

Kuban State Medical University
4, Mitrofan Sedin St., Krasnodar, Russia, 350063

E-mail: elenanik94@mail.ru

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*Zea maydis styli cum stigmatis (*Engl. *corn silk)* are well known in medical practice. In the scientific literature, a lot of information has been accumulated about the chemical composition and pharmacotherapeutic actions of this medicinal plant material. A chronological analysis of this information shows that earlier, the scope of scientific publications was mainly devoted to the study of the choleretic, diuretic, hemostatic kinds of *Zea maydis styli cum stigmatis* activity, and the identification of the active substances responsible for these types of action. Currently, there is a growing scientific interest not only in the individual natural compounds of the *Zea maydis styli cum stigmatis* composition, but also in the search for new aspects of their medical application.

The aim of the article is a review and systematization of modern scientific data in the field of the *Zea maydis styli cum stigmatis* chemical composition and their pharmacological action.

Materials and methods. For the information and analytical search, the scientific data posted on the following electronic resources were used: PubMed, Web of Science, ScienceDirect, Scopus, Google Scholar, eLibrary. The search was carried out in the period from 2005 to 2021 using the following keywords: *Zea mays* L.; *Zea maydis styli cum stigmatis*; corn silk; chemical composition; pharmacological action.

Results. The review is devoted to the generalization and analysis of modern scientific data on the *Zea maydis styli cum stigmatis* chemical composition and their pharmacological action. It has been shown that, as before, the greatest attention of scientists is attracted by flavonoids in the *Zea maydis styli cum stigmatis* chemical composition. Alongside with them, phenolcarboxylic acids, vitamin K, phytosterols, volatile compounds and polysaccharides are of no small importance for the *Zea maydis styli cum stigmatis* pharmacological activity. Modern ideas about the *Zea maydis styli cum stigmatis* pharmacological activity have been expanded by summarizing the study results of their antioxidant, anti-inflammatory, antidiabetic, hypotensive, neuro- and photoprotective activities. The data on the effectiveness of their use as parts of the complex tumor diseases therapy have been published.

Conclusion. As a result of the data analysis of modern scientific literature, it has been found out that *Zea maydis styli cum stigmatis* are still in the sphere of scientists' interest. Alongside with the flavonoids of this raw material, other groups of pharmacologically active substances are also being actively studied. It has been revealed that the information about potentially significant and confirmed types of the *Zea maydis styli cum stigmatis* therapeutic action is significantly updated. The results of this review may be useful for identifying promising directions for the development of the drugs based on *Zea maydis styli cum stigmatis*.

Keywords: *Zea maydis styli cum stigmatis*; corn silk; chemical composition; pharmachologic action

Abbreviations: ZMSS – *Zea maydis styli cum stigmatis*; MPRM – medicinal plant raw material; PASs – pharmacologically active substances; MCF-7 – an epithelial-like cell line derived from invasive human breast ductal adenocarcinoma; TBARS – Thiobarbituric acid reactive substances; mRNA – messenger ribonucleic acid.

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СОВРЕМЕННОЕ СОСТОЯНИЕ ИССЛЕДОВАНИЙ В ОБЛАСТИ ХИМИЧЕСКОГО СОСТАВА И ФАРМАКОЛОГИЧЕСКОГО ДЕЙСТВИЯ КУКУРУЗЫ СТОЛБИКОВ С РЫЛЬЦАМИ (ОБЗОР ЛИТЕРАТУРЫ)

Е.Б. Никифорова, Н.М. Бат, Н.А. Давитавян

Федеральное государственное бюджетное образовательное учреждение
«Кубанский государственный медицинский университет»
Министерства здравоохранения Российской Федерации
350063, Россия, г. Краснодар, ул. Митрофана Седина, 4

E-mail: elenanik94@mail.ru

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Кукурузы столбики с рыльцами (КСР) (лат. – *Zea maydis styli cum stigmatis,* англ. – *corn silk)* хорошо известны в медицинской практике. В научной литературе накоплено немало информации о химическом составе и фармакотерапевтическом действии данного лекарственного растительного сырья. Хронологический анализ этих сведений показывает, что ранее ландшафт научных публикаций был посвящен, главным образом, изучению желчегонной, диуретической, кровоостанавливающей активности КСР и выявлению действующих веществ, ответственных за данные виды активности. В настоящее время отмечается нарастающий научный интерес не только к отдельным индивидуальным природным соединениям в составе КСР, но и к поиску новых аспектов их медицинского применения.

Цель. Обзор и систематизация современных научных данных в области химического состава и фармакологического действия КСР.

Материалы и методы. Для информационно-аналитического поиска использовали научные данные, размещенные на электронных ресурсах PubMed, Web of Science, ScienceDirect, Scopus, Google Scholar, eLibrary. Поиск осуществляли за период с 2005 г. по 2021 г. по ключевым словам: кукуруза обыкновенная; кукурузы столбики с рыльцами; *Zea maydis styli cum stigmatis; corn silk;* химический состав; фармакологическое действие.

Результаты. Обзор посвящен обобщению и анализу современных научных данных о химическом составе и фармакологическом действии КСР. Показано, что по-прежнему наибольшее внимание ученых в составе КСР привлекают флавоноиды. Наряду с ними немаловажное значение для фармакологической активности КСР имеют фенолкарбоновые кислоты, витамин К, фитостерины, летучие соединения и полисахариды. Современные представления о фармакологическом действии КСР расширены за счет обобщения результатов исследования их антиоксидантной, противовоспалительной, антидиабетической, гипотензивной, нейро- и фотопротекторной активности. Опубликованы данные, свидетельствующие об эффективности их применения в составе комплексной терапии опухолевых заболеваний.

Заключение. В результате проведенного анализа данных современной научной литературы было установлено, что интерес ученых к КСР не ослабевает. Наряду с флавоноидами данного сырья достаточно активно изучаются и другие группы фармакологически активных веществ. Выявлено, что существенно обновились сведения о потенциально значимых и подтвержденных видах лечебного действия КСР. Результаты данного обзора могут быть полезны для определения перспективных направлений разработки лекарственных средств на основе КСР.

Ключевые слова: кукурузы столбики с рыльцами; *Zea maydis styli cum stigmatis, corn silk;* химический состав; фармакологическое действие

Список сокращений: КСР – кукурузы столбики с рыльцами; ЛРС – лекарственное растительное сырье; ФАВ – фармакологически активные вещества; MCF-7 – эпителиоподобная клеточная линия, полученная из инвазивной аденокарциномы протоков молочной железы человека; ТБК-АП – активные продукты, реагирующие с тиобарбитуровой кислотой; мРНК – матричная рибонуклеиновая кислота.

INTRODUCTION

Medicinal plant drugs do not only remain relevant, but are increasingly becoming objects of research by scientists from around the world. On the one hand, this trend is quite naturally based on a combination of the historically proven effectiveness of their use, and, on the other hand, it is based on a high level of safety in relation to toxic and side effects in comparison with synthetic drugs. In addition, the undoubted advantages of phytopreparations include a mildly increasing effect, often of a multifunctional character, as well as the possibility of using it in patients of various age groups, especially in children and the elderly.

Today, pharmacy and pharmaceutical practice have an extensive arsenal of medicinal plants. At the same time, it should be notified that both new, potentially useful plant sources of pharmacologically active substances (PAS) and those already in demand

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in medical practice, are in the field of researchers' view.

The latter category includes common corn (*Zea mays* L.), which is a producer of a well-known medicinal plant raw material (MPRM) with a pharmacopoeial status of *Zea maydis styli cum stigmatis*, often called corn silk. This raw material is traditionally used as a choleretic, diuretic, and hemostatic agent, due to the rich and diverse *Zea maydis styli cum stigmatis* chemical composition [1].

In the scientific literature, a lot of information has been accumulated about various groups of PASs presented in *Zea maydis styli cum stigmatis*, as well as about the studied and potentially significant types of their pharmacotherapeutic action [2]. A chronological analysis of this information shows that earlier, the scope of scientific publications was mainly devoted to the study of choleretic, diuretic, hemostatic activities of *Zea maydis styli cum stigmatis* and the identification of the active substances responsible for these features [3]. Currently, there is a growing scientific interest not only in the individual representatives of PASs in the *Zea maydis styli cum stigmatis* composition, but also in the study and broadening knowledge about new types of their pharmacotherapeutic action. In particular, within recent years, the results have been obtained and published. They confirmed antioxidant, anti-inflammatory, antidiabetic properties and some other established types of the *Zea maydis styli cum stigmatis* therapeutic action in the extracts and individual PASs obtained from this raw material [4].

Thus, information about the *Zea maydis styli cum stigmatis* biological effect and chemical composition have undergone significant changes. In this regard, it seems relevant to analyze and summarize the scientific information on this issue. The results of such a study, in the authors' opinion, will contribute to the formation of modern ideas about the PAS component composition, the spectrum of its pharmacological activity and the identification of possible prospects for the use of *Zea maydis styli cum stigmatis* to obtain modern effective and safe drugs.

THE AIM of the article is a review and systematization of modern scientific data in the field of the *Zea maydis styli cum stigmatis* chemical composition and their pharmacological action.

MATERIALS AND METHODS

For the information and analytical search, the scientific data posted on the following electronic resources were used: PubMed, Web of Science, ScienceDirect, Scopus, Google Scholar, eLibrary. The search was carried out in the period from 2005 to 2021 using the following keywords: *Zea mays* L.; *Zea maydis styli cum stigmatis*; corn silk; chemical composition; pharmacological action.

RESULTS AND DISCUSSION

1. Zea maydis styli cum stigmatis chemical composition

The *Zea maydis styli cum stigmatis* chemical composition is characterized by a diverse spectrum of PASs. It is represented by phenolic compounds (flavonoids, anthocyanins, and phenolcarboxylic acids), triterpene saponins, organic acids, water- and fat-soluble vitamins, fatty and essential oils, polysaccharides, phytosterols, and some other PASs [5].

1.1. Phenolic compounds

Zea maydis styli cum stigmatis phenolic compounds have a fairly representative composition, in which flavonoids are rightfully the most significant. Their role as the most likely carriers of the *Zea maydis styli cum stigmatis* pharmacotherapeutic activity was previously emphasized. Currently, it is confirmed by the requirements of regulatory documentation regarding the quantitative content of compounds of a flavonoid character [4]. According to the literature data, luteolin and apigenin derivatives are considered the predominant *Zea maydis styli cum stigmatis* flavonoids: maisin, methoxymaisin, apimaisin, vitexin, isovitexin, orientin, isoorientin, and a number of others [6–9]. The structural formulas of the main *Zea maydis styli cum stigmatis* flavonoids are shown in Fig. 1.

As for the quantitative content of flavonoids in *Zea maydis styli cum stigmatis*, according to all kinds of sources, it is about 0.5–0.7%, depending on the variety of the maize and its habitat [10–12].

Among the *Zea maydis styli cum stigmatis* flavonoids, the interest of researchers is currently attracted, first of all, by maisin and its analogs isolated from *Zea maydis styli cum stigmatis* and studied by scientists from different countries in relation to the established and potential types of these compounds' pharmacotherapeutic action [13, 14].

Alongside with the compounds of a flavonoid character, the phenolic groups of *Zea maydis styli cum stigmatis* PASs are represented by phenolcarboxylic acids, among which the presence of chlorogenic, ferulic, caffeic and hydroxycinnamic acids was confirmed in the *Zea maydis styli cum stigmatis* composition [3, 15, 16].

According to the scientific literature and the results of the authors' research, *Zea maydis styli cum stigmatis* are quite rich in polyphenolic compounds. In particular, the qualitative composition and the quantitative content of tannins in *Zea maydis styli cum stigmatis* have been studied [3, 17]. Using the method of high performance liquid chromatography, the qualitative composition of the compounds of this group was established, i.e., the presence of gallic, ellagic acids, and a number of other substances [18].

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1.2. Vitamins

Zea maydis styli cum stigmatis contain a significant amount of compounds of the vitamin origin: vitamins K, group B, ascorbic acid, etc. [2]. At the same time, vitamin K is predominant among the substances of this group.

The presence of vitamin K in the *Zea maydis styli cum stigmatis* composition was established by Prof. Mikhlin in 1941. The scientist was able to isolate a new compound with a vitamin activity from *Zea maydis styli cum stigmatis* and study it in sufficient detail; he called it vitamin K_3. The resulting substance was a complex mixture – the lipoid *Zea maydis styli cum stigmatis* fraction obtained as a result of the combined extraction from *Zea maydis styli cum stigmatis*. As a result of the studies, a correlation was notified between the level of chlorophyll content and the concentration of vitamin K3 in *Zea maydis styli cum stigmatis*: the extracts from *Zea maydis styli cum stigmatis* of the immature corn were significantly more active in comparison with those obtained from ripe yellow ones [3].

1.3. Polysaccharides

In recent years, the *Zea maydis styli cum stigmatis* polysaccharides have been actively studied. The data on their quantitative content and composition have been obtained and published [19].

The results of the studies on the extraction of this PAS group from *Zea maydis styli cum stigmatis* using enzymolysis and an ultrasonic extraction, the study of their physicochemical and pharmacological properties are presented. It was established that the selected group of PAS consisted of rhamnose, arabinose, xylose, mannose, galactose, and glucose [20, 21].

Two fractions were obtained by acid hydrolysis of the *Zea maydis styli cum stigmatis* polysaccharides, subjected to the studies by gel permeation chromatography, gas chromatography, nuclear magnetic resonance, Fourier transform infrared spectroscopy, scanning electron microscopy. The results showed that the studied polysaccharide fractions consisted of xylose, mannose, galactose, rhamnose, arabinose, and glucose [22].

With the use of 1D and 2D NMR, the structural analysis of the *Zea maydis styli cum stigmatis* polysaccharides showed that their constituents are α-D-glucose, α-L-arabinose, β-D-galactose, β-D-mannose, β-D-xylose, α-L-rhamnose [23].

The results of the studies devoted to the research of the interaction between the *Zea maydis styli cum stigmatis* polysaccharides and flavonoids, are of interest. Using molecular dynamics and thermodynamic modeling, the interaction between polysaccharides with different molecular weights and flavonoids is shown. The authors suggested that the adsorption of flavonoids on polysaccharides can be mainly due to van der Waals forces and hydrogen bonds, and the formation of such complexes can enhance the biological activity of the *Zea maydis styli cum stigmatis* polysaccharides [24].

1.4. Saponins

Qualitative reactions and a chromatographic analysis revealed the presence of triterpene saponins (oleanolic and ursolic acids) in *Zea maydis styli cum stigmatis*. In terms of oleanolic acid, the content of this PAS group, determined by using a spectrophotometric method, was about 2.5% on average [3].

1.5. Phytosterols

As mentioned above, *Zea maydis styli cum stigmatis* contain phytosterols: β-sitosterol and stigmasterol, which perform important physiological and therapeutic functions. The results of the studies devoted to the search for an effective extraction technology with solvents having different polarity, purification and crystallization of the *Zea maydis styli cum stigmatis* phytosterols using ultrasound, have been published [25].

1.6. Volatile compounds

Ion chromatography revealed various volatile *Zea maydis styli cum stigmatis* components with a quantitative predominance of alcohols (2,3-butanediol; ethanol, etc.). Alongside with them, the presence of ketones (2,3-butanedione; 3-hydroxy-2-butanone; 3-methyl-2,5-furandione, etc.), aldehydes (benzeneacetaldehyde; heptanol; hexanol, etc.), furans (furan, 2-pentylfuran, 2,3-dihydrobenzenefuran, etc.) and a number of other compounds, has been established [26].

By gas chromatography and mass spectrometry, the qualitative and quantitative composition of the compounds included in the dichloromethane *Zea maydis styli cum stigmatis* extract, was established by El-Ghorab A. et al. The main constituents of this extract were cis-α-terpineol (24.22%), 6,11-oxidoacor-4-ene (18.06%), citronellol (16.18%), trans-pinocamphone (5.86%), eugenol (4.37%), neo-iso-3-tujanol (2.59%) and cis-sabinene hydrate (2.28%) [27].

1.7. Phytohemagglutinins

The *Zea maydis styli cum stigmatis* composition also includes phytohemagglutinins (lectins), which are carbohydrate-protein complexes. The carbohydrate part of the *Zea maydis styli cum stigmatis* lectins is formed by galactose, mannose, glucose, arabinose, xylose. In addition, the traces of rhamnose, uronic acid, glucosamine, galactosamine were found. It has been established that the protein part is represented by asparagine, glutamic acid, glycine, alanine, etc. [3].

Generalized and systematized data on the chemical *Zea maydis styli cum stigmatis* composition are presented in Table 1.

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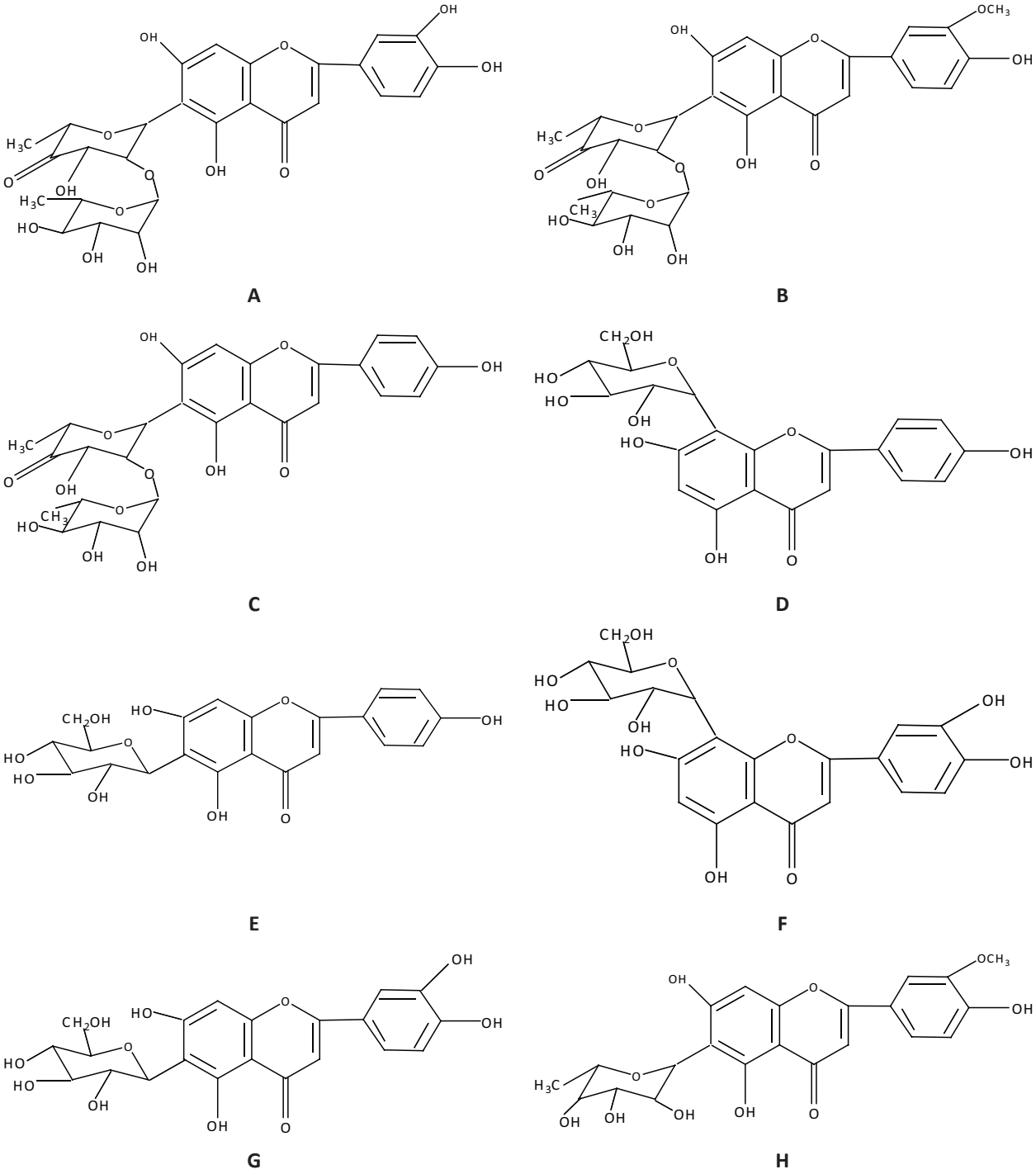

Figure 1 – Structural formulas of main *Zea maydis styli cum stigmatis* flavonoids
Note: A – maisin; B – methoxymaisine; C – apimaisin; D – vitexin; E – isovitexin; F – landmark; G – isoorientin; H – chrysoeriol 6-C-β-fucopyranoside.

2. Pharmacological action of Zea maydis styli cum stigmatis

2.1. Zea maydis styli cum stigmatis application in traditional medicine

The history of the *Zea maydis styli cum stigmatis* application for the treatment of various diseases goes back to the far past. The medicinal properties of *Zea maydis styli cum stigmatis* and a review of the empirical experience of their application are described in sufficient detail by Hager H. in "The Guide to Pharmaceutical and Medico-Chemical Practice", published in 1902–1903. [3, 4].

In folk medicine of various countries, it is recommended to use aqueous extracts from *Zea maydis styli cum stigmatis* most often for the diseases of the liver and biliary tract, kidneys and bladder. In particular, the traditional medicine of China and Japan prefers to use

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Table 1 – *Zea maydis styli cum stigmatis* chemical composition

PAS group	Individual compounds	Source
Flavonoids Phytohemagglutinins	Maisin, methoxymaisin, apimaisin, chrysoeriol 6-C-β-fucopyranoside; 4''-OH-3'-methoxymaisine molludistin, isomolludistin, vitexin, isovitexin, orientin, isoorientin, isoscoparin, isoquercitrin, quercetin-3,7-diglucoside	[6–14]
Phenolcarboxylic acids	Chlorogenic acid, ferulic acid, caffeic acid, hydroxycinnamic acid	[3, 15, 16]
Tannins	Gallic acid, ellagic acid, halocatechin, epicatechin, catechin, epigalocatechin, catechin gallate, epicatechin gallate	[3,17, 18]
Vitamins	Phylloquinone, thiamine, riboflavin, tocopherol, nicotinamide, ascorbic acid	[2, 3]
Polysaccharides	Monomer composition: rhamnose, arabinose, xylose, mannose, galactose, glucose	[20–24]
Saponins	Oleanolic acid, ursolic acid	[3]
Phytosterols	β-sitosterol, stigmasterol	[25]
Volatile compounds	Alcohols: 2,3-butanediol; ethanol; 1,2-propanediol; 2-furanmethanol; ketones: 2,3-butanedione; 3-hydroxy-2-butanone; 3-methyl-2,5-furandio; dihydro-2(3H)-furanone; 2-heptanone; 3-octanone; 2,4-pentanedione; aldehydes: benzeneacetaldehyde, heptanol, hexanol, propanol, pentanol, furfural furan derivatives: furan, 2-pentylfuran, 2,3-dihydrobenzenefuran; terpenoids and phenol derivatives: cis-alpha-terpineol, 6,11-oxidoacor-4-ene, citronellol, trans-pinocamphone, eugenol, neo-iso-3-tujanol, cis-sabinene hydrate	[26, 27]
Phytohemagglutinins	Carbohydrate part: galactose, mannose, glucose, arabinose, xylose, rhamnose, uronic acid, glucosamine, galactosamine; protein part: asparagine, glutamic acid, glycine, alanine, lysine, proline, serine, threonine, cysteine, valine, methionine, leucine, isoleucine, tyrosine, phenylalanine, histidine, arginine, cystine	[3]

a *Zea maydis styli cum stigmatis* decoction as a diuretic [2].

Along with this, folk medicine in Ukraine and Belarus uses *Zea maydis styli cum stigmatis* decoctions for uterine, pulmonary, hemorrhoidal bleeding, edema of the cardiovascular origin, and as a sedative remedy [28]. As a hemostatic agent for gynecological and nasal bleedings, a diuretic for urolithiasis, as well as for the treatment of cholangitis, cholecystitis, hepatitis, atherosclerosis, diabetes, parasitic infestations and obesity, *Zea maydis styli cum stigmatis* decoctions are used in Bulgarian folk medicine [3].

2.2. A modern view on the pharmacological action of *Zea maydis styli cum stigmatis*

At the present stage, interest in *Zea maydis styli cum stigmatis* is associated with the research aimed at studying new types of a pharmacological action of this MPRM, detailing the mechanisms of already known and newly discovered therapeutic effects of *Zea maydis styli cum stigmatis*.

2.2.1. Diuretic and nephroprotective action

Thanks to the empirical experience of traditional medicine, diuretic *Zea maydis styli cum stigmatis* actions determined their use at an early stage primarily as a diuretic. Modern researchers could not help being interested in the mechanism of the diuretic *Zea maydis styli cum stigmatis* effect on the kidney function. In particular, *in vivo*, laboratory animals were used to study the excretion of water, uric acid, potassium and sodium ions with urine when taking *Zea maydis styli cum stigmatis* aqueous extracts. It was found out that the *Zea maydis styli cum stigmatis* aqueous extract had a pronounced diuretic effect alongside with a kaliuretic effect. At the same time, a decrease in the glomerular filtration without changing the function of the proximal tubules, the excretion of sodium and uric acid was recorded [29].

In vivo studies have shown a positive *Zea maydis styli cum stigmatis* effect on the level of uric acid, which plays a significant role in the pathogenesis and development of cardiovascular diseases complications. The experiments were carried out on several groups of laboratory rats fed with the *Zea maydis styli cum stigmatis* extract, normal food, as well as food with a high salt content, and the *Zea maydis styli cum stigmatis* extract. The determination of the nitric oxide content, superoxide dismutase, glutathione peroxidase and uric acid in the vascular tissues blood of the laboratory animals made it possible to confirm significant negative changes that occurred under the influence of the increased salt intake. Alongside with this, the authors of the study notified that, due to its antioxidant properties, the therapeutic potential of the *Zea maydis styli cum stigmatis* extract may be required in case of an oxidative damage caused by a high salt and/or uric acid content [30].

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Table 2 – Main types of *Zea maydis styli cum stigmatis* pharmacological action

Kind of pharmacological action	Type of study	Object of study	Dose/ concentration	Source
Diuretic and nephro-protective actions	*in vivo*	*Zea maydis styli cum stigmatis* aqueous extract	500 mg/kg	[29]
		Zea maydis styli cum stigmatis aqueous extract	500 mg/kg	[30]
		Zea maydis styli cum stigmatis methanolic extract	200–500 mg/kg	[31]
Antihypertensive action	*in vivo*	*Zea maydis styli cum stigmatis* aqueous extract	130; 192.5; 260 mg/kg	[33]
	in vitro	*Zea maydis styli cum stigmatis* aqueous extract	10 mg/kg	[35]
Hypoglycemic and antidiabetic actions	*in vivo*	*Zea maydis styli cum stigmatis* aqueous extract	500 mg/kg	[36, 37]
		Zea maydis styli cum stigmatis aqueous extract	300, 600, 1200 mg/kg	[38]
		Zea maydis styli cum stigmatis aqueous extract	500–4000 mg/kg	[39]
		Zea maydis styli cum stigmatis alcohol extract	100, 300, 500 mg/kg	[40]
		Zea maydis styli cum stigmatis polysaccharides	200, 500, 800 mg/kg	[41]
		Zea maydis styli cum stigmatis polysaccharides	40, 100, 300 mg/ml	[42]
	in vitro	*Zea maydis styli cum stigmatis* alcohol extract	50%	[43]
		Zea maydis styli cum stigmatis alcohol extract	5–25 mg/ml	[44]
	in vivo, in vitro	*Zea maydis styli cum stigmatis* polysaccharides, phenolic compounds	10 μg/ml	[45]
		Zea maydis styli cum stigmatis polyphenolic compounds	0.001–1 mg/ml	[46]
Weight loss, lipid-lowering effect	*in vivo*	*Zea maydis styli cum stigmatis* aqueous extract	100 mg/kg	[47]
		Zea maydis styli cum stigmatis aqueous extract	100 mg/kg	[48]
		Maisin	10 mg/kg	[50]
		Zea maydis styli cum stigmatis aqueous extract	600, 800 μg/ml	[51]
Antitumor activity	*in vivo*	*Zea maydis styli cum stigmatis* polysaccharides	50, 100, 200 mg/kg	[53]
		Zea maydis styli cum stigmatis alcohol extract	10 mg/kg	[54]
	in vitro	*Zea maydis styli cum stigmatis* alcohol extract	2–10 mg/ml	[55]
		Zea maydis styli cum stigmatis methanolic extract	250, 500, 1000 mg/ml	[56]
		Maisin	200 μg/ml	[57]
	in vivo, in vitro	*Zea maydis styli cum stigmatis* polysaccharides	0–1 mg/ml	[58]
Immunotropic action	*in vitro*	*Zea maydis styli cum stigmatis* aqueous extract	2.5–70 mg/ml	[59, 60]
		Maisin	100 mg/ml	[61]
Anti-inflammatory action	*in vivo*	*Zea maydis styli cum stigmatis* aqueous extract	1, 2, 4 g/kg	[62]
Antioxidant action	*in vitro*	*Zea maydis styli cum stigmatis* alcohol extract	2%	[28]
		Zea maydis styli cum stigmatis methanolic extract	0.2–4.0 g in simulated medium	[64]
Antibacterial action	*in vitro*	*Zea maydis styli cum stigmatis* hexane extract	1024 mg/ml	[65]
Dermatoprotective action	*in vivo*	*Zea maydis styli cum stigmatis* aqueous extract	2–4 g/kg	[66]
	in vitro	*Zea maydis styli cum stigmatis* aqueous extract	0.75–1.5%	[67]
			0–1.0 mg/ml	[68]
Neuroprotective action	*in vitro*	Maisin	5–50 mg/ml	[69]
		Zea maydis styli cum stigmatis terpens	25, 50, 100 mmol	[70, 71]

A study of the *Zea maydis styli cum stigmatis* effect on the *in vivo* gentamicin-induced nephrotoxicity in laboratory rats showed that *Zea maydis styli cum stigmatis* significantly reduced serum creatinine levels. It was found out that under the influence of *Zea maydis styli cum stigmatis*, the manifestations of interstitial nephritis significantly decreased and the occurrence of acute tubular necrosis was not observed, in comparison with the control group of animals. The results obtained showed that *Zea maydis styli cum stigmatis* could reduce the phenomena of nephropathy during a long-term therapeutic use of gentamicin and related aminoglycosides [31].

The results of the herbal medicines study market were published, as a result of which *Zea maydis styli cum stigmatis* were named in the list of top 10 components of herbal medicines for the treatment of the urinary system diseases. It is recommended to use *Zea maydis styli cum stigmatis* as ones of the main components in the development of combined drugs for the treatment of urological and nephrological diseases [32].

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2.2.2. Antihypertensive action

In recent years, scientific teams in various countries of the world have been studying antihypertensive effects of *Zea maydis styli cum stigmatis*. In particular, the ability of *Zea maydis styli cum stigmatis* to normalize intraocular and blood pressure has been shown. With this objective in view, a randomized *in vivo* study of the *Zea maydis styli cum stigmatis* aqueous extract effect on these parameters in people suffering from hypertension, was conducted. The results of the experiments showed that therapy with the *Zea maydis styli cum stigmatis* aqueous extract gave a statistically significant dose-dependent decrease in the mean intraocular pressure and blood pressure within a few hours after the administration. According to the authors of the study, the achieved effect may be associated with sodium uresis and diuresis caused by a high content of potassium in the *Zea maydis styli cum stigmatis* extract [33].

Generalized data on the effectiveness of the *Zea maydis styli cum stigmatis* use for the hypertension treatment alone and in combination with synthetic drugs under review, are presented in the published scientific review. The meta-analysis covers five randomized trials involving 567 people, the results of which suggest an increase in the antihypertensive effect when *Zea maydis styli cum stigmatis* is combined with synthetic drugs [34].

The mechanism of the antihypertensive *Zea maydis styli cum stigmatis* action was studied *in vitro* using the methods of proteomics and bioinformatics. The aim of the study was to determine the *Zea maydis styli cum stigmatis* effect on the activity of angiotensine transforming enzyme (ATE) and the presence of components in this MPRM that can have such an effect. The use of proteomics and a bioinformatics analysis made it possible to identify bioactive *Zea maydis styli cum stigmatis* peptides that significantly inhibited the ATE activity and reduced blood pressure levels in a dose-dependent manner. In addition, by means of a docking analysis, the authors showed the interaction mechanism of the discovered peptides with ATE [35].

2.2.3. Hypoglycemic and antidiabetic action

As revealed in recent years, alongside with diuretic, choleretic and hemostatic activities, *Zea maydis styli cum stigmatis* have also hypoglycemic properties. The effectiveness of the aqueous *Zea maydis styli cum stigmatis* extract in diabetic nephropathy induced by streptozocin, was studied by Suzuki R. et al. *in vivo.* Urinary albumin excretion and creatinine clearance were studied to diagnose diabetic nephropathy. It was found out that the *Zea maydis styli cum stigmatis* extract prevented glomerular hyperfiltration and suppressed the progression of experimental diabetic glomerular sclerosis. Alongside with that, this group of scientists managed to isolate an individual compound with antidiabetic properties, the structure of which was shown to correspond to chrysoeriol 6-C-β-fucopyranoside. The researchers focused on the advisability of a further *Zea maydis styli cum stigmatis* study in order to expand the prospects for their use in diabetes mellitus and related diseases [36, 37].

The *in vivo* study results of the antidiabetic potential of the *Zea maydis styli cum stigmatis* extract have been published. They indicate that a 4-week application of the extract in laboratory mice significantly increased glucose tolerance and led to a marked decrease in the insulin resistance index. In addition, a decrease in hyperlipidemia, as evidenced by a decrease in total cholesterol, triglycerides, low density cholesterol and an increase in high density cholesterol, was found out. A decrease in an oxidative stress has been established by reducing the level of malondialdehyde and increasing the activity of superoxide dismutase; there was also a reduction of the lipid accumulation in the liver and the prevention of morphological changes in the liver tissue in type 2 diabetes mellitus. The results obtained confirmed the traditionally declared benefits of *Zea maydis styli cum stigmatis* in diabetes mellitus and the antidiabetic potential of *Zea maydis styli cum stigmatis*, which can become the basis for the development of affordable herbal remedies aimed at the treatment of type 2 diabetes [38].

An *in vivo* study of the *Zea maydis styli cum stigmatis* effect on glycemic metabolism in the laboratory mice with experimental diabetes induced by alloxan and adrenaline, was carried out. It was found out that after the oral administration of the *Zea maydis styli cum stigmatis* extract to mice, the level of glucose and glycosylated hemoglobin in the blood significantly decreased, while the level of insulin secretion was markedly increased. At the same time, against the background of taking the *Zea maydis styli cum stigmatis* extract, a gradual restoration of pancreatic beta cells was observed, and the body weight of the animals also increased [39].

The researchers who had studied the antidiabetic, antioxidant, and antihyperlipidemic activities of the fraction of phenolic compounds isolated from *Zea maydis styli cum stigmatis* in the experiments *in vivo*, came to a similar conclusion. The authors showed that the use of this fraction significantly reduced weight loss, water consumption and especially the concentration of glucose in the blood of mice with experimental diabetes, which indicated its potential antidiabetic activity. Alongside with this, there was a decrease in the level of malondialdehyde, total cholesterol, triacylglycerol, low density lipoproteins, and the amount of high density lipoproteins increased [40].

Pan Y. et al. focused their attention on the study of the antidiabetic effects of the polysaccharide obtained from *Zea maydis styli cum stigmatis*. *In vivo,* on the model of experimental diabetes in mice, it was found out that the use of a polysaccharide led to the stabilization of the animals' body weight, a decrease in blood glucose and serum insulin levels, and an improvement in glucose tolerance. There was a decrease in the level of glycated

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whey protein and non-esterified fatty acids, as well as a marked increase in the activity of superoxide dismutase, glutathione peroxidase and catalase. In addition, the isolated polysaccharide also showed a cytoprotective effect in histopathological observations [41].

The evaluation results of the inhibitory effect of *Zea maydis styli cum stigmatis* polysaccharides on α-glucosidase and α-amylase *in vivo* are presented. It has been shown that *Zea maydis styli cum stigmatis* polysaccharides can significantly inhibit these enzymes and increase glucose uptake by skeletal muscle cells, which allows us to consider them potentially useful for the treatment of type 2 diabetes mellitus [42].

An *in vitro* model was used to establish the inhibitory effect of the *Zea maydis styli cum stigmatis* extract on the formation of carboxymethyllysine, which is the end product of glycation and is currently considered a biological marker of diabetes. It was found out that the inhibition degree in the formation of carboxymethyllysine by the *Zea maydis styli cum stigmatis* extract was 76.57%. The authors of the studies showed that the *Zea maydis styli cum stigmatis* extract suppressed the formation of carboxymethyllysine due to the absorption of glyoxal/methylglyoxal or due to its antioxidant activity associated with the content of flavonoids in it [43].

Alongside with maisin, an antidiabetic activity is highly likely to be characteristic of *Zea maydis styli cum stigmatis* apigenin and luteolin derivatives. The antioxidant activity of the ethyl acetate fraction of *Zea maydis styli cum stigmatis* and its ability to inhibit α-amylase and α-glucosidase in enzymatic reactions were studied in the *in vitro* experiments. The results of the studies confirmed the presence of a pronounced antioxidant effect of *Zea maydis styli cum stigmatis*, which may be required for the prevention and treatment of diabetes mellitus and its complications, including diabetic nephropathy [44].

The results of the *in vivo* studies devoted to the research of the mechanism reducing the glucose level in the blood of mice under the influence of *Zea maydis styli cum stigmatis,* have been published. The ability of saccharides and phenolic compounds of *Zea maydis styli cum stigmatis* to inhibit intestinal α-glucosidases was evaluated in the work. Synthetic drugs from the group of α-glucosidase inhibitors have a number of gastrointestinal side effects and not all of them are commercially available. The results of the study showed that the polyphenolic compounds of *Zea maydis styli cum stigmatis* had an effective inhibitory action on intestinal α-glucosidases. An *"in silico"* analysis of the *Zea maydis styli cum stigmatis* polyphenols showed that maisin can be responsible for the inhibition of α-glucosidases [45].

The leading protective role against damage to endothelial cells of the vascular tissue under the conditions of high glucose levels is assigned to the polyphenolic compounds of *Zea maydis styli cum stigmatis*. The protective effect was studied *in vitro* using human umbilical vein endothelial cells, and subsequently *in vivo* in the rats with streptozocin-induced diabetes. It has been shown that the phenolic fraction of *Zea maydis styli cum stigmatis* can have a positive effect on patients with diabetes and play a significant role in preventing the development and progression of diabetic complications such as diabetic nephropathy and atherosclerosis [46].

2.2.4. Weight loss, lipid-lowering effect

Alongside with the anti-diabetic effect of *Zea maydis styli cum stigmatis* shown in various studies, there are reports of their ability to reduce body weight. One of such *in vivo* studies showed that the oral administration of the *Zea maydis styli cum stigmatis* extract high in maisin to mice, resulted in the inhibition of the expression of the genes involved in adipocyte differentiation, reduced fat accumulation and synthesis, and promoted the expression of the genes involved in lipolysis and fat oxidation [47].

The results of the *Zea maydis styli cum stigmatis* extract effect on cholesterol metabolism in the *in vivo* experimental model in mice on a high-fat diet have been published. It has been found that the addition of the *Zea maydis styli cum stigmatis* extract alongside with the diet enriched with fats, improves the level of adipocytokines secretion and glucose homeostasis. Alongside with this, the *Zea maydis styli cum stigmatis* extract has been shown to be effective in lowering the cholesterol pool in the liver, consistent with lowering blood and liver cholesterol levels [48].

In the review study, Wang B. et al. emphasized that the lipid-lowering properties of *Zea maydis styli cum stigmatis* are extremely relevant for the prevention and treatment of the metabolic syndrome, including obesity, hypertension, hyperglycemia, and abnormal levels of triglycerides and high-density lipoprotein cholesterol [49].

An *in vivo* study of the potential anti-obesity activity of maisin was carried out by Lee C. et al. in several groups of mice that received food with different fat contents. In the animals, body weight and body fat were measured, as well as mRNA expression levels of proteins involved in adipocyte differentiation, fat accumulation, fat synthesis, lipolysis, and fat oxidation in the adipose tissue and liver. It was found out that maisin reduced the level of intracellular lipid droplets and triglycerides, and suppressed lipid accumulation and adipocyte differentiation. In addition, maisin has been shown to cause apoptotic death of preadipocytes, which may ultimately lead to a decrease in adipose tissue mass. Alongside with this, weight gain and fat mass in the mice decreased, the levels of thyroglobulin, total cholesterol, low-density cholesterol and glucose in blood serum decreased. On the whole, the effects obtained made it possible to suggest that maisin had anti-obesity effects *in vivo,* and this compound could be used as a functional food ingredient or as a drug for the prevention and treatment of obesity [50].

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Similar results were found out when studying the effect of the *Zea maydis styli cum stigmatis* extract and phytosterol on adipocyte growth factors. The oral administration of the subjects under *in vivo* study showed a significant reduction in weight and a decrease in the number of adipocytes in the liver and adipose tissue. The combined use of the *Zea maydis styli cum stigmatis* extract and phytosterol has demonstrated the ability to effectively reduce preadipocyte differentiation by inhibiting the activity of adipocyte growth factors [51].

The effect of the *Zea maydis styli cum stigmatis* decoction on the lipid profile was studied *in vivo* in patients with angina pectoris. A meta-analysis of several randomized trials has been published, indicating that the use of the *Zea maydis styli cum stigmatis* decoction contributed to the normalization of high-density lipoprotein levels and the reduction of total cholesterol and low-density lipoprotein in patients with angina pectoris. The authors suggested that the *Zea maydis styli cum stigmatis* decoction alone, as well as in the combination with a traditional drug treatment, might have a beneficial effect on blood lipids [52].

2.2.5. Antitumor activity

The recent studies devoted to the research of the possible *Zea maydis styli cum stigmatis* antitumor activity are of undoubtful interest.

The results of *in vivo* studying the *Zea maydis styli cum stigmatis* effect on tumor growth and immunological parameters in mice with experimental hepatocarcinoma, have been published. The study demonstrated that *Zea maydis styli cum stigmatis* could not only suppress tumor growth, but also increase the survival time of mice. In addition, the introduction of *Zea maydis styli cum stigmatis* contributed to an increase in body weight, a number of peripheral leukocytes, and a number of other indicators of the immune system functioning [53].

The data that give evidence of the significant effectiveness of the *Zea maydis styli cum stigmatis* extracts for the treatment of benignant and malignant prostate gland diseases have been presented. In particular, a study has been conducted to investigate the effect of the *Zea maydis styli cum stigmatis* extract on benignant prostatic hyperplasia. The experiments were carried out *in vivo* on male rats divided into groups receiving hormone therapy with testosterone, and combination therapy with testosterone and the *Zea maydis styli cum stigmatis* extract. It was found out that this treatment with the *Zea maydis styli cum stigmatis* extract led to a noticeable decrease in the weight of the prostate gland and alleviated the symptoms of the disease [54].

The *in vitro* study results of the antioxidant and antitumor activities of the *Zea maydis styli cum stigmatis* phenolic compounds of different corn varieties in relation to breast carcinoma cells have been obtained. The results of the study showed a correlation between the total content of phenolic compounds, the antioxidant activity and cytotoxicity against breast carcinoma cells [55].

The antitumor properties of the *Zea maydis styli cum stigmatis* extract have been investigated in relation to human breast cancer. The cytotoxicity of the extract was evaluated *in vitro* on MCF-7 breast cancer cells in comparison with normal human mesenchymal cells. The results of the studies led to the conclusion that the *Zea maydis styli cum stigmatis* extract reduced the viability of malignant cells and increased their apoptosis in a dose-dependent manner [56].

A potential antitumor activity of maisin isolated from the *Zea maydis styli cum stigmatis,* was evaluated *in vitro* on androgen-independent human prostate cancer cells. It turned out that maisin dose-dependently reduced the viability of cancer cells and significantly induced their apoptotic death. It has been shown that the combined treatment with maisin and other antitumor agents synergistically enhances the death of malignant cells. These results show for the first time that maisin can have a pronounced therapeutic potential for the treatment of chemoresistant or androgen-independent human prostate cancer [57].

The ability to significantly inhibit the proliferation of pancreatic cancer cells *in vitro* and *in vivo* has been established for the crude *Zea maydis styli cum stigmatis* polysaccharide. The studies have shown that this polysaccharide can induce apoptosis of pancreatic cancer cells, stop the cell cycle, and prevent migration and invasion of pancreatic cancer cells [58].

2.2.6. Immunotropic action

Attention is drawn to the studies' results of the *Zea maydis styli cum stigmatis* effect on immunity parameters. Scientists of the Korean Immunological Center Kim K.A., Choi S.K., Choi H.S. found out *in vitro* that the *Zea maydis styli cum stigmatis* extracts alter the activity of mouse macrophages, stimulating the production of cyclooxygenase and oxidase synthase. The involvement of *Zea maydis styli cum stigmatis* components in immunological reactions has also been reported previously, in particular, their ability to suppress a tumor necrosis factor and adhesion of bacterial lipopolysaccharides on cell walls [59, 60].

It has been supposed that the immunological properties of *Zea maydis styli cum stigmatis*, as well as some other types of activity of this raw material, may be due to the presence of maisin. In particular, the ability of maisin to activate macrophages was evaluated *in vitro* using mouse cells. It has been found out that maisin dose-dependently increased the secretion of a tumor necrosis factor and the production of nitric oxide synthase by 11.2 and 4.2 times, respectively, compared with untreated control cells. These results make it possible to prognose that maisin can be a new immunomodulator that enhances an early innate immunity [61].

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2.2.7. Anti-inflammatory action

Zea maydis styli cum stigmatis attracted scientists' attention in terms of studying their possible anti-inflammatory action. Experimental carrageenan-induced pleurisy in rats was used as a model for studying this type of activity *in vivo*. It was found out that pretreatment with the *Zea maydis styli cum stigmatis* extract reduced the volume of exudate, the number of leukocytes in the focus of inflammation, the level of an oxidative stress, and the values of other markers of the inflammatory process [62].

For the treatment of the oral cavity diseases, the drug "Insadol", which included the *Zea maydis styli cum stigmatis* extract, was previously used. "Insadol" was registered as an anti-inflammatory drug with the ability to stimulate mucosal repair, reduce pain, reduce gums bleeding and was previously used to treat the oral cavity diseases [63].

2.2.8. Antioxidant action

Various groups of scientists have suggested a possible relationship between the established types of the *Zea maydis styli cum stigmatis* activity and the antioxidant properties of this MPRM. The work by Maksimovic Z.A. and Kovacevic N. is devoted to a focused study of the antioxidant effect of *Zea maydis styli cum stigmatis*. The scientists obtained a methanol *Zea maydis styli cum stigmatis* extract and fractionated it using mixtures of different polarity solvents. The isolated fractions were studied for the presence of the antioxidant activity *in vitro* using the TBARS test, which makes it possible to assess the degree of lipid peroxidation. The maximum activity was found out in lipophilic fractions, the components of which were phenolic acids, flavonoid aglycones (flavones, flavonols, and methylated flavones), as well as flavonoid monosides [64].

The antioxidant properties of the *Zea maydis styli cum stigmatis* extract made it possible to consider it as a potential remedy for the treatment of toxic hepatitis. On the model of experimental acute toxic hepatitis induced in laboratory rats by the exposure to the trichloromethane solution, it was found out that the dry extract of *Zea maydis styli cum stigmatis* demonstrates hepatoprotective properties and is a low-hazard substance. It has been shown *in vitro* that the mechanism of the studied extract's action is due to its antioxidant activity [28].

Antioxidant effects *in vivo* and *in vitro* are summarized and described in the work by Hasanudin K. et al., for heteropolar fractions obtained from *Zea maydis styli cum stigmatis.* Herewith, ethyl alcohol, methanol, dichloromethane and acetone were used. The same authors drew attention to the anti-inflammatory effect and a number of other *Zea maydis styli cum stigmatis* properties [2].

2.2.9. Antibacterial action

Quite recently, for the first time, the data were published regarding the evaluation of the antibacterial activity and the antibiotic-modulating action of the hexane extract of *Zea maydis styli cum stigmatis*. The studies have shown that the extract exhibited an antimicrobial activity against the strains of *Escherichia coli, Staphylococcus aureus,* and *Pseudomonas aeruginosa* [65].

2.2.10. Dermatoprotective action

Zea maydis styli cum stigmatis have good prospects to be used for suppressing skin pigmentation.

In particular, photoprotective effects of *Zea maydis styli cum stigmatis* have been studied *in vivo* by the oral administration of the aqueous extract of *Zea maydis styli cum stigmatis* to laboratory mice. The study showed that experimental therapy led to a decrease in the photoaging process, as evidenced by the positive dynamics of biological markers of this process. They are: a decrease in the thickness of the epidermis and the formation of wrinkles, a decrease in the expression of anti-inflammatory genes, a decrease in the level of lipid peroxidation of skin lipids and blood DNA, etc.). The authors suggested that these effects are achieved due to the content of compounds with a potential antioxidant and an anti-inflammatory activity in *Zea maydis styli cum stigmatis* [66].

The study of the *Zea maydis styli cum stigmatis* inhibitory effect on the production of melanin *in vitro* revealed that the *Zea maydis styli cum stigmatis* extract applied to the cells of pigmented melanocytes (melan-A) obtained from normal epidermal melanoblasts of C57BL inbred mouse embryos, reduced melanin production by 37.2% without any manifestation of cytotoxicity [67].

There is scientific evidence that also confirms the photoprotective properties of *Zea maydis styli cum stigmatis*. An *in vitro* study was conducted to research the prophylactic effect of the *Zea maydis styli cum stigmatis* extract on human keratinocytes. The cells were pretreated with the *Zea maydis styli cum stigmatis* extract and then exposed to ultraviolet. The results showed that the survival of keratinocytes after pre-treatment with the *Zea maydis styli cum stigmatis* extract was markedly increased. The *Zea maydis styli cum stigmatis* extract statistically significantly reduced intracellular damage caused by ultraviolet rays and slowed down the apoptosis reaction due to the stabilization of the mitochondrial membrane potential [68].

2.2.11. Neuroprotective action

The *in vitro* study results of the neuroprotective effect of maisin isolated from *Zea maydis styli cum stigmatis* are presented. The scientists found out that maisin pretreatment reduced the cytotoxic effect of hydrogen peroxide on neuroblastoma cells, weakened their apoptosis, and significantly and dose-dependently increased the levels of antioxidant enzymes. The obtained data suggested that maisin has a neuroprotective effect due to its antioxidant properties [69].

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Neuroprotective properties have also been studied for terpene compounds isolated from *Zea maydis styli cum stigmatis*. The studies were carried out *in vitro* on model human bone marrow neuroblastoma cells damaged by the exposure to hydrogen peroxide. The results showed that some of the studied substances inhibited apoptosis and had a statistically significant protective effect in relation to the experimental cell culture [70,71].

Thus, as a result of the conducted studies, it was revealed that at the present stage, scientific information on the pharmacological action of *Zea maydis styli cum stigmatis* has been significantly expanded. Generalized data on the main types of the *Zea maydis styli cum stigmatis* pharmacological action, established on the basis of information and analytical search, are presented in Table 2.

CONCLUSION

Generalization and analysis of modern scientific literature data made it possible to establish that *Zea maydis styli cum stigmatis* are still in the sphere of scientists' interest, as evidenced by the information replenished and expanded in recent years, on their chemical composition and spectrum of pharmacological action. Alongside with the *Zea maydis styli cum stigmatis* flavonoids, other groups of PASs in this raw material are also actively studied. It was revealed that knowledge about potentially significant and confirmed types of *Zea maydis styli cum stigmatis* therapeutic actions has been significantly updated. In addition to the traditionally known choleretic, diuretic, hemostatic effects of *Zea maydis styli cum stigmatis*, their antioxidant, anti-inflammatory, antidiabetic, immunotropic, neuroprotective, antitumor, photoprotective and a number of other pharmacological effects significant for medicine, have been established. The results of this review may be useful for identifying promising directions for the development of drugs based on *Zea maydis styli cum stigmatis*.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

Elena B. Nikiforova – determination of the aim and objectives of the study, information and analytical search on the topic of the study, writing the sections "Chemical composition of *Zea maydis styli cum stigmatis*", "Pharmacological properties of *Zea maydis styli cum stigmatis*"; Nafiset M. Bat – writing the sections "Introduction", "Conclusion"; Naira A. Davitavyan – preparation of references.

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AUTHORS

Elena B. Nikiforova – Candidate of Sciences (Pharmacy), Associate Professor, Acting Head of the Department of Pharmacy, Kuban State Medical University. ORCID ID: 0000-0001-7081-3523. E-mail: elenanik94@mail.ru

Nafiset M. Bat – Doctor of Sciences (Pharmacy), Professor, Dean of the Pharmaceutical Faculty, Kuban State Medical University. ORCID ID: 0000-0003-3593-2302. E-mail: batnm@mail.ru

Naira A. Davitavyan – Candidate of Sciences (Pharmacy), Associate Professor, Associate Professor of the Department of Pharmacy, Kuban State Medical University. ORCID ID: 0000-0001-8151-0587. E-mail: davitavyan08@mail.ru

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OBESITY AS A REFLECTION OF PSYCHO-EMOTIONAL DISORDERS: FOCUS ON PHARMACOTHERAPY

V.N. Shishkova

National Medical Research Center for Therapy and Preventive Medicine
Bld. 3, 10, Petroverigsky Ln., Moscow, Russia, 101000

E-mail: veronika-1306@mail.ru

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Obesity is a vivid example of a multifactorial disease. In its development, not only the discrepancy between the energy intake and its expenditure but also various neurohumoral mechanisms, as well as external socio-economic and psychosocial factors, play important roles. One of the frequent options for combining psychoemotional disorders with obesity is the development of certain types of eating disorders in a patient. In this regard, the actual problem is to find the optimal therapeutic and prophylactic strategy in the management of such patients.

The aim of the work is to analyze the literature data on the features of the pathogenetic mechanisms of the obesity development against the background of psychoemotional disorders that are realized through eating disorders, and to identify the possibilities of using sibutramine to carry out a pharmacological correction of these pathological conditions.

Materials and methods. In the process of selecting materials for writing a review article, the following databases were uses: PubMed, Scopus, Web of Science, Google Scholar, ScienceDirect, etc. The search carried out, was based on the publications for the period from 2009 to 2020. The following words and phrases were chosen as parameters for the literature selection: obesity, psycho-emotional disorders, eating disorders, sibutramine.

Results. This review summarizes the main pathogenetic aspects that unite both the development of psychoemotional and metabolic disorders. A modern classification of obesity, taking into account the latest domestic and international recommendations of professional communities, is given. Eating disorders are considered in detail, their socio-psychological and psychiatric classifications are given. The prospect of choosing a therapeutic and prophylactic strategy for managing such patients is assessed, depending on the presence of psycho-emotional and eating disorders.

Conclusion. Thus, the combined drug containing sibutramine and metformin registered in the Russian Federation is effective, safe and can be used in patients with alimentary obesity and eating disorders, taking into account contraindications.

Keywords: obesity; psychoemotional disorders; eating disorders; sibutramine

Abbreviations: CNCD – chronic non-communicable diseases; RF – Russian Federation; WHO – World Health Organization; BMI – body mass index; WC – waist circumference; HC – hip circumference; CVD – cardiovascular diseases; NA – anorexia nervosa; BN – bulimia nervosa; PO – pathological overeating; GIT – gastrointestinal tract; AH – arterial hypertension; BP – blood pressure; DM2 – type 2 diabetes mellitus; CI – confidence interval.

ОЖИРЕНИЕ В ЗЕРКАЛЕ ПСИХОЭМОЦИОНАЛЬНЫХ НАРУШЕНИЙ: ФОКУС НА ФАРМАКОТЕРАПИЮ

В.Н. Шишкова

Федеральное государственное бюджетное учреждение «Национальный медицинский исследовательский центр терапии и профилактической медицины»
Министерства здравоохранения Российской Федерации
101000, Россия, г. Москва, Петроверигский пер., д. 10, стр. 3

E-mail: veronika-1306@mail.ru

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Ожирение представляет собой яркий пример многофакторного заболевания, в развитии которого важную роль играет не только несоответствие между поступлением и расходом энергии, но и различные нейрогуморальные механизмы, а также внешние социально-экономические и психосоциальные факторы. Одним из частых вариантов сочетания психоэмоциональных расстройств с ожирением является развитие у пациента тех или иных типов нарушений пищевого поведения. В связи с этим, актуальной проблемой является поиск оптимальной терапевтической и профилактической стратегии в ведении таких пациентов.

Цель. Проанализировать данные литературы об особенностях патогенетических механизмов развития ожирения на фоне психоэмоциональных расстройств, реализующихся посредством нарушений пищевого поведения, и обозначить возможности применения сибутрамина с целью фармакологической коррекции данных патологических состояний.

Материалы и методы. В процессе подбора материала для написания обзорной статьи использовали такие базы данных, как: PubMed, Scopus, Web of Science, Google Scholar, ScienceDirect, и др. Поиск осуществлялся по публикациям за период с 2009 по 2020 гг. Параметрами для отбора литературы были выбраны следующие слова и словосочетания: ожирение, психоэмоциональные нарушения, расстройства пищевого поведения, сибутрамин.

Результаты. В настоящем обзоре обобщаются основные патогенетические моменты, объединяющие как развитие психоэмоциональных, так и метаболических нарушений. Дается современная классификация ожирения, с учетом последних отечественных и международных рекомендаций профессиональных сообществ. Подробно рассматриваются нарушения пищевого поведения, дается их социально-психологическая и психиатрическая классификации. Оценивается перспектива выбора терапевтической и профилактической стратегии ведения таких пациентов в зависимости от наличия психоэмоциональных расстройств, нарушений и пищевого поведения.

Заключение. Таким образом, зарегистрированный в Российской Федерации комбинированный препарат, содержащий сибутрамин и метформин, представляет собой эффективное и безопасное лекарственное средство, которое может применяться у пациентов с алиментарным ожирением и нарушениями пищевого поведения при учете противопоказаний.

Ключевые слова: ожирение; психоэмоциональные нарушения; расстройства пищевого поведения; сибутрамин

Список сокращений: ХНИЗ – хронические неинфекционные заболевания; РФ – Российская Федерация; ВОЗ – Всемирная Организация Здравоохранения; ИМТ – индекс массы тела; ОТ – окружность талии; ОБ – окружность бедер; ССЗ – сердечно-сосудистые заболевания; НА – нервная анорексия; НБ – нервная булемия; ПП – патологическое переедание; ЖКТ – желудочно-кишечный тракт; АГ – артериальная гипертензия; АД – артериальное давление; СД2 – сахарный диабет 2 типа; ДИ – доверительный интервал.

INTRODUCTION

Modern development rates of high-tech types of medical care and the obvious progress in the field of pharmacology have made the long-standing dream of mankind to overcome many serious and incurable ailments possible. However, the further progress goes, the more obvious it becomes that some diseases, which have not been previously given much attention to, are now acquiring such threatening forms that they have begun an active offensive against humanity. Obesity, included in the international classification of diseases only in 1950, but spreading around the world at an incredible speed and carrying with it a huge amount of physical and psycho-emotional suffering for a person, can be attributed to such insidious pathologies [1].

Over the past decades, the number of diagnosed patients with obesity or overweight worldwide has increased severalfold. So, in 2016, already about 40% of the adult population was overweight, and 13% were obese[1]. According to experts, with continuing trends, by 2050, 45% of our planet population, will be overweight, and 16% will be obese [1]. According to the latest data, in 2019 obesity rose to the fifth place in the structure of risk factors for premature death worldwide, and the number of people who die annually from the consequences of obesity, is almost 3 million people [2].

Obesity is a significant risk factor for the development of the most common chronic non-communicable diseases (CNCD) in humans, such as cardiovascular, cerebrovascular, oncological, diabetes mellitus, pathologies of the respiratory system and the musculoskeletal system. In addition, the presence of obesity significantly affects the course and prognosis of a number of infectious diseases, including a new coronavirus infection. Thus, all of the above mentioned can lead to an increase in the burden on healthcare systems in all countries worldwide, and will require huge investments to prevent and overcome these consequences [2, 3].

In Russia, the prevalence of obesity in the new millennium was studied in the epidemiological research "ECVD-RF" (Epidemiology of Cardiovascular Diseases and their risk factors in the regions of the Russian Federation). According to the results, it amounted to 29.7%, which, in comparison with the data gained at the beginning of the nineties of the last century, turned out to be several times higher. The researchers also notified that woman over 45 are more likely to be obese in Russia, and overweight is associated with a high socioeconomic status [4].

Thus, in the Russian Federation, as well as throughout the world, the situation with obesity has ceased to be a background problem and is increasingly coming to the fore in the study of topical issues of treatment and prevention of the most common CNCDs, that occupy a leading position in the incidence and mortality of the population [5].

[1] World Health Organization. Obesity and overweight. Available from: https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight.

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THE AIM of the work is to analyze the literature data on the features of the pathogenetic mechanisms of the obesity development against the background of psychoemotional disorders that are realized through eating disorders, and to identify the possibilities of using sibutramine to carry out a pharmacological correction of these pathological conditions.

MATERIALS AND METHODS

In the process of selecting materials for writing a review article, the following databases were uses: PubMed, Scopus, Web of Science, Google Scholar, ScienceDirect, etc. The search carried out, was based on the publications for the period from 2009 to 2020. The following words and phrases were chosen as parameters for the literature selection: obesity, psycho-emotional disorders, eating disorders, sibutramine.

RESULTS AND DISCUSSION

Definition and classification

According to the definition of the World Health Organization (WHO), obesity is an abnormal or excessive fat accumulation in the body, which can lead to adverse consequences for human health[2]. In the latest version of clinical guidelines for the diagnosis and treatment of patients with obesity, the Association of Endocrinologists of the Russian Federation provides a more complete definition of this pathology. Obesity is a chronic disease characterized by excessive accumulation of adipose tissue in the body, which poses a threat to health and is a major risk factor for several other chronic diseases, including type 2 diabetes mellitus (DM2) and cardiovascular diseases [6].

For a long time, the only indicator that the doctor was guided by when diagnosing overweight or obesity, was the body mass index (BMI). BMI is calculated as the ratio of body weight in kilograms to the square of height in meters (kg/m^2). At the same time, according to the opinion of WHO experts[3], overweight is established if the BMI is greater than or equal to 25, and obesity – if the BMI is greater than or equal to 30, and then the degree of obesity is specified (Table 1). However, despite the apparent convenience and ease of the BMI indicator use, it is currently only considered an indirect criterion, since it has certain limitations for use in children, the elderly, athletes and pregnant women. It may also correspond to a different degree of developing complications risk, which was the reason for revising this classification and creating a new one (Table 2) [6, 7].

Considering that the main morphological substrate of adverse cardio- and metabolic obesity risks is associated with an excessive accumulation of visceral adipose tissue, current domestic and international clinical recommendations of professional communities of endocrinologists, cardiologists, internists, bariatric surgeons, etc. emphasize the need to measure, along with BMI, waist circumference (WC) and the ratio of WC to hip circumference (HC). Thus, the obesity phenotype, and hence a lot of risks associated with it, can be assessed [6–10]. So WC ≥ 94 cm in men and ≥ 80 cm in women is a diagnostic criterion for visceral obesity, and the WC/HC ratio > 0.9 in men and > 0.85 in women is a metabolically unhealthy obesity phenotype, regardless of the BMI value [8, 9].

According to the etiological principle, obesity is classified into primary (exogenous-constitutional or alimentary) and secondary (symptomatic, associated with other diseases or conditions) [6]. Thus, the secondary type of obesity includes obesity as a part of known genetic syndromes; cerebral obesity (due to the brain damage); obesity due to other diseases of the endocrine system; obesity against the background of taking drugs that contribute to weight gain. However, the most common etiological type of obesity today is primary alimentary or exogenous-constitutional obesity. Thus, the issues of pathogenesis, or causes of overnutrition in most obese patients, become very relevant, given the possibility of developing effective methods of prevention and treatment.

Obesity and psycho-emotional disorders

According to modern concepts, obesity is a vivid example of a multifactorial disease, in the development of which an important role is played not only by the discrepancy between the energy intake and its expenditure, but also by various neurohumoral mechanisms, as well as by external socio-economic and psychosocial factors [11, 12].

It should be notified that the increase in the prevalence of obesity over the past decades may be closely related to the growth rate of the prevalence of mental health disorders, especially the affective spectrum (depression and anxiety disorders), among the population of many developed countries [13, 16]. "The Global Burden of Diseases, Injuries, and Risk Factors Study", conducted in 2017, demonstrated an increase in the prevalence of affective disorders worldwide, which are among the top three causes of disability in both men and women [14]. In the Russian Federation, the prevalence of affective disorders among the general population is as follows: depression comprises 8.8% and anxiety disorders – 18.1%. Among the patients with CNCDs seeking for medical care in outpatient and inpatient health care facilities, they are almost 50% [15, 16]. The conducted studies and meta-analyses have shown that affective violations increase the risk of developing both the CNCDs themselves (especially CVDs) and unfavorable cardiovascular outcomes [17–24]. At the same time, pathogenetic dysfunction of monoaminergic systems, mainly serotonin-, norepinephrine- and dopaminergic ones, in the central nervous system underlies the development of both affective disorders (depression and anxiety disorders) and obesity [25]. It is known that serotonin regulates the rate of satiety development, affects appetite and the choice of nutrients, suppresses the desire to overeat foods rich in carbohydrates and fats, and

[2] Ibid.
[3] Ibid.

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its deficiency in depression, on the contrary, increases craving for such food sources, the intake of which not only reduces the vegetative symptoms of depression but also promotes weight gain. Norepinephrine is also involved in the regulation of food intake, hereby, it influences neuropeptide Y and leptin levels, and the stimulation of dopamine receptors is accompanied not only by a decrease in the volume and number of meals, but it also regulates the energy consumption [26]. It should be notified that the severity of affective disorders also correlates with the severity of obesity and comorbid CVDs [27–29]. However, in real clinical practice, internists (therapists, endocrinologists, cardiologists, etc.) tend to overlook such relationships due to insufficient awareness of the pathogenetic mechanisms and risks associated with mental health [30, 31].

Another option for combining psycho-emotional disorders with obesity is the development of certain variants of eating disorders in the patient. It is known that the majority of obese patients are not aware of the presence of any disorders associated with eating, explaining the excess in nutrition by addiction to tasty and favorite dishes or products. However, at the same time, in most obese patients, difficulties gradually begin to arise with an independent cessation of food intake, the control over the amount of food eaten gets lost, the feeling of fullness disappears or lags, contributing to constant overeating. Thus, a daily consumption of excess calorie food, with gradually increasing portion sizes and the formation of circadian eating disorders is often accompanied by chronic psycho-emotional stresses and physical inactivity. These factors contribute to the rapid progression of obesity, most fully illustrating undiagnosed eating disorders. It should be emphasized that the comorbidity of eating disorders with depressive or anxiety disorders can develop long before the formation of clinically significant obesity [32–34].

The response to various stresses that accompany human life in abundance in the modern world is one of the important causes of obesity, which is realized through the development of eating disorders. Often, the beginning of the formation of eating disorders is preceded by a traumatic situation, accompanied by the development of an affective disorder of the anxiety or depressive spectrum. Next, the hyperphagic reaction joins, i.e. overeating, as a form of relieving a psycho-emotional stress, which is a simplified behavioral response that gives an imaginary feeling of protection and calm. This is how a stereotyped compensatory response to situations of psycho-emotional stress is launched and consolidated by overeating, which gives only a short-term relief. However, in the future, due to excess weight gain, patients begin to develop secondary depressive or anxiety disorders associated with a negative perception of their appearance and feelings about their helplessness in controlling food intake [35]. Psychoemotional tension or stress can contribute to the development of primary obesity not only due to hyperphagic reactions and poor nutrition, but also by increasing the risk of alcohol abuse and enhancing the effects of physical inactivity. There is also a relationship between the severity of depressive disorders, the severity of eating disorders and the progression of obesity [35, 36]. In the studies by E.M. Pisetsky et al., it was shown that eating disorders can further increase the risk of suicide attempts, including the situations after the relief of the main depression symptoms [37]. Due to this close relationship, eating and psycho-emotional disorders, on the one hand, contribute to weight gain, and on the other hand, progression of obesity worsens the mood and psycho-emotional state of a person, strengthening and perpetuating eating disorders [38].

Table 1 – Classification of obesity by BMI[4]

Body mass	BMI, kg/m^2	Risk of concomitant diseases
Underweight	<18.5	Low (increased risk of other diseases)
Normal body weight	18.5–24.9	Usual
Overweight	25.0–29.9	Higher
Obesity I degree	30.0–34.9	High
Obesity II degree	35.0–39.9	Very high
Obesity III degree	≥40	Extremely high

Table 2 – Classification of obesity by stages

Diagnosis	Anthropometric data	Clinical data
Overweight	BMI ≥ 25.0–29.9 kg/m^2	No complications associated with obesity
Obesity stage 0	BMI ≥ 30.0 kg/m^2	No complications associated with obesity
Obesity stage 1	BMI ≥ 25.0 kg/m^2	One or more moderate complications associated with obesity
Obesity stage 2	BMI ≥ 25.0 kg/m^2	One or more severe obesity-related complications

Note: the presence or absence of concomitant diseases, the course of which is directly associated with obesity (i. e., DM2, arterial hypertension, non-alcoholic fatty liver disease, etc.), their severity determines the stage of obesity and, accordingly, the choice of therapy.

[4] Ibid.

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Table 3 – Summary of data from studies on the use of drugs containing sibutramine and sibutramine in combination with metformin

Sl. No.	Detected connection	Sample	Referent	Research results	Reference
1.	Sibutramine therapy is associated with a reduced risk of cardiovascular complications in patients without contraindications	Obese adults N=23,927	Orlistat N=77047	Against the background of orlistat therapy, the risk of myocardial infarction or cerebrovascular accident HR – 1.69, at p=95%, CI 1.12–2.56. Against the background of sibutramine therapy, the risk of myocardial infarction or cerebrovascular accidents HR – 1.52, p=95%, CI 0.92–2.48.	[49]
2.	Therapy with sibutramine (Reduxin®) for 12 months is associated with persistent and clinically significant weight loss and WC, regardless of gender, age and the presence of concomitant diseases	Adult patients with obesity and without comorbid pathology, N=98,774	–	For 12 months of therapy: a clinically significant decrease in body weight by 10-20%, 20% or more in 52.1% and 42.1% of patients, respectively. The mean reduction in waist circumference over 3, 6, and 12 months of therapy was 6.3±4.31 cm, 10.6±6.30 cm, and 16.0±8.94 cm, respectively (p<0.001).	[50]
3.	Weight loss during therapy with the combined drug sibutramine + metformin is associated with the achievement of metabolic health compensation parameters, improved prognosis and quality of patients' lives	Adult patients with obesity and disorders of carbohydrate metabolism, N=55	–	For 6 months of therapy, a decrease in body weight by 10% or more – in 91% of patients; a decrease in TG levels by 0.73±1.0 mmol/l (25%); total cholesterol (TC) – by –0.97±4.8 mmol/l (17%); LDL – by –0.67±1.0 mmol/l (20%) and an increase in HDL by 0.24±43.8 mmol/l (16%) from the initial level; a decrease in FFA from 0.54±0.28 to 0.43±0.25 meq/l, by 20.3% (p<0.001). Achievement of normal values of glycemic parameters observed in 93.2% of patients	[45]
4.	Adding a combination of sibutramine and metformin to the base hypoglycemic therapy in patients with type 2 diabetes and obesity provides effective and safe weight loss, and also improves the effectiveness of treatment, including reaching the target values of carbohydrate and lipid metabolism	Adult patients with obesity and type 2 diabetes, N= 5812	–	A decrease in BMI for 3 and 6 – 3.4±1.5 kg/m^2 (on average 9.5±4.2 kg) and 5.4±2.3 kg/m^2 (15.1±6.4 kg), respectively. The average change in glucose level: –2.0±1.6 mmol/l, the average change in the level of glycated hemoglobin was –1.2±1.1%.	[47]
5.	Therapy with the combined drug sibutramine + metformin (Reduxin® Forte) for 12 months was accompanied by a decrease in body weight, lipotoxicity, normalization of carbohydrate and lipid metabolism, a decrease in the level of leptin and postprandial ghrelin, and normalization of eating habits.	Adult patients with obesity and disorders of carbohydrate metabolism, N=78	–	Weight loss – 21.0±4.62 kg; a reduction in waist circumference – 16.8±3.2 cm; a decrease in TG levels by 0.43±0.3 mmol/l (14%); total cholesterol – by 0.78±0.67 mmol/l (13%); LDL – by 0.61±0.5 mmol/l (19.6%) and an increase in HDL by 0.15±0.2 mmol/l (14%), a decrease in fasting blood glucose by 0.82±0.6 mmol/l (13.1%) (p<0.001); a decrease in glycated hemoglobin – 0.42±0.05% (p<0.001); achievement of normal values of glycemia parameters – 99.3%; improving the quality of patients' lives.	[48]

Thus, obesity is formed as a result of complex pathogenetic and clinical interactions between psychoemotional and metabolic disorders. Due to all the reasons mentioned above, a detailed study of the eating disorders phenomenon, the mechanisms of its formation and consolidation, can help in choosing the most effective tactics for the treatment and prevention of weight gain in most patients with obesity.

Eating disorder

The interpretation of the term "eating disorder" from the standpoint of a psychiatrist and an endocrinologist or a psychologist is somewhat different. So, within the framework of socio-psychological typology, which is used in the practice of endocrinologists and nutritionists, as well as psychologists, eating disorders are understood as the use of such an amount of nutrients that does not correspond to the energy needs of the body. In modern society, unfortunately, eating disorders are considered as a socially acceptable variant of addictive behavior, in contrast to drug addiction, alcoholism or cigarette smo-

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king[5]. The essence of addictive or dependent behavior is the desire to escape from reality, artificially change one's psycho-emotional state, which gives the illusion of security and tranquility. Therefore, psychological personality traits, primarily pronounced personal anxiety, impulsivity, low self-esteem, and some psycho-emotional infantilism, are essential for the formation of obesity [34].

There are three main types of addictive eating behavior: external, emotional, restrictive [35]. The external type refers to eating that is not caused by hunger or low blood glucose, but by external causes. Such reasons include tasty-smelling and appetizing-looking dishes, a well-laid table, and the appearance of people eating, various advertising posters or videos with food. Accepting an invitation to "have a bite for company's sake", a hearty meal at a party or at a festive table; buying a large amount of food in a supermarket are also in the area of the responsibility of external eating behavior. From the situations that contribute to overeating with this type of eating behavior, internal causes become apparent and prompt, often in the field of interpersonal communication, when eating is associated with a means of establishing trusting relationships and encouragement. The external type of eating behavior gradually contributes to the formation of increased appetite and delayed satiety, which is often felt by such patients as mechanical fullness in the stomach or "abdominal discomfort" from overeating [35].

For the emotional type of eating behavior, as the term itself makes it clear, the reason for eating is not hunger either, but emotions make that person "comfort eat", calming himself in this way. The main triggers for emotional overeating can be a severe stress, fear, longing, anxiety, grief, feelings of loneliness and even boredom. As in the mechanism of the formation of affective disorders, with emotional eating behavior, an important role is played by the innate imbalance of neurotransmitter monoamines, which a person seeks to compensate for with a plentiful meal. A similar formation mechanism is also characteristic of other variants of addictive behavior, such as drug or alcohol addiction. Therefore, this type of eating behavior is also called "food drunkenness" [35, 38].

Restrictive eating behavior is characterized by adherence to meaningless and excessive dietary restrictions. Compliance with strict diets is accompanied by a strong feeling of hunger; it is replaced by a breakdown and overeating, the development of "dietary depression", which leads to the formation of a vicious circle. It must be emphasized that in most cases, combinations of different types of eating disorders can be identified in one patient at different times. Therefore, for example, a transition from an external or restrictive to an emotional type of eating disorder is possible against the background of a prolonged exposure to stressful factors. The degree of obesity does not correlate with the type of eating behavior [12, 36].

It should be emphasized that without establishing the type of eating disorder, it is impossible to build an effective treatment strategy and achieve long-term results. It is also important to know that in approximately 30% of patients, eating disorders first occur against the background of irrational diets, which lead to the development of emotional discomfort and cause them to refuse therapy. In addition, already existing disorders can be exacerbated by diet therapy [6, 12].

On the other hand, the practice of psychiatrists also uses its own classification of eating disorders, in accordance with the new International Classification of Diseases, 11th revision (ICD-11)[6]. According to this manual, eating disorders and eating behaviors are included in section L1-6B8. However, only eating disorders include abnormal eating behavior accompanied by marked concerns about weight and body shape. These conditions include anorexia nervosa (6B80), bulimia nervosa (6B81), binge eating (6B82), and a pathological preferential-restrictive eating disorder (6B83). However, it should be emphasized that not all of these conditions are associated with obesity[7].

Anorexia nervosa (AN) is clinically characterized by a gradual decrease in body weight to a degree that does not correspond to normative values, which cannot be explained by another health disorder and is not associated with the inaccessibility of food. Patients with AN intentionally prevent the recovery and maintenance of their normal body weight through persistent restriction of food intake or cleansing behavior (vomiting, use of laxatives and diuretics), as well as through excessive physical exertion. Low weight or body shape is central to self-assessment in the AN patients, and normal weight is mistakenly perceived as being overweight[8].

Bulimia nervosa (BN) is characterized by recurrent episodes of uncontrollable food consumption. At the moment of such overeating, a patient completely loses control over his eating behavior, eats noticeably more than usual and cannot stop eating or limit the amount of food eaten. Further, compensatory behaviors aimed at preventing weight gain develop – inducing vomiting, using laxatives and intense sports. Patients with (BN) are preoccupied with their weight and figure, which have a strong effect on their self-esteem[9].

Binge eating (BE) is also characterized by frequent episodes of uncontrollable food consumption, but unlike BN, bouts of overeating are not necessarily followed by compensatory behavior. However, these attacks are experienced as very unpleasant and are often accompanied by negative emotions such as feelings of guilt or disgust.

[5] Malkina-Pykh IG. Terapiya pishchevogo povedeniya [Eating behavior therapy]. Psychology. M.: Eksmo; 2007:1040 p. Russian

[6] ICD-11. Chapter 06 Statistical classification. M.: "KDU", "University Book", 2021. – 432 p. Russian

[7] Ibid.

[8] Ibid.

[9] Ibid.

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Pathological preferential-restrictive eating disorder is characterized by avoidance or restriction of food intake, which can lead to significant weight loss, clinically significant nutritional deficiencies up to the need for increased supplementation or tube feeding. This type of eating behavior, unlike AN, is not dictated by preoccupation with weight and figure. It is important to note that for the diagnosis of most of the listed mental disorders, an important requirement is the presence of severe distress, i.e. painful experiences, anxiety, discomfort due to a mental or behavioral deviation, and impaired functioning in one or more significant areas of life (personal, family, social, educational, professional, etc.).

Thus, in the clinical practice of internists, there may be patients with mental eating disorders, however, the severity of the clinical picture and the characteristic patterns of behavior of such patients should attract the attention of the attending physician and serve as a reason for referral to a psychiatrist.

Therapeutic strategies

Traditional approaches in the treatment of obesity are establishing a trusting relationship with the patient and discussing a realistic goal of weight loss; long-term and gradual changes in nutrition and the rejection of starvation diets; increasing and intensifying daily physical activities. It is optimal to make up an individual nutrition and training plan together with qualified specialists – a nutritionist and a physiotherapist, followed by a constant support for a long time [10, 11].

Recognizing the obviousness of any intervention aimed at reducing excess weight and maintaining optimal weight, cannot be considered as a one-time or short-term therapeutic intervention, the formation of proper eating behavior is important. Given the frequency of obesity comorbidity with a variety of psychoemotional disorders that have a mutually aggravating effect and prevent constant adherence to recommendations for lifestyle changes and nutrition, some patients require a consultation with a psychologist, psychotherapist or psychiatrist to select psychopharmacotherapy [10].

Prescribing drugs for the treatment of obesity is recommended for patients with a BMI ≥ 30 kg/m^2 (or a BMI ≥27 kg/m^2 in the presence of comorbid cardio-metabolic diseases) when they cannot achieve weight loss of 5–10% within 6 months against the background of the use of all non-drug methods or at the stage of maintaining the achieved result [6, 7, 10]. Currently, the following drugs for the treatment of obesity are registered in the Russian Federation: orlistat, liraglutide, sibutramine, including sibutramine + microcrystalline cellulose and sibutramine + metformin in the form of combinations. Each of the presented drugs has been briefly characterized in the light of solving the problem of eating disorders in obese patients.

Being an inhibitor of gastrointestinal lipase and having a therapeutic effect only within the gastrointestinal tract (GIT), orlistat prevents the splitting and subsequent absorption of fats and fat-soluble vitamins from food. Direct consequences of the mechanism of the orlistat action are such frequent side effects as fatty stools, oily discharge from the rectum, imperative urges to defecate, increased defecation and fecal incontinence, abdominal pains, gas with some amount of discharge from the intestine. These factors make the use of this drug somewhat difficult in the patients with eating disorders who cannot completely refuse fatty foods [6, 10]. The drug orlistat has also such registered contraindications as acute pancreatitis and the diseases accompanied by diarrhea; chronic malabsorption syndrome and cholestasis (since orlistat increases the likelihood of gallstone formation, leading to a decrease in gallbladder motility). It should be emphasized that the therapeutic effect of this drug is modest, and currently, there are no positive data to judge the effect of orlistat on the overall mortality or mortality from CVDs [10].

Liraglutide is an analog of human glucagon-like peptide-1. For a long time, being a hypoglycemic agent, it has been used only in the treatment of type 2 diabetes mellitus. The mechanism of liraglutide action is due to the reinforced feeling of stomach fullness and satiety, without increasing a 24-hour energy expenditure. It has been shown that liraglutide has a positive effect on cardiometabolic risk factors against the background of weight loss [11]. However, just as with orlistat, its side effects are direct acting and the most common are nausea, vomiting, diarrhea, constipation, dry mouth, dyspepsia, gastritis, gastroesophageal reflux, upper abdominal pains, bloating, belching, and cholelithiasis. The drug has also strict contraindications: with a history of medullary thyroid cancer, including family members; with the syndrome of multiple endocrine neoplasia type II; depression; suicidal thoughts or suicidal behavior, incl. in history, severe renal and hepatic insufficiency; chronic heart failure of the 4th functional class; to patients aged ≥75 years. Its use is not recommended to patients with inflammatory bowel disease and diabetic gastric paresis, it is recommended to take the drug in patients with mild to moderate hepatic insufficiency, thyroid disease and a history of acute pancreatitis with caution [10]. Thus, in addition to the impact on the quality of patients' lives due to the frequency of side effects, it has a wide range of contraindications. Liraglutide has an unfavorable effect associated with a possible negative effect on the psycho-emotional status of a person, which makes it impossible to recommend it as a drug of choice for the treatment of obesity to most patients [39].

In our country, sibutramine is the only currently registered drug for the treatment of obesity that has a central effect, being an inhibitor of the reuptake of serotonin, norepinephrine and, to a lesser extent, dopamine in the synapses of the central nervous system. Sibutramine has a dual mechanism of action: on the one hand, it accelerates the onset of satiety, significantly reducing

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the intake of calories from food; on the other hand, it increases the energy consumption of the body due to the activation of thermogenesis. As a whole, they lead to a more effective weight loss, regardless of gender, age, and the presence of concomitant diseases [40]. Moreover, it has been notified that the higher the initial BMI is, the more intense the decrease in body weight will be [41].

It should be notified that sibutramine therapy is accompanied by a feeling of "energy boost" and improved mood, which makes it easier to endure any food restrictions in the process of choosing the right diet [40]. It has also been shown that sibutramine not only provides effective weight loss, but also improves cardio-metabolic parameters. Thus, against the background of sibutramine therapy, the state of atherogenic dyslipidemia and the insulin resistance significantly improves (the levels of glycated hemoglobin, uric acid, triglycerides, total cholesterol, low-density lipoproteins decrease, and the content of anti-atherogenic high-density lipids increases) [41].

It should be notified that due to the sympathetic activation against the background of sibutramine therapy, a slight change in hemodynamic parameters is possible. Therefore, all patients need to control the pulse and blood pressure (BP) levels before starting therapy, then from the 1st to the 3rd month – once every 2 weeks; from the 4th to the 6th month – once a month; from the 6th to the 12th month – once every 3 months. The drug cannot be prescribed to the patients with uncontrolled arterial hypertension, i.e. if the blood pressure is constantly above 145/90 mm Hg, coronary heart disease, decompensated chronic heart failure, cardiac arrhythmias, previous cerebrovascular diseases (stroke, transient cerebrovascular accident), occlusive diseases of peripheral arteries in patients over the age of 65 years. However, in real clinical practice, in patients with obesity and controlled hypertension, sibutramine has shown itself to be an effective and safe drug that effectively reduces body weight [42]. The side effects such as loss of appetite, dry mouth, some agitation and an increased activity, sweating are usually mild, notified only at the beginning of treatment, are transient and, as a rule, do not require discontinuation of therapy. The use of sibutramine in combination with metformin increases the therapeutic efficacy of both components, both in patients with and without carbohydrate metabolism disorders [43].

Over the past decades, much experience has been gained in the clinical use of sibutramine and metformin used for treatment of obese patients. The expediency of combination therapy using sibutramine and metformin, and later the creation of a combination and its fixed form (Reduxin® Forte), was dictated by the need to obtain a more pronounced therapeutic effect through the active influence on various links in the obesity pathogenesis. In the international study that took place in medical centers in England, Canada, France and Belgium, the effect of sibutramine on body weight, metabolic control and blood pressure, was evaluated in obese patients treated with metformin and suffering from type 2 diabetes. The study included 195 patients (44% men) with DM2 and BMI >27 kg/m^2. The improvement in glycemic control in patients has been shown to occur in parallel with weight loss. The researchers concluded that sibutramine is an effective adjunct to the treatment of patients with obesity and T2DM with metformin [44].

According to Russian researchers, therapy with Reduxin® Forte for 24 weeks was also accompanied by significant changes in anthropometric parameters, i.e.: weight loss by 5% or more in 6 months of therapy was achieved in 94% of patients, while 91% of patients managed to reduce weight by 10% or more. Given the severity of weight loss, which was accompanied by a significant decrease in waist circumference, conclusions, regarding the effectiveness of the fixed combination (metformin + sibutramine) for weight loss, as well as the reduction of visceral fat, which helps to reduce the risk of complications, were drawn by A.S. Ametov et al. [45].

It has also been shown that the combined use of sibutramine with metformin not only increases the effectiveness of weight loss, but also reduces the level of chronic inflammation and the risk of complications associated with overweight, including CVDs and T2DM, in combination with a comprehensive restoration of metabolic health. These data are especially relevant in the context of reducing cardiovascular risks and improving the prognosis of such patients [46].

An analysis of the AURORA program results, which included a sample of 5812 patients taking a combination drug containing sibutramine and metformin, demonstrated clinically significant weight loss and a decrease in waist circumference in more than 90% of patients with T2DM, regardless of the underlying hypoglycemic therapy. In addition, the combination of sibutramine and metformin has been shown to have a positive effect on carbohydrate and lipid metabolism, contributing to an additional decrease in glycated hemoglobin and normalization of the atherogenic index in more than 50% of patients. It should be emphasized that the incidence of adverse events did not exceed 5.1%. All of the above mentioned, makes it possible to draw conclusions about the clinical feasibility of including combination therapy (sibutramine + metformin) in the therapy of patients with DM2 and obesity, since its use is effective and safe, regardless of the presence of polymorbid pathology and the use of various concomitant drugs [47].

In another study based on using a fixed combination of metformin + sibutramine, it was found out that during 12 months of therapy, the insulin resistance index normalized in 100% of patients. The dynamics of the decrease in BMI for 3 months of therapy was 3.6±1.1 kg/m^2 (at an average of 9.8±4.2 kg); for 6 months – 5.5±2.6 kg/m^2 (15.9±5.4 kg); for 2 months – 7.1±3.02 kg/m^2 (21.0±4.62 kg)

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(p<0.001). The decrease in WC for 3, 6 and 12 months of therapy, on average, amounted to 7.3±2.8, 13.1±6.4 and 16.8±3.2 cm, respectively, which indicates the advisability of taking the combination drug metformin + sibutramine to reduce the amount of visceral fat and reduce the risk of complications such as type 2 diabetes and CVD.

It is important to notify that during the therapy, a correction of the patients' eating behavior was observed: the ongoing treatment reduced the number of patients with the emotiogenic type of pathological overeating by 4.7 times, by 5.8 times with the external type, and by 1.9 times with the restrictive PO. At the end of taking the drug, 75.7% of patients observed a rational type and developed correct eating habits. The decrease in caloric intake during ad libitum food intake for 3 months of therapy was 36% (1648±852 kcal). Most patients notified a decrease in the frequency of unplanned meals, the disappearance of the need for evening meals. Approximately 89% of patients managed to achieve and maintain individual target values of daily caloric intake during the year of therapy [48].

Thus, taking into account the favorable cardio-metabolic profile of the therapeutic metformin action [49], which has been used for a long time in the treatment and prevention of all obesity-associated diseases, as well as its concomitant effects with sibutramine, one can be confident in the positive synergy of the double combination – sibutramine + metformin (Reduxin® Forte[10]). This makes it possible to achieve a significant reduction in body weight, compensation for metabolic disorders and correction of the eating habits of patients with obesity, which is the key to successful obesity therapy.

So, in October 2021, the Ministry of Health of the Russian Federation decided to amend the instructions for use of the drug Reduxin® Forte. Now, in addition to use in people with obesity, prediabetes and additional risk factors for developing DM2, the drug is also indicated for all patients with alimentary obesity (BMI ≥ 30) who do not have contraindications. This drug gives hope for the improvement in the situation with the treatment of obesity in most patients in our country. The summarized data from the studies of the efficacy and safety of the drugs containing sibutramine and its combination with metformin in routine clinical practice are presented in Table 3. It is interesting to conduct further studies to identify additional pleiotropic effects of this combination in obese patients.

CONCLUSION

Thus, the combined prevalence of obesity and psycho-emotional disorders worldwide is steadily increasing. Given the negative impact of eating disorders on the ability to effectively reduce and maintain optimal body weight, an earlier prescription of drug therapy that can affect both conditions is necessary. The combined drug containing sibutramine and metformin (Reduxin® Forte) registered in the Russian Federation is an effective and safe drug that can be used in all patients with alimentary obesity, taking into account contraindications.

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CONFLICT OF INTERESTS

The author declares no conflict of interest.

AUTHOR'S CONTRIBUTION

Veronika N. Shishkova – design development, material recruitment, analysis and interpretation of the results, writing and editing the text.

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AUTHOR

Veronika N. Shishkova – Doctor of Sciences (Medicine), Head of the Department for the Prevention of Cognitive and Psychoemotional Disorders, National Medical Research Center for Therapy and Preventive Medicine. ORCID ID: 0000-0002-1042-4275. E-mail: veronika-1306@mail.ru

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ОРИГИНАЛЬНАЯ СТАТЬЯ

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FEATURES OF QUANTITATIVE ESTIMATION OF FLAVONOID CONTENT IN *JUGLANS NIGRA* L. BARKS PREPARATIONS

V.A. Kurkin, N.I. Zimenkina

Samara State Medical University
89, Chapaevskaya St., Samara, Russia, 443099

E-mail: v.a.kurkin@samsmu.ru

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The aim of the research is the development of quantification procedures of flavonoids in *Juglans nigra* L. barks preparations using modern instrumental analytical techniques (spectrophotometry, high performance liquid chromatography).
Materials and methods. The subjects of research were tincture and dry extract of *Juglans nigra* L. bark, the samples of which were prepared in March and April 2020 in the Botanical Garden of Samara State Medical University (Samara); the standard samples (SS) of myricitrin, myricetin. The registration of the electronic spectra was carried out with a spectrophotometer «Specord 40» (Analytik Jena, Germany). The chromatographic analysis was carried out by the method of reversed-phase HPLC on a microcolumn liquid chromatograph "Milichrom-6" (NPAO "Nauchpribor", Russia).
Results. Using differential spectrophotometry, methods for the quantitative determination of the total amount of flavonoids in terms of myricitrin in the tincture and dry extract of *Juglans nigra* L. bark, has been developed. It has been determined that the content of the total amount of flavonoids in terms of myricitrin in the tincture and dry extract of *Juglans nigra* L., is 0.84±0.07% and 12.38±0.24%, respectively. The error of a single determination of the total amount of flavonoids in terms of myricitrin in the tincture and dry extract of *Juglans nigra* L. bark with a confidence probability of 95%, is ± 8.91% and ± 2.10%, respectively. Methods for the quantitative determination of myricitrin in the tincture and dry extract of *Juglans nigra* L. bark by HPLC has been developed. The content of the dominant flavonoid – myricitrin (myricetin-3-O-α-L-rhamnopyranoside) – in the tincture and dry extract of *Juglans nigra* L., was 0.42±0.06% and 8.45±0.24%, respectively. The error of the single determination of myricitrin in the tincture and dry extract of *Juglans nigra* L. with a confidence probability of 95% is ± 15.04% and ±2.96%, respectively.
Conclusion. The developed methods for the quantitative determination of flavonoids in the preparations of *Juglans nigra* L. barks L. can be used in solving the problems of standardization of *Juglans nigra* L. preparations.
Keywords: *Juglans nigra* L.; bark; UV spectrophotometry; HPLC; myricitrin; flavonoids
Abbreviations: MPRM – medicinal plant raw materials; HP – herbal preparation; HPLC – high-performance liquid chromatography; SS – standard sample.

ОСОБЕННОСТИ КОЛИЧЕСТВЕННОЙ ОЦЕНКИ СОДЕРЖАНИЯ ФЛАВОНОИДОВ В ПРЕПАРАТАХ КОРЫ ОРЕХА ЧЕРНОГО

В.А. Куркин, Н.И. Зименкина

Федеральное государственное бюджетное образовательное учреждение высшего образования «Самарский государственный медицинский университет» Министерства здравоохранения Российской Федерации
443099, Россия, г. Самара, ул. Чапаевская, 89

E-mail: v.a.kurkin@samsmu.ru

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RESEARCH ARTICLE

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Цель. Разработка методик количественного определения флавоноидов в препаратах коры ореха черного с помощью современных инструментальных методов анализа (спектрофотометрия, микроколоночная высокоэффективная жидкостная хроматография).

Материалы и методы. Объектами исследования являлись настойка и сухой экстракт коры ореха черного (*Juglans nigra* L.), образцы которой были заготовлены в марте-апреле 2020 года на территории Ботанического сада ФГБОУ ВО СамГМУ Минздрава России (г. Самара), стандартные образцы мирицитрина, мирицетина. Регистрацию УФ-спектров проводили с помощью спектрофотометра «Specord®40» (Analytik Jena) методом дифференциальной спектрофотометрии. Хроматографический анализ осуществляли методом обращенно-фазовой ВЭЖХ на микроколоночном жидкостном хроматографе «Милихром-6» (НПАО «Научприбор»).

Результаты. Разработана методика количественного определения суммы флавоноидов в пересчете на мирицитрин в настойке и сухом экстракте коры ореха черного (*Juglans nigra* L.) с помощью метода дифференциальной спектрофотометрии. Установлено, что содержание суммы флавоноидов в настойке и сухом экстракте коры ореха черного составляет 0,84±0,07% и 12,38±0,24% (в пересчете на мирицитрин) соответственно. Ошибка единичного определения суммы флавоноидов в пересчете на мирицитрин в настойке и сухом экстракте коры ореха черного с доверительной вероятностью 95% составляет ±8,34% и ±2,10% соответственно.

Разработана методика количественного определения мирицитрина в настойке и сухом экстракте коры ореха черного (*Juglans nigra* L.) методом ВЭЖХ. Содержание доминирующего флавоноида – мирицитрина (мирицетин-3-О-α-L-рамнопиранозид) в настойке и сухом экстракте коры ореха черного составляет 0,42±0,03% и 8,45±0,24% соответственно. Ошибка единичного определения мирицитрина в настойке и сухом экстракте коры ореха черного с доверительной вероятностью 95% составляет ±7,14% и ±2,96% соответственно.

Заключение. Разработанные методики количественного определения флавоноидов в настойке и сухом экстракте коры ореха черного могут быть использованы для решения вопросов стандартизации препаратов указанного лекарственного растительного сырья.

Ключевые слова: орех черный; *Juglans nigra*; кора; УФ-спектрофотометрия; ВЭЖХ; мирицитрин; флавоноиды

Список сокращений: ЛРС – лекарственное растительное сырье; ЛРП – лекарственный растительный препарат; ВЭЖХ – высокоэффективная жидкостная хроматография; СО – стандартный образец.

INTRODUCTION

At present, the search for herbal preparations with a high content of biologically active compounds and a pharmacological activity is a hot topic in pharmacy. Representatives of the genus *Juglans* L. species of the *Juglandaceae* family have the indicated features and, therefore, are promising species of medicinal plant raw materials to be used in medical practice. Representatives of the *Juglans* genus L. are potential sources of naphthoquinones as an important class of biologically active compounds [1–4]. About eight species of plants of the genus *Juglans L.* tend to be cultivated in the territory of the Russian Federation. Medicinal plants *Juglans regia* L., *Juglans nigra* L. and *Juglans cinerea* L. are very interesting for research [5].

The authors' opinion, the bark of *Juglans nigra* L. can serve as a promising object for the production of new herbal formulations [6, 7]. The previous studies have shown that the bark of *Juglans nigra* L. contains various derivatives of naphthoquinone and other compounds: nitrogenous matters, triterpenes and phenolic compounds, including flavonoids [8–13]. A variety of chemical composition, including the presence of a large number of phenolic compounds, determine a wide range of pharmacological activity of the genus *Juglans* L. representatives (*Juglans nigra* L., *Juglans regia* L. and *Juglans cinerea* L) [14–18]. The known antimicrobial, general tonic, anti-inflammatory and antioxidant activities of *Juglans* L. preparations which are present in the pharmological market, may be due to the substances of a flavonoid identity [19–23]. These data indicate the relevance of studying the flavonoids of the *Juglans nigra* L. bark and preparations based on this medicinal plant.

Regardless of the fact that standardization of the bark and *Juglans nigra* L. preparations is carried out according to the content of naphthoquinones (in terms of juglone), has been was found out that the dominant and diagnostically significant compound was flavonoid myricitrin (myricetin-3-O-α-L-rhamnopyranoside). This compound revealed anti-inflammatory, antinociceptive and neurotropic effects [24–26]. Consequently, *Juglans nigra* L. bark preparations are promising for a further study not only in the field of pharmacology, but also in the context of their quality control [27–30].

THE AIM of the research is the application of methods of UV spectrophotometry and microcolumn high performance liquid chromatography (HPLC) to control the content of flavonoids, as well as the analysis of the content of the total amount of flavonoids (UV spectrophotometry) and myricitrin (HPLC) in the obtained tincture and dry extract of the *Juglans nigra* L. bark.

MATERIALS AND METHODS

The objects of research were *Juglans nigra* L. bark tincture and dry extract. These samples were prepared in March-April 2020 in the territory of the Botanical Garden of Samara University (Samara). The tincture and dry extract of *Juglans nigra* L. bark were analyzed using standard samples of myricitrin and myricetin (Fig. 1) by methods of UV spectrophotometry and HPLC.

For HPLC, acetonitrile, c. p. acetic acid ("Component-reactive" LLC, Russia), the water obtained using a

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system for obtaining purified water with a multi-stage purification system (adsorption, reverse osmosis, membrane filtration) and tested for purity under the chromatographic analysis conditions.

The registration of UV spectra was carried out using the spectrophotometry «Specord 40» (Analytik Jena, Germany). The spectral characteristics of these standard samples of myricitrin and myricetin are presented below.

Miricitrin (myricetin-3-O-α-L-rhamnopyranoside) is a yellow with a cream tint crystalline substance with melting points of 203-205 C (aqueous alcohol)[1], UV spectrum (EtOH, λ_{max}, nm): 212, 260, 358; + NaOAc 268, 366; + NaOAc + H_3BO_3 260, 382; + $AlCl_3$ 278, 416; +$AlCl_3$ + HCl 270, 406.

NMR^{1}H (300 MHz, DMSO-d_6, δ, ppm, J/Hz.): 12.68 (1H, s, 5-OH group), 9.23 (2H, br.s , 7-OH-group and 4'-OH-group), 6.88 (2H, s, H-2 'and H-6'), 6.36 (1H , d, 2.5 Hz, H-8), 6.19 (1H, d, 2.5 Hz, H-6), 5.20 (1H, d, 1.5 Hz, H-1'' of rhamnose), 3.1–5.0 (m, 4H of rhamnose), 0.84 (3H, d, 6 Hz, CH_3 of rhamnose).

NMR^{13}C (126.76 MHz, DMSO-d_6, δC, ppm): C-4 (177.85), C-7 (164.24), C-5 (161.37), C-4› (157.57), C-9 (156.49), C-2 and C-3 (145.83), C-3' and C-5' (145.83), C-1 '(119.70), C-2' and C-6 '(108.00), C-10 (104.12), C-1›› of rhamnose (102.00), C-6 (98.41), C-8 (94.30), C-2› (116.21), C-3›› (71.03), C-5 '' (70.62), C-4'' (70.47), C-2'' (70.08), C-6'' (CH_3) (17.57).

Mass spectrum (HR-ESI-MS, 180°C, m/z): m/z 465.1016 [M + H]+, m/z 487.0830 [M + Na]+, m/z 503.0560 [M + K]+.

Myricetin (3,5,7,3',4',5'-hexahydroxyflavone) is a turtle green crystalline substance with melting points of 357°C (aqueous alcohol), UV spectrum (EtOH, λ_{max}, nm): 254, 377; + NaOAc 275, 382; + NaOAc + H_3BO_3 258, 392; + A1C1$_3$ 266, 440; +A1C1$_3$+ HCl 266, 440.

NMR^{1}H (399.78 MHz, DMSO-d_6, δ, ppm, J/Hz): 12.45 (1 H, s, 5-OH group), 10.73 (1H s, 7-OH- group), 9.28 (1H, s, 4'-OH-group), 9.17 (2H, s, 3'-OH-group and 5'-OH-group), 8.75 (1H, s, 3-OH-group), 7.20 (2H, s, H-2 'and H-6'), 6.32 (1H, d, 2.2 Hz, H-8), 6.14 (1H, d, 2.2 Hz, H-6).

NMR^{13}C (100.52 MHz, DMSO-d6, δ_c, ppm): C-4 (176.29), C-7 (164.39), C-5 (161.25), C-9 (156.59), C-4' (147.36), C-3' and C-5' (146.23), C-2 and C-3 (136.38), C-1' (121.30), C-2' and C-6' (107.68), C-10 (103.49), C-8 (93.71), C-6 (98.67).

Based on the spectral data, since the dominant flavonoid myricitrin has an absorption maximum at 360 ± 2 nm in the long-wavelength region of the electronic spectrum, 360 nm wavelength for the detection of analytes during HPLC analysis was chosen.

Preparation of work solutions for analysis by UV spectrophotometry

Juglans nigra L. bark tincture was obtained from the *Juglans nigra* L. bark using a 70% water-ethanolic solution at the ratio of «raw material- extracting solvent» 1:5 using the method of fractional maceration. A part of the *Juglans nigra* L. bark fluid extract 1:1 was used to obtain a solid extract, and then the dry extract of *Juglans nigra* L. bark. The thick extract was obtained by removing the extractant from the liquid extract under vacuum. The thick extract was then dried in a hot-air oven to obtain a dry extract.

Sample processing of *Juglans nigra* L. bark tincture. 1.00 ml of *Juglans nigra* L. bark tincture was placed in a 25 ml capacity measuring flask, the volume of the solution was adjusted by a 70% water-ethanolic solution (sample solution A_1). 1 ml of the sample solution A_1 was placed in a 50 ml capacity measuring flask, 2 ml of 3% ethanolic solutions of aluminum chloride was added and the volume of the solution was adjusted by a 96% ethanolic solution (sample solution B_1). The reference solution was prepared by the following methods: 1 ml of the sample solution A_1 was placed in a 50 ml capacity measuring flask and the volume of the solution was adjusted by a 96% ethanolic solution.

Sample processing of *Juglans nigra* L. bark dry extract. About 0.2 g of the dry *Juglans nigra* L. bark extract (an accurately weighed quantity) was placed in a 25 ml capacity measuring flask, dissolved in 15 ml of a 70% water-ethanolic solution by heating in a water bath and the volume of the solution was adjusted by the same solvent (sample solution A_2). 1 ml of the sample solution A_2 was placed in a 50 ml capacity measuring flask, 2 ml of 3% ethanolic solutions of aluminum chloride was added, and the volume of the solution was adjusted by a 96% ethanolic solution (sample solution B_2). The reference solution was prepared in the following way: 1 ml of the sample solution A_2 was placed in a 50 ml capacity measuring flask and the volume of the solution was adjusted by a 96% ethanolic solution.

Preparation of a standard myricitrin solution for UV spectrophotometry. About 0.0025 g of myricitrin (an accurately weighed quantity) was placed in a 25 ml capacity measuring flask, dissolved in 15 ml of 80% water-ethanolic solutions by heating in a water bath. The contents of the capacity measuring flask was cooled down to room temperature, the volume of the solution was adjusted by a 80% water-ethanolic solution (myricitrin standard solution A_3). 5 ml of myricitrin standard solution A_3 was placed in a 50 ml capacity measuring flask, 2 ml of 3% ethanolic solutions of aluminum chloride was added and the volume of the solution was adjusted by a 96% ethanolic solution (myricitrin standard solution B_3).

Method of quantitative determination of total amount of flavonoids in *Juglans nigra* L. bark tincture

About 1.00 ml of *Juglans nigra* L. bark tincture (an accurately weighed quantity) was placed in a 25 ml capacity measuring flask, the volume of the solution was

[1] USA National Library of Medicine National Institutes of Health. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Myricitrin.

adjusted by a 70% water-ethanolic solution (sample solution A_1). 1 ml of sample solution A_1 was placed in a 50 ml capacity measuring flask, 2 ml of 3% ethanolic solutions of aluminum chloride was added and the volume of the solution was adjusted by a 96% ethanolic solution (sample solution B_1). The reference solution was prepared in the following way: 1 ml of the sample solution A_1 was placed in a 50 ml capacity measuring flask and the volume of the solution was adjusted by a 96% ethanolic solution.

The content of the total amount of flavonoids in terms of myricitrin and absolutely dry raw materials in percent (X) was calculated by the formula:

$$x = \frac{A * m_0 * 25 * 50 * 5 * 100}{A_0 * V * 25 * 1 * 25},$$

where: A – the absorption of the test solution; A_0 – the absorption of the myricitrin standard solution; V – the volume of the tincture for analysis, ml; m_0 – mass of the myricitrin standard sample, g.

If a standard sample of myricitrin is absent, the theoretical value of the specific absorbance – 432 at the wavelength of 416 nm – can be used.

$$x = \frac{A * 25 * 50}{V * 432},$$

where: A – the absorption of the test solution; V – the volume of the tincture for the analysis, ml; 432 – specific absorbance ($E^{1\%}_{1cm}$) of myricitrin at 416 nm.

Method of quantitative determination of total amount of flavonoids in *Juglans nigra* L. bark dry extract

About 0.2 g of dry *Juglans nigra* L. bark extract (an accurately weighed quantity) was placed in a 25 ml capacity measuring flask, dissolved in 15 ml of a 70% water-ethanolic solution by heating in a water bath and the volume of the solution was adjusted by the same solvent (sample solution A_2). 1 ml of the sample solution A_2 was placed in a 50 ml capacity measuring flask, 2 ml of a 3% ethanolic solution of aluminum chloride was added and the volume of the solution was adjusted by a 96% ethanolic solution (sample solution B_2). The reference solution was prepared in the following way: 1 ml of the sample solution A_2 was placed in a 50 ml capacity measuring flask and the volume of the solution was adjusted by a 96% ethanolic solution.

The content of the total amount of flavonoids in terms of myricitrin and absolutely dry raw materials in percent (X) was calculated by the formula:

$$x = \frac{A * m_0 * 25 * 50 * 5 * 100}{A_0 * m * 25 * 1 * 25},$$

where: A – the absorption of the test solution; A_0 – the absorption of the myricitrin standard solution; m – mass of the dry extract, g; m_0 – mass of the myricitrin standard sample, g.

If a standard sample of myricitrin is absent, the theoretical value of the specific absorbance – 432 at the wavelength of 416 nm can be used.

$$x = \frac{A * 25 * 50}{m * 432},$$

where: A – the absorption of the test solution; m – mass of the dry extract, g; 432 – the specific absorbance ($E^{1\%}_{1cm}$) of myricitrin at 416 nm.

Preparation of sample solutions for HPLC analysis

Sample processing for *Juglans nigra* L. bark tincture. 5.00 ml of *Juglans nigra* L. bark tincture (an accurately weighed quantity) was placed in a 25 ml capacity measuring flask. The volume of the solution was adjusted by a 96% ethanolic solution (sample solution A_4). The sample solution A_4 was decanted using a Milipore membrane filter (0.45 µm).

Sample processing of dry *Juglans nigra* L. bark extract. About 0.2 g of dry *Juglans nigra* L. bark extract (an accurately weighed quantity) was placed in a 25 ml capacity measuring flask, dissolved in 15 ml of a 70% water-ethanolic solution by heating in the water bath and the volume of the solution was adjusted by the same solvent (sample solution A_5). 5 ml of the sample solution A_5 was placed in a 25 ml capacity measuring flask. The volume of the solution was adjusted by a 96% ethanolic solution (sample solution B_5). The sample solution A_5 was decanted using a Milipore's membrane filter (0.45 µm).

Preparation of myricitrin sample solution for HPLC. About 0.02 g of myricitrin (an accurately weighed quantity) was placed in a 50 ml capacity measuring flask, dissolved in a 70% water-ethanolic solution, and the volume of the solution was adjusted by the same solvent.

Preparation of a myricetin standard solution for HPLC. About 0.02 g of myricetin (an accurately weighed quantity) was placed in a 50 ml capacity measuring flask, dissolved in a 70% water-ethanolic solution, and the volume of the solution was adjusted by the same solvent.

Chromatographic conditions

A chromatographic analysis was carried out by reverse-phase high performance liquid chromatography (RP-HPLC) on a microcolumn liquid chromatograph "Milichrom-6" by using the following conditions: isocratic mode, steely column "KAH-6-80-4". The mobile phase was acetonitrile: a 1% solution of acetic acid in water at the ratio of 2:8, the elution rate was 100 µL/min. The volume of the eluent was 2500 µL. The substances were detected at the wavelength of 360 nm. The volumes of the injected samples were 4 µl for the tincture and dry extract of *Juglans nigra* L. bark, myricitrin, myricetin.

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Suitability assessment of the developed chromatographic system

To assess the suitability of the chromatographic system, 5-fold chromatography of the test solution of *Juglans nigra* L. bark tincture was carried out. Subsequently, the following indicators were calculated: column performance, resolution between peaks, and the asymmetry factor. Based on the calculations, the following results were obtained (Table 1).

Quantitative determination method of myricitrin in *Juglans nigra* L. bark tincture

About 5.00 ml of *Juglans nigra* L. bark tincture (an accurately weighed quantity) was placed in a 25 ml capacity measuring flask. The volume of the solution was adjusted by a 96% ethanolic solution (sample solution A_4). The sample solution A_4 was decanted using a Milipore's membrane filter (0.45 μm).

4 μl of the sample solution was injected in the liquid chromatograph "Milichrom-6" with a UV detector. Chromatography was carried out by reverse-phase high performance liquid chromatography in the isocratic mode on a steel column "KAH-6-80-4" (No. 2; 2 mm x 80 mm; Separon-C18 7 μm). The eluent system was acetonitrile – water in the ratio of 2:8 with the addition of 1% acetic acid, the elution rate – 100 μl/min, the eluent volume – 2500 μl. The operating wavelength was 360 nm, the sensitivity range – 0.5.

Parallely, 4 μl of the myricitrin standart sample was introduced into the chromatograph and chromatographed as described above. At least 3 parallel determinations for the myricitrin tincture test solution and the myricitrin standard solutions were carried out, as described above. The peak of myricitrin was identified on the chromatograms of the test solution. The average area of the myricitrin peak on the chromatograms of the myricitrin sample solution was calculated according to the results of 3 determinations.

The content of myricitrin in the *Juglans nigra* L. bark tincture was calculated in terms of absolutely dry raw materials in percent (X) by the formula:

$$x = \frac{S * m_0 * 0,98 * V * V_2 * 100}{S_0 * V_t * V_0 * V_1},$$

where: S – the average value of the the myricitrin peak area in the chromatogram of the working solution; S_0 – the average value of the myricitrin peak area in the chromatogram of the standard sample solution; V – the volume of the working solution, ml; V_2 – the volume of the injected sample of the working solution, μl; V_0 – the volume of the sample of the myricitrin standard solution, ml; V_2 – the volume of the injected sample of the myricitrin standard solution, μl; V_t – the volume of the tincture for the analysis, ml; m_0 – mass of the myricitrin standard sample, g; 0,98 – substance assay in 1.0 g of the myricitrin standard sample.

Methods of myricitrin quantitative determination in *Juglans nigra* L. bark dry extract

0.2 g of *Juglans nigra* L. bark dry extract (an accurately weighed quantity) was placed in a 25 ml capacity measuring flask, dissolved in 15 ml of a 70% water-ethanolic solution by heating in a water bath and the volume of the solution was adjusted by the same solvent (sample solution A_5). 5 ml of the sample solution A_5 was placed in a 25 ml capacity measuring flask. The volume of the solution was adjusted by a 96% ethanolic solution (sample solution B_5). The sample solution A_5 was decanted using a Milipore's membrane filter (0.45 μm).

On a microcolumn liquid chromatograph "Milichrom-6" with a UV detector, 4 μl of the sample solution was injected. Chromatography was carried out by reverse-phase high performance liquid chromatography in isocratic mode on a steely column "KAH-6-80-4". The mobile phase was acetonitrile: a solution of 1% acetic acid in water at the ratio of 2:8, elution rate was 100 μL/min. The eluent volume was 2500 μL. The operating wavelength was 360 nm, the sensitivity range was 0.5. 4 μl of the resulting solution was injected in the liquid chromatograph "Milichrome-6" (NPAO "Nauchpribor", Russia) with a UV detector. It was chromatographed under reversed-phase chromatography in the isocratic mode on the steel column "KAH-6-80-4" (No. 2; 2 mm x 80 mm; Separon-C18 7 μm), the eluent system was acetonitrile – water in the ratio of 2: 8 with the addition of 1% acetic acid, the elution rate was 100 μl/min, the eluent volume was 2500 μl. The operating wavelength was 360 nm, the sensitivity range was 0.5.

The content of myricitrin in the *Juglans nigra* L. bark dry extract in terms of absolutely dry raw materials in percent (X) was calculated by the formula:

$$x = \frac{S * m_0 * 0,98 * V * V_2 * 100}{S_0 * m_e * V_0 * V_1},$$

where: S – the average value of the myricitrin peak area in the chromatogram of the working solution; S_0 – the average value of the myricitrin peak area in the chromatogram of the standard sample solution; V – the working solution volume, ml; V_2 – the injected sample volume of the working solution, μl; V_0 – the solution volume of the myricitrin standard sample, ml; V_2 – the injected sample volume of the myricitrin standard sample, μl; m_e – mass of the dry extract, g; m_0 – mass of the myricitrin standard sample, g; 0,98 – the substance assay in 1.0 g of the myricitrin standard sample.

Metrological characteristics of the developed methods

To carry out the calibration procedure, a series of dilutions of myricitrin (250–2500 μg/mL) were chromatographed under the described conditions. Based on the data obtained, a graph was plotted in the coordinates "concentration, μg/mL – peak area" and the linear regression equation ($Y = aX + b$), the value of the coefficient of

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determination (r^2), and the standard deviation were calculated using Microsoft Excel 2013. Statistical processing of the experimental data on the intermediate precision of the developed methods in the analysis of 11 samples of the test solutions of the tincture and dry extract (P = 95%) was carried out. Herewith, Student's t-test to calculate the boundary values of the confidence interval of the average result and determine the error of a single determination (SP RF XIV, GPM 1.1 .0013.15)[2] was used. The stability of the methods was determined by the sample of the *Juglans nigra* L. bark tincture analyzing it 2, 4, 8, 12, 24, 48, and 72 hours after the first analysis. The correctness of the methods was determined at the sample of the *Juglans nigra* L. bark tincture and the standard myricitrin solution in the amount of 25% to 75% of the original content using the standard addition method.

RESULTS AND DISCUSSION

Proceeding from the literature data, there are several approaches to the standardization of medicinal plant materials of the genus *Juglans* species including the *Juglans nigra* L. bark.

The colleagues from the Pyatigorsk Medical and Pharmaceutical Institute, the branch of Volgograd State Medical University, proposed an approach to the standardization of medicinal plant and herbal preparations of the genus *Juglans*. This approach consisted in using naphthoquinones (in particular, juglone) as the analyzed biologically active substance group [4, 28, 29].

To quantify the total amount of naphthoquinones in terms of juglone in the herbal preparations of the genus Juglans species, the developed methods of the photocolorimetric determination was used. In this case, the extraction was obtained by the double extraction method with a 20% water-ethanolic solution, followed by the concentration, and a triple extraction with diethyl ether [4, 28, 29].

In addition to polyphenols, such as phenylpropanoids, flavonoids and tannins, as well as terpenoids, they can also act as the analyzed biologically active substance group for the quantitative determination methods. The analysis of these compounds was carried out by spectrophotometry, HPLC, liquid chromatography, mass spectrometry with an ion trap, GC-MS [29, 31].

Previously, the studies by the Department of Pharmacognosy with Botany and foundations of Phytotherapy of Samara State Medical University showed the possibility of quantifying the total amount of flavonoids in the *Juglans nigra* L. bark by differential spectrophotometry in terms of myricitrin; the analytical wavelength corresponded to 416 nm [7]. Based on the studies carried out, it can be concluded that further research is needed in terms of standardization of medicinal herbal preparations of *Juglans nigra* L.

A comparative study of the tested solutions electronic spectra of *Juglans nigra* L. bark preparations (tincture and dry extract) made it possible to establish two absorption maxima of about 260 nm and 360 nm, which were characteristic of flavonoids (flavonols) and this was confirmed by the bathochromic shift of the long-wavelength band in the presence of $AlCl_3$, as well as facts of differential spectra with an absorption maximum of 414–416 nm (Fig. 2B-2E).

The authors found out that myricitrin in *Juglans nigra* L. makes a significant contribution to the nature of the absorption spectrum of the hydroalcoholic extract from the *Juglans nigra* L. bark, therefore, this is a dominant and diagnostically significant substance for this type of raw materials. Taking into account the fact that the absorption maxima of the solution of the standard myricitrin sample and the aqueous-alcoholic extract of the *Juglans nigra* L. bark are in the region of 416 nm (differential variant), it is advisable to determine the content of the total amount of flavonoids in terms of myricitrin at the wavelength of 416 nm (Fig. 2E and 2E).

During the development of quantitative determination methods for the *Juglans nigra* L. bark tincture and dry extract, the optimal parameters of the sample processing and the analytical wavelength for the quantitative analysis (416 nm) were determined.

The dependence of the optical density on the myricitrin concentration was described by a linear regression in the concentration range from 10 to 50 μg/mL (Fig. 3).

The metrological characteristics of the quantitative determination methods of the total flavonoids content in the preparations of *Juglans nigra* L. bark are presented in Table 2. The results of the intermediate precision assessment of the results of the experiments carried out, indicate a satisfactory reproducibility of the analysis results. The error of a single determination of the flavonoids amount in the *Juglans nigra* L. bark tincture and dry extract with a confidence level of 95% is ±8.34% and ±2.10%, respectively (Table 2).

The validation assessment of the developed methodology was carried out according to the following indicators: specificity, linearity, correctness. The specificity of the method was determined by the correspondence of the absorption maxima of the *Juglans nigra* L. bark flavonoid complex and the myricitrin standard reference with aluminum chloride. The linearity of the method for a series of the standard myricitrin solutions (with the concentrations ranging from 10 to 25 μg/ml) was determined. The coefficient of determination was 0.99988.

The methods correctness was determined by the standard addition method. A myricitrin solution with a known concentration (25%, 50% and 75%) was added to the test tincture solution. A relative error of the analysis was ±3.19%. The experiments with the addition of myricitrin to the samples of raw materials showed that the analysis error is within the error of a single determination, which indicates the absence of a systematic error in the developed method (Table 3).

[2] State Pharmacopoeia of the Russian Federation. XIV edition. Vol. 4. Moscow, 2018. – 1832 p. Available from: http://resource.rucml.ru/feml/pharmacopia/14_4/HTML/index.html.

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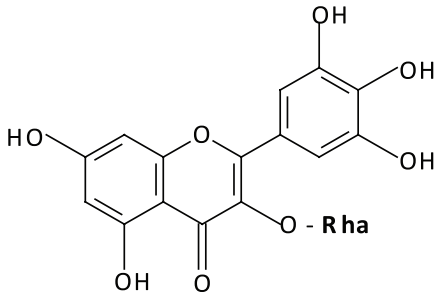

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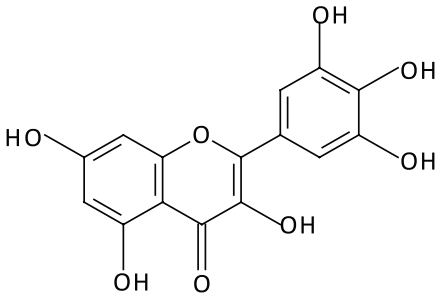

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Table 1 – Determination of chromatographic column suitability

Chromatographic column parameter	Meaning	Standard indicator
Column performance	5115	Not below 2000 theoretically perfect plates
Resolution between peaks	1.65	Not below 1.5
Asymmetry factor	1.35	Not Below 1.5

Table 2 – Metrological characteristics of quantitative determination methods of the flavonoids total amount in *Juglans nigra* L. bark preparations

Sample	n	f	$\overline{X}$	S	$S_{\overline{x}}$	P (%)	T (P, t)	±ΔX	E,%
Tincture of *Juglans nigra* L. bark	11	10	0.84	0.03357	0.01012	95	2.23	± 0.07	± 8.34
Dry extract of *Juglans nigra* L. bark	11	10	12.38	0.10650	0.03211	95	2.23	± 0.24	± 2.10

Table 3 – The content of flavonoids total amount ~~of~~ in the *Juglans nigra* L. bark tincture depending on the addition of myricitrin

Initial content of flavonoids total amount, mg/g	Addition of myricitrin, mg/g	The content of flavonoids total amount, mg/g		Error	
		Injected quantity	Detected quantity	Absolute, mg	Relative, %
5.0	1.25	6.25	6.05	–0.2	–3.2
5.0	2.5	7.5	7.9	+0.4	+5.33
5.0	3.75	8.75	8.45	–0.3	–3.43

Table 4 – The content of the flavonoids total amount in *Juglans nigra* L. bark samples

No.	Sample raw material	Absorbance, A	The content of total of flavonoids calculated on myricitrin and absolutely dry raw materials, %
1	Tincture of *Juglans nigra* L. bark	0.29	0.84 ± 0.07
2	Dry extract of *Juglans nigra* L. bark	0.85	12.38 ± 0.24

Table 5 – Retention times of peaks in *Juglans nigra* L. bark flavonoids

Flavonoid	Retention time on the chromatogram, min		
	Standard sample	Tincture	Dry extract
Miricitrin (1)	7.326	6.951	6.741
Myricetin (2)	14.211	18.909	17.277

Table 6 – Results of determining the methods correctness

Initial content of myricitrin, mg/g	Quantity of added of myricitrin, mg/g	Content of myricitrin, mg/g		Error	
		Injected quantity	Detected quantity	Absolute, mg	Relative, %
4.20	1.05	5.25	4.93	–0.32	–6.09
4.20	2.10	6.30	6.01	+0.29	+4.60
4.20	3.15	7.35	7.12	–0.23	–3.14

Table 7 – The content of myricitrin in the samples of *Juglans nigra* L. bark preparations of determined with HPLC

No.	Sample	Myricitrin content (%)
1	Tincture of *Juglans nigra* L. bark	0.42 ± 0.06
2	Dry extract of *Juglans nigra* L. bark	8.45 ± 0.25

Table 8 – Evaluation of the inter-assay precision of the methods for the quantitative determination of myricitrin in *Juglans nigra* L. bark

Sample	*f*	$\overline{X}$	S^2	*S*	*P,%*	*t (P,f)*	$\Delta\overline{X}$	*E,%*	F (P, f_1, f_2) (table)	$F_{estim.}$
Tincture of *Juglans nigra* L. bark	10	0.42	0.000436	0.02089	95	2.23	±0.04	±8.45	4.80	1.14
Tincture of *Juglans nigra* L. bark	10	0.36	0.000496	0.02228	95	2.23	±0.05	±13.87		
Dry extract of *Juglans nigra* L. bark	10	8.45	0.00377	0.06139	95	2.23	±0.21	±2.06	4.80	2.74
Dry extract of *Juglans nigra* L. bark	10	8.31	0.01035	0.1017	95	2.23	±0.23	±2.73		

The content of the flavonoids total amount in *Juglans nigra* L. bark preparations, determined by the differential spectrophotometry method at the analytical wavelength of 416 nm, is presented in Table 4.

The content of the flavonoids total amount for the test sample of *Juglans nigra* L. bark tincture was 0.84±0.07%. The content of the flavonoids total amount for the samples of the *Juglans nigra* L. bark dry extract was 12.38±0.24% (in terms of myricitrin).

The analysis by high-performance liquid chromatography showed that under the indicated chromatography conditions and using the "acetonitrile-water" system in the ratio of 2:8 in the tested solutions of the tincture and dry extract, it is possible to identify the analyzed component – myricitrin (Fig. 3B, 3C, 3D).

The retention times of the myricitrin and myricetin peaks on the chromatogram of the myricitrin standard sample, as well as in the working solutions of *Juglans nigra* L. bark tincture and dry extract, are presented in Table 5.

Adding of myricitrin (1) and myricetin (2) solutions into the test solutions of *Juglans nigra* L. bark tincture and dry extract, manifests itself on the chromatogram as the increase in the intensity of myricitrin and myricetin peaks, respectively, compared to that of myricitrin and myricetin in the initial test solution (Fig. 5A and 5B).

Taking into account a low content of myricetin in the extract in comparison with myricitrin, it could be reasonable to carry out a quantitative analysis basing on only myricitrin. The dependence of the chromatographic peak area on the concentration of myricitrin was described by the linear regression equation in the concentration range from 250 to 1500 μg/ml (Fig. 6).

The correctness of the methods was determined by the standard addition method. Solutions of myricitrin with known concentrations (25%, 50% and 75%) were added to the test solution of the tincture (Table 6). A relative error was ±4.19%. A permissible error determined for the samples with additives of standard samples was within the error of a single determination, which indicates the absence of a systematic error.

The content of myricitrin in the samples of *Juglans nigra* L. bark preparations, determined by reversed-phase HPLC, is presented in Table 7.

To assess the index of inter-assay precision, the relative standard deviation, variance, Student's test and Fisher's F-test were calculated (Table 8). The evaluation of the inter-assay precision of the tincture and dry extract samples was carried out on two Milichrom-6 devices. For each sample, the studies were carried out in the amount of eleven experiments (Table 8). The results of calculating the value of the relative standard deviation did not exceed 2%, the error of a single determination of the myricitrin content in the samples of tincture carried out on "Milichrome-61" and "Milichrome-62", were 8.45% and 13.87%, respectively. The errors of a single determination of the myricitrin content in the dry extract samples were 2.06% and 2.73%, respectively (Table 8).

The calculation of the Fisher criterion allows us to state that the average results of the analysis of the tincture samples and of dry extract on different chromatographs are statistically significant (P=95%) and do not differ from each other. Table 8 shows that the calculated value of Fisher's F-test in the analysis of tinctures and the dry extract is less than the table value. Therefore, the variances of the analysis results of both chemists are statistically equivalent (Table 7). Thus, the developed method meets the validation requirements in terms of the intermediate precision.

The results of assessing the inter-assay precision of the developed technique when analyzing 11 trials of the tincture and dry extract samples, indicate a satisfactory reproducibility of the analysis results.

CONCLUSION

So, the results of the performed spectral and chromatographic studies have indicated possibility to standardize the *Juglans nigra* L. bark preparations by determining the total amount of flavonoids calculated in terms of myricitrin and using the method of UV spectrophotometry at the wavelength of 416 nm. Standardization can take place by determining the content of the dominant and diagnostically significant flavonoid – myricitrin – and using the HPLC method at the wavelength of 360 nm too.

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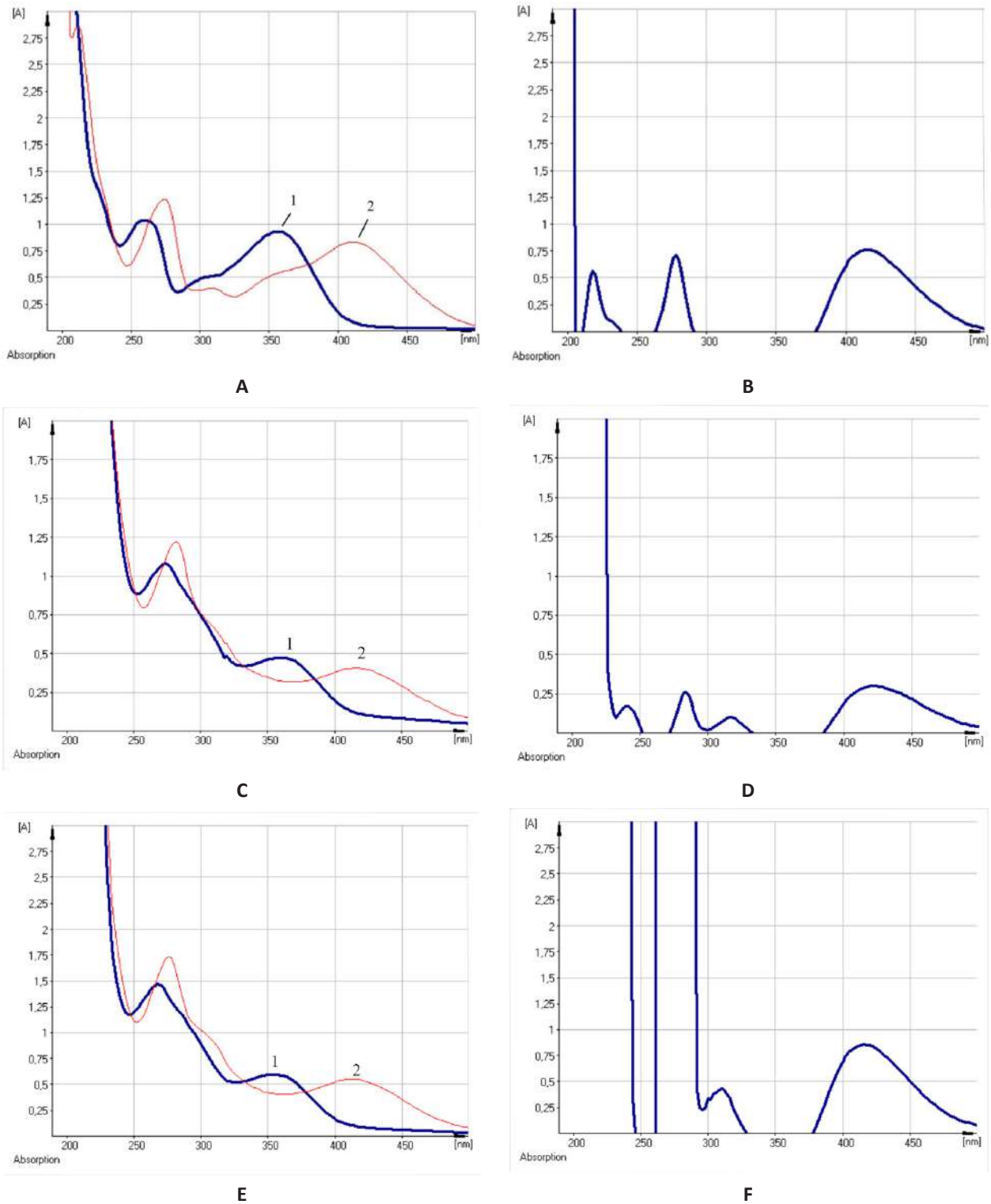

Figure 2 – Electronic spectra of test solutions of *Juglans nigra* L. bark preparations

Notes: A – Electronic spectra of ethanolic solutions of myricitrin; B – Electronic spectra of ethanolic solutions of myricitrin (differential option); C – Electronic spectra of test solution of *Juglans nigra* L. bark tincture; D – Electronic spectra of test solution of tincture of *Juglans nigra* L. bark (differential option); D – Electronic spectra of the test solution of *Juglans nigra* L. bark dry extract; E – Electronic spectra of the test solution of *Juglans nigra* L. bark dry extract (differential option). 1 – initial solution; 2 – solution with the addition of aluminum chloride.

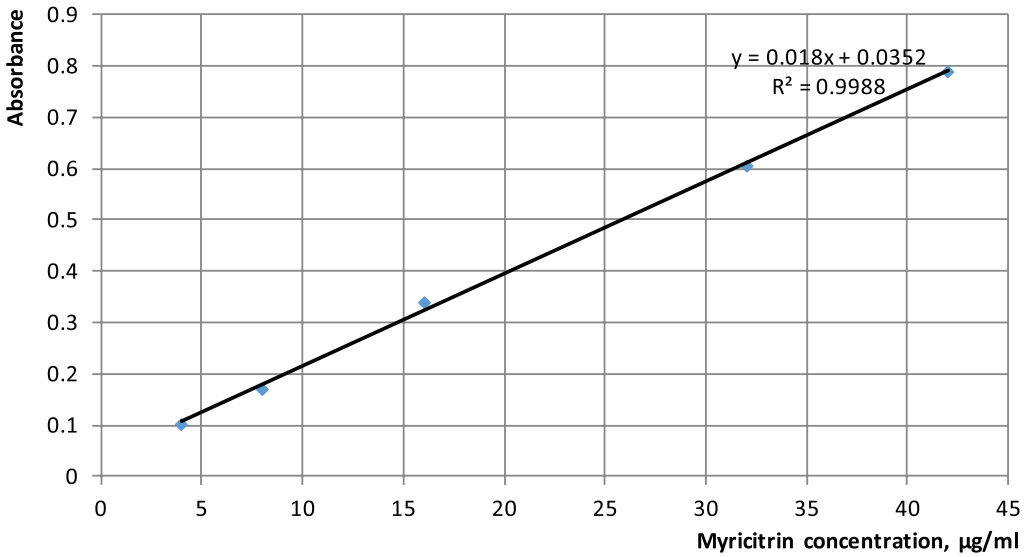

Figure 3 – Graph of the absorbance dependence on the myricitrin concentration in the sample and the linear regression equation

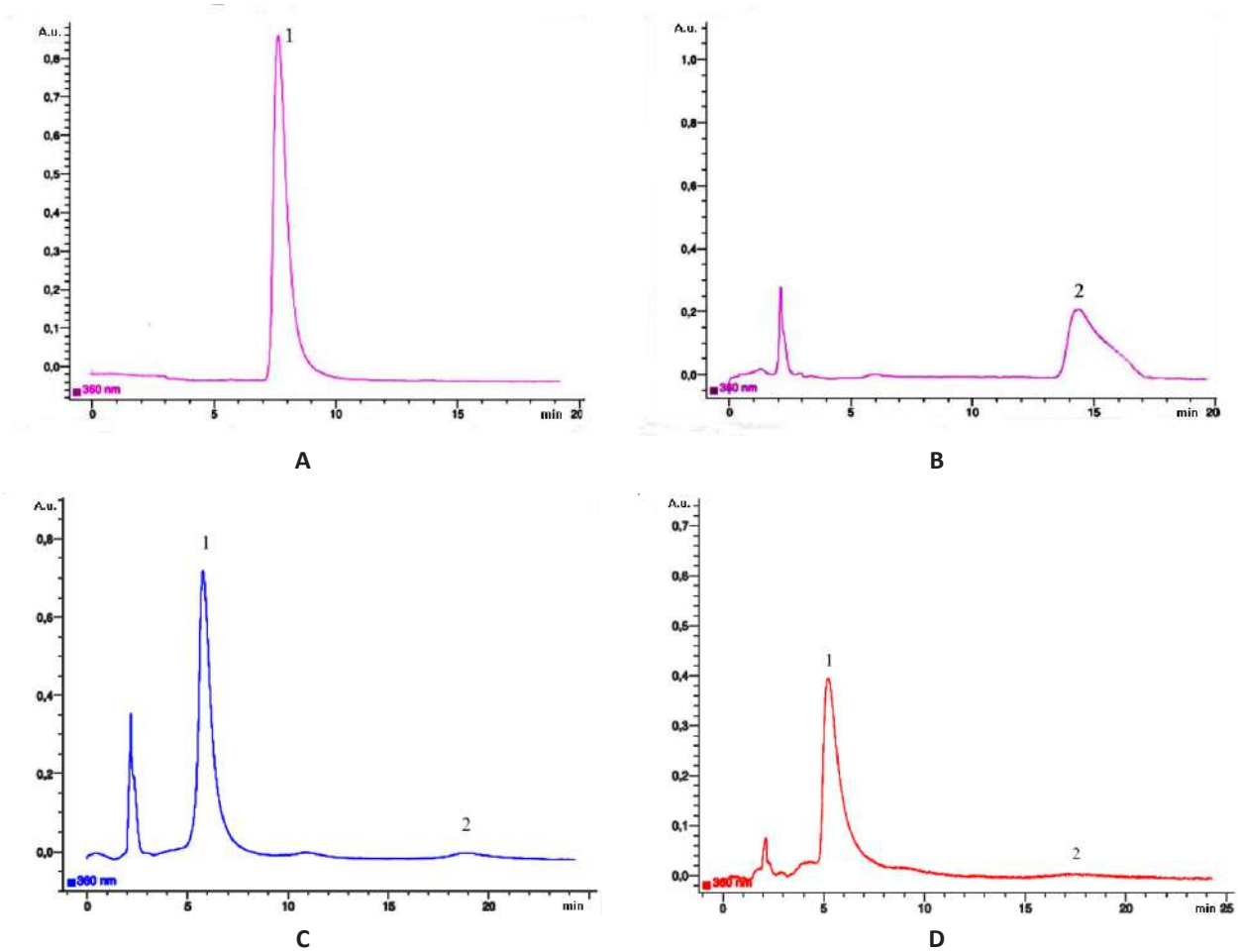

Figure 4 – HPLC chromatograms of test and standard solutions of *Juglans nigra* L. bark preparations

Notes: A – HPLC chromatogram of myricitrin; B – HPLC chromatogram of myricetin; C – HPLC chromatogram of the test solution of *Juglans nigra* L. bark tincture; D – HPLC chromatogram of the test solution of Juglans nigra L. bark dry extract. 1 – myricitrin; 2 – myricetin.

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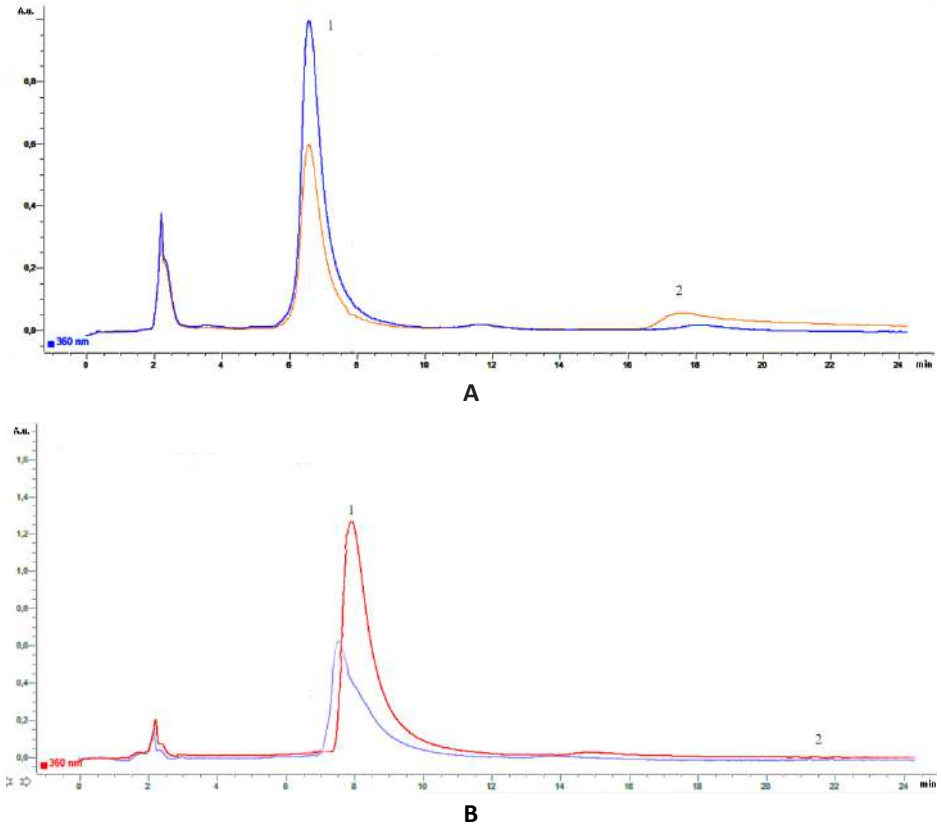

Figure 5 – HPLC chromatogram of test solution of *Juglans nigra* L. bark preparation with addition of myricitrin solution

Notes: A – HPLC chromatogram of the test solution of Juglans nigra L. bark tincture with myricitrin and myricetin addition; B – HPLC chromatogram of the test solution of Juglans nigra L. bark dry extract with myricitrin and myricetin addition. 1 – myricitrin; 2 – myricetin.

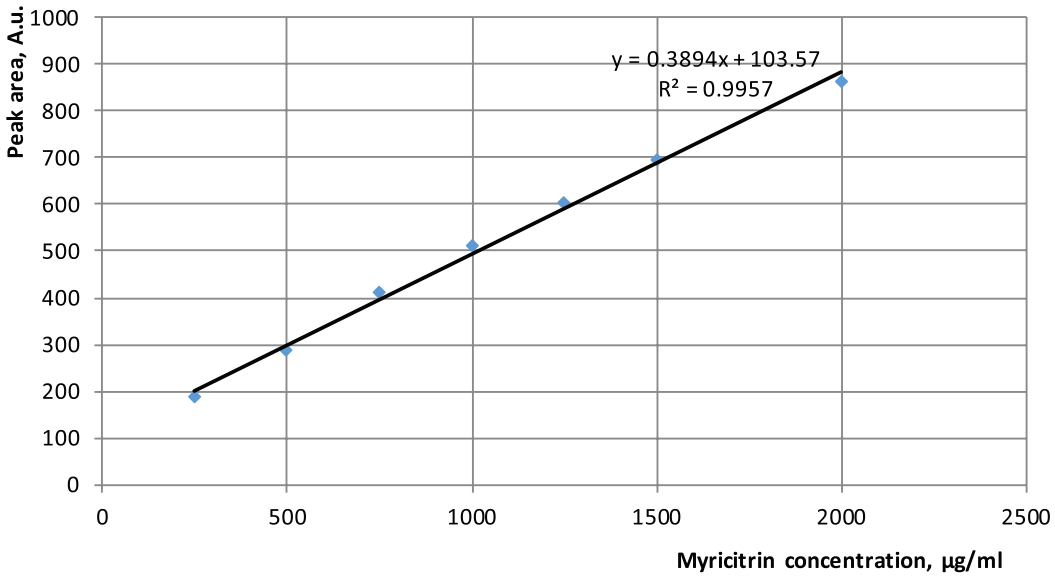

Figure 6 – Graph of the peak area dependence on the concentration of myricitrin in the sample and the linear regression equation

The content of the total amount of flavonoids in terms of myricitrin in the *Juglans nigra* L. bark tincture was 0.84±0.07%. The content of the total amount of flavonoids in terms of myricitrin in the samples of the *Juglans nigra* L. bark dry extract was 12.38±0.24%. The error of a single determination of the total amount of flavonoids in the tincture and dry extract of *Juglans nigra* L. bark by the confidence coefficient of 95% were ±8.34% and ±2.10%, respectively. The content of myricitrin in the samples of the tincture of *Juglans nigra* L. bark was 0.42 ± 0.06%. The content of myricitrin in the samples of the dry extract of *Juglans nigra* L. bark was 8.45±0.21%. The error of a single determination of the total of flavonoids in the tincture and dry extract of *Juglans nigra* L. bark by the confidence coefficient of 95% were ±7.14% and ±2.96%, respectively.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest.

CONTRIBUTION OF AUTHORS

Vladimir A. Kurkin – concept and design of research, editing; Natalya I. Zimenkina – material collection and processing, text writing and compiling the references, statistical processing of measurement results.

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AUTHORS

Vladimir A. Kurkin – Doctor of Sciences (Pharmacy), Professor, Head of the Department of Pharmacognosy with Botany and Basics of Phytotherapy, Samara State Medical University. ORCID ID: 0000-0002-7513-9352. E-mail: v.a.kurkin@samsmu.ru

Natalya I. Zimenkina – postgraduate student of the Department of Pharmacognosy with Botany and Basics of Phytotherapy, Samara State Medical University. ORCID ID: 0000-0003-1334-6046). E-mail: n.i.zimenkina@samsmu.ru

RESEARCH ARTICLE

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STUDY OF ORGANIC ACIDS PROFILE OF GENUS *PERSICARIA* MILL SPECIES

A.S. Chistyakova, A.A. Gudkova, A.I. Slivkin, E.E. Chupandina

Voronezh State University,
1, Universitetskaya Sq., Voronezh, Russia, 394018

E-mail: anna081189@yandex.ru

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The genus *Persicaria* Mill. species belonging to the buckwheat family (*Polygonaceae* Juss.) and widespread in Russia, are weeds. The chemical composition's main components of the genus *Persicaria* Mill. species**,** are flavonoid glycosides (rutin, avicularin, hyperoside, etc.). The data concerning a comparative study of the organic acids in the herb representatives of genus *Persicaria* Mill., have not been detected in the available literature.

The aim of the research is a comparative study of the organic acids qualitative and quantitative composition in the genus *Persicaria* Mill. species growing in the Voronezh region.

Materials and methods. The objects of the study were dried herb samples of the genus *Persicaria* Mill. species. All the species were harvested in the Voronezh region during the blooming period. The quantitative content of ascorbic acid and the amount of organic acids in terms of malic (hydroxy-succinic) acid was carried out according to the titrimetric methods of the Russian Federation State Pharmacopeia, the XIVth ed. The study of the qualitative composition of the organic acids profile and their quantitative content assessment in the studied objects, the herbs, was carried out by the method of capillary electrophoresis ("Kapel", St. Petersburg, Russia).

Results. With the help of pharmacopoeial titrimetric methods, it was established that the highest content of the organic acids total amount is characteristic of the *Persicaria maculosa* Mill. herb (5,60%), the lowest one – of the *Persicaria tomentósa* (Schrank) E. P. Bicknell herb (4.03%). *Persicaria maculosa* S. F. Gray and *Persicaria hydropiper* (L.) Delarbre are the richest in ascorbic acid (0.17% and 0.15%, respectively). Using the method of capillary electrophoresis, the composition of the total amount of the studied organic acids has been established. It is represented by oxalic, formic, citric, malic, wine, propionic, lactic, benzoic and other acids.

Conclusion. The study of the organic acids of the genus *Persicaria* Mill. species has been carried out. It has been established that in the studied species, the organic acids total amount in terms of malic acid and the amount of ascorbic acid are similar. By the method of capillary electrophoresis, a complete composition of organic acids has been studied, and the quantitative content of each component has been established. In all the studied *Persicaria* Mill. species, the predominance of oxalic, formic and malic acids has been revealed.

Keywords: *genus Persicaria*; *Persicaria*; medicinal herbal raw materials; organic acids; capillary electrophoresis; titrimetry

Abbreviations: AsA – ascorbic acid; SP – State Pharmacopoeia; OAs – organic acids; GPM – General Pharmacopoeia Monograph; PM – Pharmacopoeial Monograph; BASs – biologically active substances.

ИЗУЧЕНИЕ ПРОФИЛЯ ОРГАНИЧЕСКИХ КИСЛОТ ВИДОВ РОДА ГОРЕЦ (*PERSICARIA* MILL.)

А.С. Чистякова, А.А. Гудкова, А.И. Сливкин, Е.Е. Чупандина

Федеральное государственное бюджетное образовательное учреждение высшего образования «Воронежский государственный университет» Министерство науки и высшего образования Российской Федерации
394018, Россия, г. Воронеж, Университетская пл., д. 1

E-mail: anna081189@yandex.ru

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ОРИГИНАЛЬНАЯ СТАТЬЯ

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Виды рода горец (*Persicaria* Mill. (L.), относящиеся к семейству гречишных (*Polygonaceae* Juss.), широко распространены на территории России, являются сорняками. Основными компонентами химического состава травы видов рода горец являются флавоноидные гликозиды (рутин, авикулярин, гиперозид и др.). Данных, касающихся сравнительного изучения органических кислот в траве представителей рода горец, в доступной литературе не обнаружено.

Цель. Сравнительное изучение качественного и количественного состава органических кислот видов рода горец (*Persicaria* Mill. (L.), произрастающих в Воронежской области.

Материалы и методы. Объектами исследования служили высушенные образцы травы видов рода горец. Все виды были заготовлены в Воронежской области во время цветения. Количественное содержание аскорбиновой кислоты и суммы органических кислот в пересчете на яблочную (гидроксибутандовую) кислоту проводили согласно титриметрических методик, рекомендованных Государственной Фармакопеей Российской Федерации XIV изд. Изучение качественного состава профиля органических кислот и оценку их количественного содержания в траве изучаемых объектов проводили методом капиллярного электрофореза (Капель, СПб, Россия).

Результаты. С помощью фармакопейных титриметрических методик выявлено, что наибольшее содержание суммы органических кислот характерно для травы горца почечуйного (5,60%), наименьшее для травы горца войлочного (4,03%). Аскорбиновой кислотой наиболее богаты горцы почечуйный и перечный (0,17% и 0,15% соответственно). При использовании метода капиллярного электрофореза был установлен состав суммы органических кислот изучаемых растений, представленный щавелевой, муравьиной, лимонной, яблочной, янтарной, пропионовой, молочной, бензойной и другими кислотами.

Заключение. Проведено исследование органических кислот видов рода горец. Установлено, что содержание суммы органических кислот в пересчете на яблочную кислоту и количество аскорбиновой кислоты в изучаемых видах сходно. Методом капиллярного электрофореза изучен полный состав органических кислот и установлено количественное содержание каждого компонента. Выявлено преобладание щавелевой, муравьиной и яблочной кислот во всех изучаемых видах рода горец.

Ключевые слова: род горец; *Persicaria*; лекарственное растительное сырье; органические кислоты; капиллярный электрофорез; титриметрия

Список сокращений: АсК – Аскорбиновая кислота; ГФ – Государственная Фармакопея; ОК – органические кислоты; ОФС – общая фармакопейная статья; ФС – фармакопейная статья; БАВ – биологически активные вещества.

INTRODUCTION

Organic acids (OAs) of plants are active metabolites [1]; they are involved in the synthesis of amino acids [2] and detoxification of heavy metals in plants [2, 3]. Such OAs as ascorbic acid (AsA or vitamin C), citric, malic acids are important for normal functioning of a human body [4–6], take part in metabolic processes, regulate the activity of the digestive system, have bactericidal [1] and antioxidant effects, promote the reduction the inflammatory response, accelerate tissue regeneration [7–10], activate cellular respiration and protein synthesis [4, 5].

Such a wide range of pharmacological activities explains the interest of Russian and foreign authors in the study of the qualitative and the quantitative content of OAs in plants [1, 14–16]. However, despite the significant list of positive OAs effects, there are also negative ones. Therefore, the scientists DO Bokov, et al. [17] notify that a high content of oxalic acid in the human diet can lead to the development of urolithiasis caused by metabolic disorders (the most important factor is a violation of the acid-base balance). Oxalic acid, which enters the urine, forms compounds with calcium ions. This fact leads to the formation of oxalate crystals.

The genus *Persicaria* Mill. species belonging to the buckwheat family (*Polygonaceae* Juss.), are promising study objects. According to the latest data from the international information base "The Plant List"[1], *genus Persicaria* includes about 66 species that are widespread in both hemispheres. Numerous works are devoted to the study of the taxonomy and quantitative composition of the genus *Persicaria* Mill. species (S. Hassannejad, et al: GI Vysochina; FA Vagabova, et al.) [18–20], and some of them touched on the issues of species chemosystematics, developed on the study of the flavonoid composition. Based on the research data, the genus *Persicaria* Mill. species were divided into 4 ranges: (*Persicariaeformes* Kom., *Hydropiperiformes* Kom., *Lapathiiformes* Worosh., *Amphibiae* Kom. However, given the peculiarities of the genus *Persicaria* Mill. species, which, growing in the same territory, can interbreed with each other and form various polymorphic forms, there are still disagreements among scientists. These disagreements regard the self-dependence of some species (for example, in the Lapathiiformes range, the probability of singling out *Persicaria tomentósa* (Schrank) into a separate species is doubtful.

In the officinal medicine, *Persicaria maculosa* S.F. Gray and *Persicaria hydropiper* (L.) Delarbre included in the State Pharmacopoeia of the Russian Federation of the XIV[th] ed.[2], are certified and recommended for use as hemostatic agents. Other representatives of the genus are considered impurity plants and cannot be harvested as medicinal plant materials. However, as a result of assessing their pharmacological activity, anti-inflamma-

[1] The Plant List. Available from: http://www.theplantlist.org.

[2] State Pharmacopoeia of the Russian Federation, 14th ed. 4 volumes. Available from: http://femb.ru/femb/pharmacopea.php.

tory, antioxidant and membrane-protective effects have been established [21]. The main components of the genus *Persicaria* Mill. species herb chemical composition are believed flavonoid glycosides (rutin, avicularin, hyperoside, glycosides of kaempferol, quercetin, etc.) [18, 19, 22–24], tannins, phylloquinone [25], calcium salts [26]. The data concerning a comparative study of the organic acids in the herb representatives of genus *Persicaria* Mill., have not been detected in the available literature.

THE AIM of the research is a comparative study of the organic acids qualitative and quantitative composition in the genus *Persicaria* Mill. species growing in the Voronezh region.

The experimental part of this work is aimed at solving two problems. The first task is aimed at assessing the quantitative content of the total amount of organic acids in terms of malic acid, as well as AsA in the genus *Persicaria* plants using generally available pharmacopoeial methods (titrimetry). The second task is devoted to a detailed study of the qualitative composition and the quantitative content of organic acids using a modern method of analysis (capillary electrophoresis).

MATERIALS AND METHODS

Raw materials

The objects of the study were the dried herb samples of *Persicaria maculosa* S.F. Gray, *Persicaria lapathifólia* (L.) Delarbre harvested self-dependently in the village of Uglyanets (30 km north-eastward of Voronezh, the territory of the Voronezh region; *Persicaria tomentósa* (Schrank) E.P. Bicknell), collected in the Kozo-Polyansky Botanical Garden, within the city of Voronezh; *Persicaria hydropiper* (L.) Delarbre), *Persicaria minor* (Huds.) Opiz, growing in the village of Rybachy (within the city of Voronezh); two forms of *Polygonum amphibium* – terrestrial (*Persicaria amphibia var. terréstris* (Leyss.) Munshi & Javeid) and aquatic (*Persicaria amphibia* (L.) Delarbre), harvested in the coastal zone of the Voronezh River (70 km north-eastward of Voronezh). The studied species were harvested annually from the same habitats during 2016–2018. The objects were subjected to air-shadow drying. The identification of the studied species was carried out using herbarium specimens and guides to plants of the Botany and Mycology Department, Voronezh State University.

Microscopic research

The research of microscopic characteristics of the species under study was carried out according to General Pharmacopoeia Monograph.1.5.3.0003.15 "Microscopic and microchemical research techniques of medicinal plants and herbal medicinal products" (the Russian Federation State Pharmacopeia, the XIV[th] ed.[3]) on the "Biomed 6" microscope at ×100 magnification. Visualization of diagnostic characteristics was carried out using a Levenhuk C310 NG digital video camera (China).

Quantitation

The content of AsA and the amount of organic OAs in terms of malic acid was carried out according to the titrimetric methods represented in the Russian Federation State Pharmacopeia, the XIV[th] ed. (General Pharmacopoeia Monographs "Rosehip *(Rosa canina)* fruits" and "Rowan-tree *(Sorbus aucuparia)* fruits"[4].

The analysis of the quantitative content of individual organic acids was carried out by capillary electrophoresis (Kapel, Russia). The separation conditions were represented by phosphate buffer. Capillary was: L_{eff} / L_{tot} = 40/50 cm, ID = 50 µm. The sample injection was 300 mbar s. The voltage was −17 kV. The temperature was +20°C. The detection was indirect, 190 nm[5] [5, 27].

Reagents

The reagents of chemically pure and analytically pure grades (JSC "Vekton", St. Petersburg, Russia) were used. The calculation of all quantitative characteristics was carried out in terms of absolutely dry plant materials.

RESULTS AND DISCUSSION

In the studied genus *Persicaria* Mill. species, at the first stage of the research by pharmacopoeial titrimetric methods, the content of AsA and the total amount of OAs in terms of malic acid were determined. The results are shown in Table 1.

It has been found out that among the species under study, *Persicaria maculosa* S.F. Gray and *Persicaria hydropiper* (L.) Delarbre contain a greater amount of AsA (0.17±0.01 and 0.15±0.01%, respectively). The lowest AsA content is typical for the herb of *Persicaria tomentósa* (Schrank) E.P. Bicknell and *Persicaria amphibian var. terréstris* (Leyss.) (0.07±0.006 and 0.08±0.005%, respectively). According to the WHO[6] and taking into account the data on the content of AsA in the studied plants, a daily consumption of AsA is 60–80 mg/day (0.06–0.08 g/day). These plants can serve as additional sources of this compound, which must be taken into account when obtaining medicinal herbal preparations based on them.

[3] Ibid.
[4] Ibid.
[5] Komarova NV, Kamentsev YaS. Prakticheskoe rukovodstvo po ispol'zovaniyu sistem kapillyarnogo elektroforeza Kapel' [A practical guide to the use of capillary electrophoresis systems Kapel']. St. Petersburg: Veda, 2006. – 213 p. Russian
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Table 1 – Content of ascorbic acid and the amount of OAs in terms of malic acid (P>95%, n = 7)

Characteristic value	Range of *Persicariae formes*	Range of *Lapathiiformes*		Range of *Hydropiperiformes*		Range of *Amphibiae*	
	Persicaria maculosa S.F. Gray	*Persicaria tomentósa* (Schrank)	*Persicaria lapathifólia* (L.)	*Persicaria hydropiper* (L.)	*Persicaria mínor* (Huds.) Opiz	*Persicaria amphibia var. terréstris* (Leyss.)	*Persicaria amphibia* (L.)
Ascorbic acid, %	0.170±0.010	0.070±0.006	0.110±0.007	0.150±0.010	0.100±0.010	0.080±0.005	0.110±0.010
Amount of organic acids in terms of malic acid,%	5.60±0.20	4.03±0.12	5.47±0.30	5.16±0.20	4.47±0.16	5.28±0.18	4.73±0.11

Table 2 – Contents of organic acids in genus *Persicaria* Mill. species (P>95%, n = 3)

Object under study	Range of *Persicariae-formes*	Range of *Lapathiiformes*		Range of *Hydropiperiformes*		Range of *Amphibiae*	
Organic acids,%	*Persicaria maculosa* S.F. Gray	*Persicaria lapathifólia* (L.) Delarbre	*Persicaria tomentósa* (Schrank) E.P. Bicknell	*Persicaria hydropiper* (L.) Delarbre	*Persicaria mínor* (Huds.) Opiz	(*Persicaria amphibia var. terréstris* (Leyss.)	*Persicaria amphibia* (L.) Delarbre
oxalic	3.36±0.06	0.35±0.02	1.70±0.03	7.36±0.14	2.13±0.04	1.19±0.02	0.48±0.004
formic	< 0.15	2.84±0.03	< 0.15	4.71±0.09	4.47±0.08	6.69±0.12	< 0.15
fumaric	< 0.005	0.014±0.002	0.023±0.0001	0.017±0.0001	< 0.005	< 0.005	0.008±0.0002
amber	< 0.05	< 0.05	< 0.15	< 0.05	< 0.05	0.067±0.001	< 0.05
malic	0.130±0.003	0.044±0.001	0.062±0.0001	0.055±0.0001	0.073±0.0002	0.28±0.005	0.66±0.01
citric	0.07±0.001	0.28±0.005	0.20±0.004	0.25±0.005	0.12±0.002	0.20±0.002	0.72±0.01
propionic	<0.15	0.22±0.004	0.16±0.002	0.17±0.002	0.03±0.0001	0.16±0.003	< 0.15
lactic	< 0.12	< 0.12	< 0.12	< 0.12	0.29±0.006	< 0.12	< 0.12
benzoic	0.006±0.0001	0.03±0.0001	< 0.005	0.02±0.0005	0.007±0.0001	0.008±0.0001	< 0.005
sorbic	< 0.025	0.12±0.002	< 0.025	< 0.025	0.04±0.0008	< 0.025	< 0.025
wine	< 0.005	0.50±0.005	0.46±0.003	0.76±0.007	0.50±0.004	2.15±0.043	1.79±0.035
acetic	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Total amount	3.56±0.07	4.47±0.09	2.61±0.05	13.42±0.27	7.66±0.15	10.77±0.21	3.66±0.08

Table 3 – Determination of the dependence of oxalic acid content on frequency and size of calcium oxalate druses

Object under study	Range of *Persicariae-formes*	Range of *Lapathiiformes*		Range of *Hydropiperiformes*		Range of *Amphibiae*	
Parameter under study	*Persicaria maculosa* S.F. Gray	*Persicaria lapathifólia* (L.)	*Persicaria tomentósa* (Schrank)	*Persicaria hydropiper* (L.) Delarbre	*Persicaria mínor* (Huds.) Opiz	*Persicaria amphibia var. terréstris* (Leyss.)	*Persicaria amphibia* (L.) Delarbre
Oxalic acid content,%	3.36±0.07	0.35±0.08	1.70±0.03	7.36±0.15	2.13±0.04	1.19±0.02	0.48±0.004
Frequency of occurrence, pieces (1 mm²)	70±20	120±45	200±30	130±25	150±20	140±30	–
Druses diameter, μm	11.6–41.9	11.5–34.9	49.0–81.5	9.3–23.3	11.5–69.9	11.6–34.9	–

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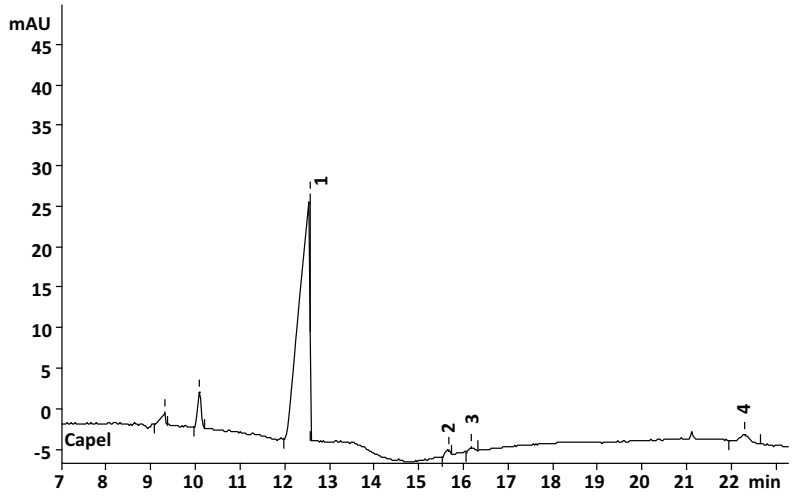

Figure 1 – Electropherogram of organic acids of *Persicaria maculosa* (S.F. Gray) herb
Note: 1 – oxalic acid; 2 – malic acid; 3 – citric acid, 4 – benzoic acid.

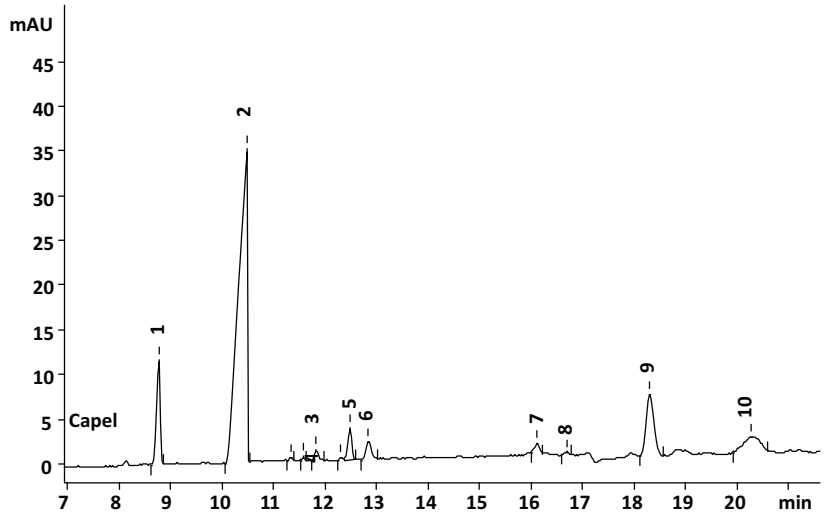

Figure 2 – Electropherogram of organic acids of *Persicaria lapathifólia* (L.) herb
Note: 1 – oxalic, 2 – formic, 3 – fumaric, 4 – malic, 5 – wine, 6 – citric, 7 – propionic, 8 – lactic, 9 – benzoic, 10 – sorbic.

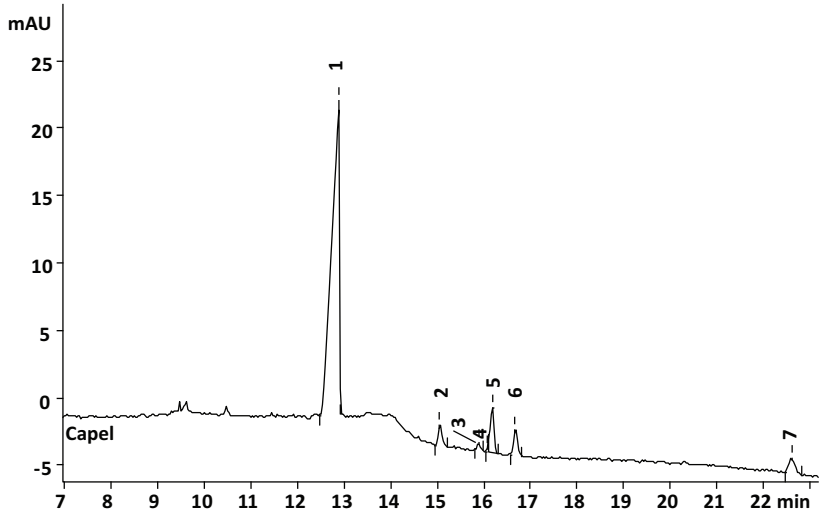

Figure 3 – Electropherogram of organic acids of *Persicaria tomentósa* (Schrank) herb
Note: 1 – oxalic, 2 – fumaric, 3 – amber, 4 – malic, 5 – wine, 6 – citric, 7 – benzoic.

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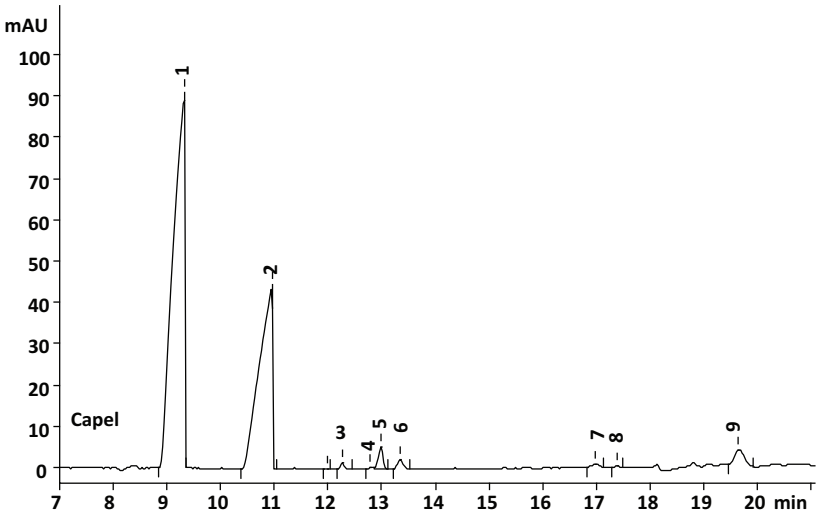

Figure 4 – Electropherogram of organic acids of *Persicaria hydropiper* (L.) herb
Note: 1 – oxalic, 2 – formic, 3 – fumaric, 4 – malic, 5 – wine, 6 – citron, 7 – propionic, 8 – lactic, 9 – benzoic.

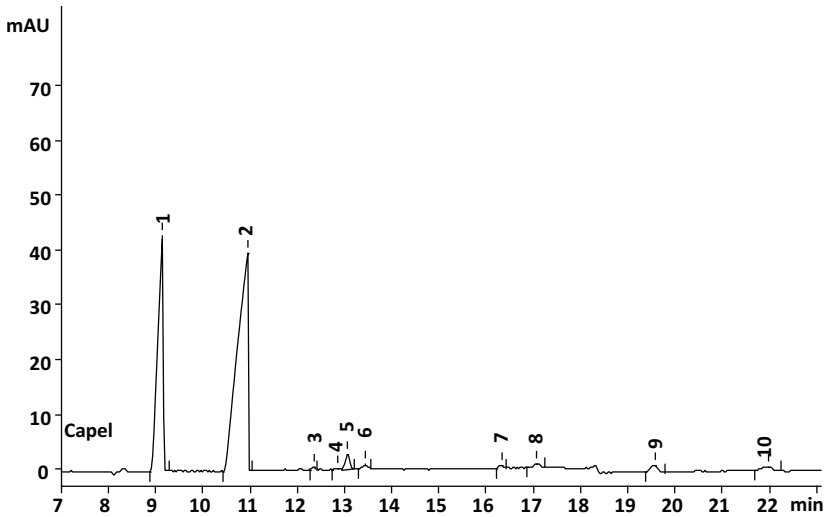

Figure 5 – Electropherogram of organic acids of *Persicaria mínor* (Huds.) herb
Note: 1 – oxalic, 2 – formic, 3 – fumaric, 4 – malic, 5 – wine, 6 – citric, 7 – propionic, 8 – lactic, 9 – benzoic, 10 – sorbic.

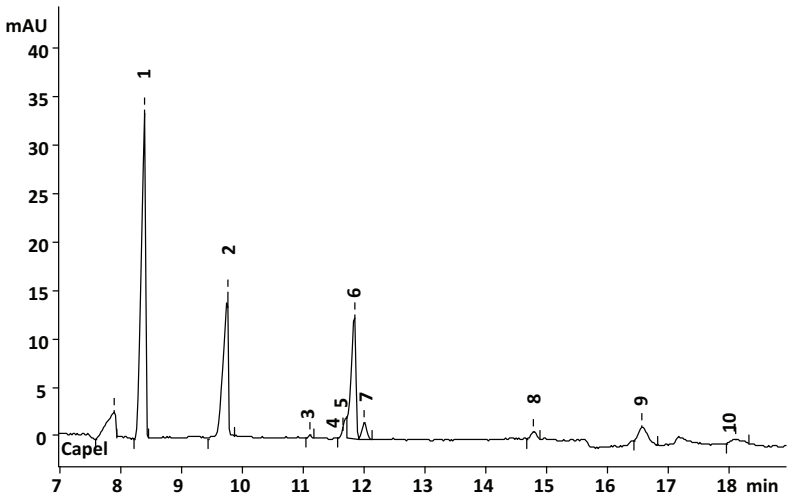

Figure 6 – Electropherogram of organic acids of *Persicaria amphibian var. terréstris* (Leyss.) herb
Note: 1 – oxalic, 2 – formic, 3 – fumaric, 4 – amber, 5 – malic, 6 – wine, 7 – citric, 8 – propionic, 9 – benzoic, 10 – sorbic.

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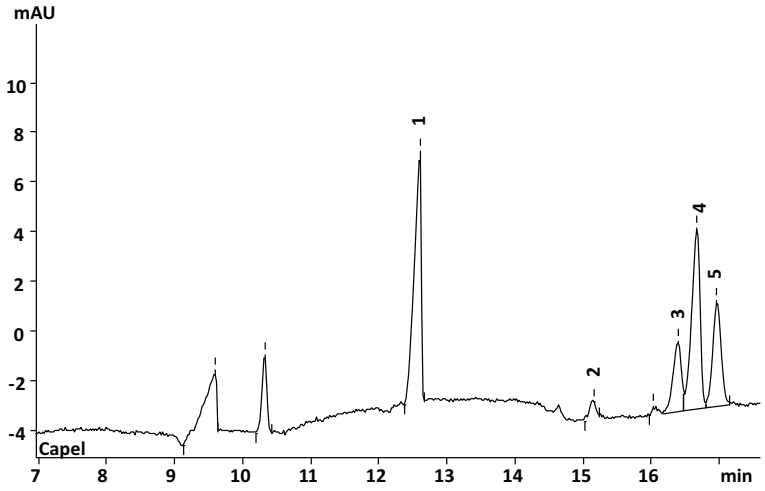

Figure 7 – Electropherogram of organic acids of the *Persicaria amphibia* (L.) herb
Note: 1 – oxalic, 2 – fumaric, 3 – malic, 4 – wine, 5 – citric.

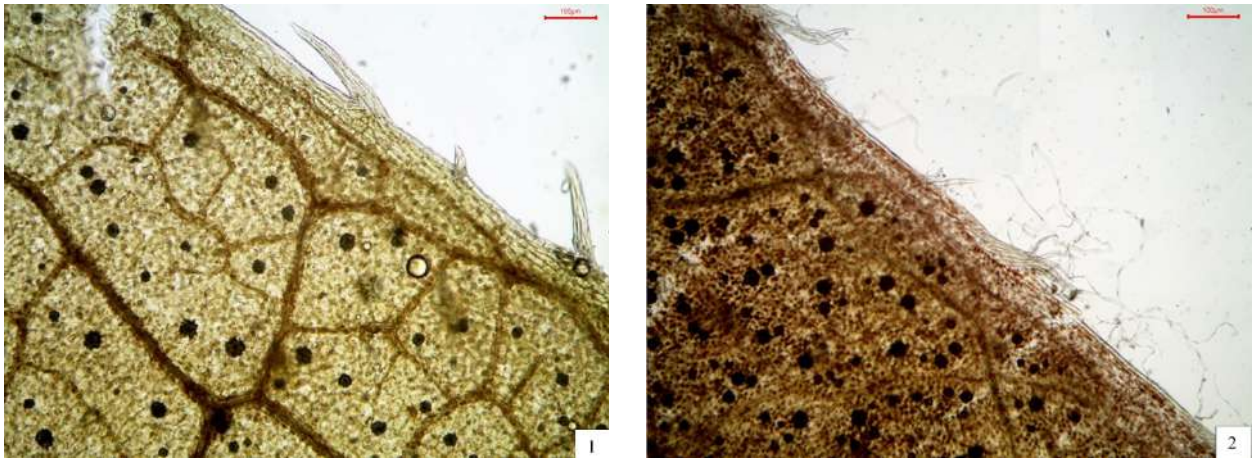

Figure 8 – Druses of calcium oxalate on micropreparations of *Persicaria maculosa* S.F. Gray (1) and *Persicaria tomentósa* (Schrank) (2) leaves

Analyzing the total amount of OAs in terms of malic acid in the studied genus *Persicaria* Mill. species, it was found out that their greater content is observed in the herb of *Persicaria maculosa* S.F. Gray and *Persicaria lapathifólia* (L.) Delarbre. In the herb of *Persicaria hydropiper* (L.) Delarbre, this indicator is 13% higher (5.16±0.20%) than in *Persicaria mínor* (Huds.) Opiz (4.47±0.16%), which is the main impurity in harvesting *Persicaria hydropiper* (L.) raw materials. The difference in the quantitative content of the both as the amount of OAs and the content of AsA is observed within two forms of one species of *Persicaria amphibia* (L.) *Delarbre.* In *Persicaria amphibia var. terréstris* (Leyss.), the amount of AsA is 27% less (0.08±0.005%), and the amount of OAs is 10% more (5, 28±0.18) than in *Persicaria amphibia* (L.) *Delarbre* (4.73±0.11).

The study of OAs and AsA in plant raw materials by pharmacopoeial methods has a number of disadvantages, in particular, malic acid, for which it is recommended to recalculate the content of the OAs amount, is not always present in the raw material in a prevailing amount. It can be in a free form and in the form of potassium and calcium salts. The pharmacopoeial method does not make it possible to establish the qualitative composition of OAs present in plant raw materials both in a free form and in the form of salts [5, 14, 28].

One of the most often used methods in the OAs analysis of plants, is capillary electrophoresis, which combines simplicity, rapidity, reliability and a low resource consumption compared to chromatographic methods of analysis [1, 5, 28–32]. The next stage of the work was the study of the complete composition of OAs (both in a free form and in the form of salts) by the method of capillary electrophoresis. Herewith, oxalic, formic, fumaric, amber, malic, citric, propionic, lactic, benzoic, sorbic, wine and acetic acids were identified in the studied *Persicaria* species, and their quantitative contents have been determined. The obtained data are

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shown in Table 2, the electrophoregrams are presented in Figures 1–7.

The analysis of the data obtained in the course of the study showed that despite the close relationship of the genus *Persicaria* Mill (L.) species, there is a heterogeneity in the qualitative and quantitative composition of the OAs of the plants under study. Evaluating the picture of the OAs profiles of the species under study and in accordance with their division into ranges, one can see striking differences between the *Persicariaeformes* range and others. In particular, four OAs (oxalic, malic, citric, benzoic) were reliably identified in the *Persicaria maculosa* S.F. Gray herb. Within the *Lapathiiformes* range, the differences in the composition of OAs between closely related species should be notified. In the *Persicaria lapathifólia* (L.) herb there are 9 acids (oxalic, formic, fumaric, malic, citric, propionic, benzoic, sorbic, wine), in the *Persicaria tomentósa* (Schrank) herb there are 6 acids (oxalic, fumaric, malic, citric, propionic, wine). The same situation is observed in the species of the Hydropiperiformes range: in the *Persicaria hydropiper* (L.) Delarbre herb, there are 8 acids (oxalic, formic, fumaric, malic, citric, propionic, benzoic, wine), in the *Persicaria mínor* (Huds.) Opiz herb, there are 9 acids (oxalic, formic, malic, citric, propionic, lactic, benzoic, sorbic, wine). Within the range of *Amphibiae*, in the *Persicaria amphibia var. terréstris* (Leyss.) herb, there are 8 acids (oxalic, formic, amber, malic, citric, propionic, benzoic, wine), in the *Persicaria amphibian* (L.) herb, there are 5 acids (oxalic, fumaric, malic, citric, wine). Such a difference in the patterns of the OAs profile observed in the two forms of the same species, is explained by the influence of the adaptive mechanism and a plant habitat on the synthesis of biologically active substances (BASs). Analyzing the data in Table 2, it is possible to notify the similarity in the qualitative composition of the OAs in the *Persicaria hydropiper* (L.) and *Persicaria amphibia var. terréstris* (Leyss.) herbs, which makes it possible to assume the genetic relationship between these species.

The total amount of OAs, determined by the method of capillary electrophoresis, is typical for the *Persicaria hydropiper* (L.) herb (13.42%), *Persicaria amphibia var. terréstris* (Leyss (10.77%) and *Persicaria mínor* (Huds.) Opiz (7.66%).

Oxalic acid is the main OA in the composition of the *Persicaria maculosa* S.F. Gray, *Persicaria hydropiper* (L.) Delarbre, *Persicaria tomentósa* (Schrank) herbs. In the *Persicaria maculosa* S. F. Gray herb, the content of oxalic acid was 94% of the total amount of OAs; in the *Persicaria tomentósa* (Schrank) herb it was 65%, in the *Persicaria hydropiper* (L.) herb it was 55%. One of the forms in which oxalic acid can be found in plants, is crystalline inclusions. One of the features of the buckwheat family representatives, i.e. genus *Persicaria*, is the presence of rather large and numerous druses of calcium oxalate, which are found in great numbers in idioblasts in the mesophyll of leaves, along the conductive bundles of the stem, near the base of corolla petals. In addition to determining the qualitative composition and the quantitative content of OAs in the studied species, the presence of a relationship between the quantitative content of oxalic acid, the frequency of occurrence and the size of calcium oxalate druses, revealed as a result of microscopic analysis of the studied objects, was analyzed. Fig. 8 shows, as an example, a picture of the microscopic structure of *Persicaria maculosa* S. F. Gray (1) and *Persicaria tomentósa* (Schrank) E.P. Bicknell (2) leaves, where the presence of a large amount of calcium oxalate druses on the *Persicaria tomentósa* (Schrank) E. P. Bicknell leaf is clearly visible.

Table 3 shows the results of calculating the frequency of calcium oxalate druses occurrence and determining their diameter using a Levenchuk eyepiece micrometer (China). The highest content of oxalic acid is characteristic of *Persicaria hydropiper* (L.), while the diameter of the druses, in comparison with the rest of the objects, is the smallest (9.3–23.3 microns) with an average frequency of occurrence (130±25 pieces/mm^2). The greatest number of large-diameter druses is observed in *Persicaria tomentósa* (Schrank) (200±30 pieces/mm^2), however, the content of oxalic acid in the raw material is low (1.7±0.03%). With the use of a microscopic method of analysis, calcium oxalate druses were not found out in *Persicaria amphibia* (L.), and the amount of oxalic acid, established by capillary electrophoresis, was not high (0.48±0.004%). The results obtained show that no relationship was found out between the content of oxalic acid, the frequency of occurrence and the size of calcium oxalate druses. In the studied objects, oxalic acid is found mainly in a free form, and only a small part of it – in the form of calcium salts and other compounds.

A prevailing amount of formic acid is characteristic of *Persicaria lapathifólia* (L.), *Persicaria mínor* (Huds.) Opiz, and *Persicaria amphibia var. terréstris* (Leyss.). Among the studied species, a higher content of formic acid (6.69%) is characteristic of *Persicaria amphibia var. terréstris* (Leyss.), which is 62% of the total OAs. In *Persicaria hydropiper* (L.) and *Persicaria mínor* (Huds.) Opiz, the content of formic acid is similar (4.71±0.09 and 4.47±0.08%, respectively), which is 35 and 58% of the total OAs in the plants. A smaller amount is observed in *Persicaria lapathifólia* (L.) (2.84%), however, the percentage of the total OAs is quite high and amounted to 63%. The presence of formic and oxalic acids in such high quantities explains the appearance of a not-critical irritation when plant sap comes into contact with the skin surface, which must be taken into account when harvesting raw materials.

Citric and malic acids are found in greater quantities in *Persicaria amphibia* (L.) (0.72% and 0.66%, respectively), while the content of malic acid is 50%, and citric acid

is 70% higher than in *Persicaria amphibia var. terréstris* (Leyss.) of this species (0,28% and 0.2%, respectively). It should be notified that citric acid is unevenly distributed within the limits allocated to the genus *Persicaria* Mill. species. The content of citric acid in the *Persicaria mínor* (Huds.) Opiz herb is 50% less (0.12%) than in the *Persicaria hydropiper* (L.) herb (0.25%); it is about the same in the *Persicaria lapathifólia* (L.) herb (0.28%) and *Persicaria tomentósa* (Schrank) (0.2%)), which is 65% higher than in *Persicaria maculosa* S.F. Gray (0.07%). Malic acid plays an important role in the metabolic activity of cells and contributes to the production of ATP by the body, supports the immune system, and is a chelator of toxic metals. The pharmaceutical industry produces a number of preparations containing malic acid belonging to the group of metabolites, rehydrating agents (Sterofundin isotonic), plasma substitutes (Ionehes), antiseptics (Acerbin)[7].

A small amount of amber acid was reliably found in *Persicaria amphibia var. terréstris* (Leyss.) (0.067%) and, presumably, is a marker component for this species, since in other species its content is less than the detection limit of the device. Amber acid is an important endogenous intracellular metabolite of the Krebs cycle, which performs a universal energy-synthesizing function in the cells of the body. On the basis of amber and ascorbic acid, potentiating the action of each other, the pharmaceutical industry produces the Yantavit dietary supplement, which has general tonic, angioprotective, metabolic, antihypoxic, antioxidant properties[8].

Propionic acid (0.22%) in larger quantities is characteristic of oxalate *Persicaria lapathifólia* (L.), and lactic acid is characteristic of *Persicaria mínor* (Huds.) Opiz (0.29%).

The amount of wine acid in the *Persicaria amphibia var. terréstris* (Leyss.) herb is almost twice higher (2.15%) than in *Persicaria amphibia* (L.) (1.79%): in other plants, the amount of wine acid is low. Within the ranges of *Persicaria lapathifólia* (L.) and *Persicaria tomentósa* (Schrank), its content is similar. *Persicaria maculosa* S.F. Gray contains wine acid in the amount less than the detection limit of the device, which can be a feature of the plant and also act as an additional chemotaxonomic feature of the raw material.

Such OAs as fumaric, benzoic, sorbic, are present in the plant raw materials of *genus Persicaria* in insignificant quantities. The content of acetic acid is next to nothing, below the maximum capability of the device, which may be due to a partial loss of the substance as a result of a sample preparation (acetic acid and some others belong to the class of volatile OAs).

Thus, the carried out study made it possible to establish the qualitative composition and quantitative content of OAs in the herbs of the genus *Persicaria* Mill (L.) species and to reveal the prospects of using this group of plants as additional sources of compounds important for the vital activity of the organism.

CONCLUSION

For the first time, a comparative study of OAs in the genus *Persicaria* herbs has been carried out. With the help of pharmacopoeial methods, in the species under study, the quantitative content of the amount of OAs in terms of malic and AsA has been established: *Persicaria maculosa* S. F. Gray and *Persicaria lapathifólia* (L.) are the closest in the quantitative content of these compounds.

By the method of capillary electrophoresis, the complete composition of OAs has been studied, and their quantitative content has been established. Despite a close relationship between the herbs of the genus *Persicaria* Mill. species, a heterogeneity in the qualitative and quantitative composition of the OAs of the studied plants has been revealed. The predominance of oxalic, formic and malic acids in all the studied genus *Persicaria* Mill. species has been shown. The greatest amount of organic acids is characteristic of *Persicaria hydropiper* (L.) and *Persicaria amphibia var. terréstris* (Leyss.). It has been revealed that a characteristic feature of a number of *Amphibiae* is a higher content of malic and wine acids than in the other studied species. Amber acid acts as an identification compound of *Persicaria amphibia var. terréstris* (Leyss.). Due to the presence of a large amount of formic acid in the *Persicaria hydropiper* (L.) herb, *Persicaria mínor* (Huds.) and *Persicaria amphibia var. terréstris* (Leyss.), it is recommended to use personal protective equipment when working with these objects in order to avoid skin irritation. On the basis of the study, a possible genetic relationship between *Persicaria hydropiper* (L.) and *Persicaria amphibia var. terréstris* (Leyss.) was presupposed. During the experiment, no relationship was established between the content of oxalic acid, the frequency of occurrence and the size of calcium oxalate druses. In the studied objects, oxalic acid is found mainly in a free form, and only a small part of it – in the form of calcium salts and other compounds.

The carried out research has shown that the studied genus *Persicaria* Mill. species are promising sources of OAs. The data obtained can be used in the pharmaceutical analysis when carrying out the standardization of plant materials. The information on the qualitative composition and quantitative content of the individual components of the OAs profile in the species under study will make it possible to adjust the consumption rates of herbal medicinal products based on them.

[7] Register of medicines of Russia: reference book of medicines. Available from: https://www.rlsnet.ru.
[8] Ibid.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

Anna S. Chistyakova – collecting literature data, experiment conducting, interpreting the results obtained, preparing the draft manuscript; Alevtina A. Gudkova – research planning, harvesting and drying plant materials, experiment conducting, processing the results obtained, preparing the manuscript, participating in the development of the concept and research design; Alexey I. Slivkin — manuscript publishing approval, critical review of intellectual content; Elena E. Chupandina – implementation of the experimental part of the work, discussion of the results.

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AUTHORS

Anna S. Chistyakova – Candidate of Sciences (Pharmacy), Associate Professor, Department of Pharmaceutical Chemistry and Pharmaceutical Technology, Faculty of Pharmacy, Voronezh State University. ORCID ID: 0000-0002-8291-9904. E-mail: anna081189@yandex.ru

Alevtina A. Gudkova – Doctor of Sciences (Pharmacy), Associate Professor, Docent, Department of Pharmaceutical Chemistry and Pharmaceutical Technology, Faculty of Pharmacy, Voronezh State University. ORCID ID: 0000-0002-1275-5000. E-mail: alinevoroneg@mail.ru

Alexey I. Slivkin – Doctor of Sciences (Pharmacy), Professor, Head of the Department of Pharmaceutical Chemistry and Pharmaceutical Technology, Faculty of Pharmacy, Voronezh State University. ORCID ID: 0000-0001-6934-0837. E-mail: slivkin@pharm.vsu.ru

Elena E. Chupandina – Doctor of Sciences (Pharmacy), Professor, Head of the Department of Management and Economics of Pharmacy, Voronezh State University. ORCID ID: 0000-0003-2310-4198. E-mail: chupandina@vsu.ru

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DEVELOPMENT OF NITROFURAN DERIVATIVE: COMPOSITION AND TECHNOLOGY OF EFFERVESCENT TABLETS WITH SOLID DISPERSIONS

A.O. Elagina[1], A.V. Belyatskaya[1], I.I. Krasnyuk (Jr.)[1], I.I. Krasnyuk[1], O.I. Stepanova[1], T.V. Fateeva[2], E.A. Smolyarchuk[1], S.V. Kozin[1], O.N. Plakhotnaya[1], O.V. Rastopchina[1], J.V. Rau[1,3]

[1] Sechenov First Moscow State Medical University
Bldg. 2, 8, Trubetskaya St., Moscow, Russia, 119991
[2] All-Russian Research Institute of Medicinal and Aromatic Plants
Bldg. 7, Grin St., Moscow, Russia, 117216
[3] Istituto di Struttura della Materia, Consiglio Nazionale delle Ricerche (ISM-CNR)
Via del Fosso del Cavaliere, 100-00133 Rome, Italy

E-mail: a.o.elagina@gmail.com

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Taking into account the current Product specification file, **the aim** of the work was to develop the composition and technology for obtaining effervescent tablets based on solid dispersions of furazolidone in the form of an aqueous solution for external use.

Materials and methods. The used substances were: furazolidone, anhydrous sodium carbonate (chemically pure), polyvinylpyrrolidone-24000±2000 (chemically pure), malic acid (analytically pure), tartaric acid (chemically pure), citric acid (chemically pure), sodium benzoate (chemically pure), ethyl alcohol 96% (chemically pure), purified water. Preparation of granulates is separate wet granulation in a fluidized bed (Mycrolab, BOSCH, Germany). Obtaining tablets is the process of pressing on a manual hydraulic test press ("PRG", VNIR, Russia). The dependence of disintegration, abrasion capacity and crushing resistance on compacting pressure was investigated. Technological parameters of granulates, epy obtained effervescent tablets, shelf life and storage conditions were investigated according to the State Pharmacopoeia of the Russian Federation XIVth ed.

Results. Two compositions of effervescent tablets containing solid dispersions of furazolidone as an active substance were obtained, which, when dissolved in 100 ml of water at room temperature (20°C), form a solution of furazolidone with a concentration of 0.004% in less than 5 minutes. The method of quantitative determination of the furazolidone content in the effervescent tablets was validated. A complex of physicochemical methods for the analysis of tablets was carried out. Quality standards have been developed. The developed compositions stability of instant tablets during storage during accelerated and long-term tests has been experimentally confirmed. The preliminary shelf life and storage conditions have been determined.

Conclusion. The result of technological and chemical-pharmaceutical research is the creation and evaluation of the quality of a new instant furazolidone dosage form as effervescent tablet formulations.

Keywords: furazolidone; effervescent tablets; instant tablets; solid dispersions; solubility; dissolution rate; polyvinylpyrrolidone

Abbreviations: FZ – furazolidone; SD – solid dispersions; AS – active substance; DF – dosage form; PVP – polyvinylpyrrolidone; SP RF XIV – State Pharmacopoeia of the Russian Federation XIV edition; Es – excipients; PSF – Product Specification File; GL – granulation liquid.

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РАЗРАБОТКА СОСТАВА И ТЕХНОЛОГИИ ШИПУЧИХ ТАБЛЕТОК С ТВЕРДОЙ ДИСПЕРСИЕЙ ПРОИЗВОДНОГО НИТРОФУРАНА

А.О. Елагина[1], А.В. Беляцкая[1], И.И. Краснюк (мл.)[1], И.И. Краснюк[1], О.И. Степанова[1], Т.В. Фатеева[2], Е.А. Смолярчук[1], С.В. Козин[1], О.Н. Плахотная[1], О.В. Растопчина[1], Д.В. Рау[1,3]

[1] Федеральное государственное бюджетное образовательное учреждение высшего образования «Первый Московский государственный медицинский университет имени И.М. Сеченова» Министерства здравоохранения Российской Федерации (Сеченовский Университет)
119991, Россия, г. Москва, ул. Трубецкая, д. 8, стр. 2
[2] Федеральное государственное бюджетное научное учреждение «Всероссийский научно-исследовательский институт лекарственных и ароматических растений»
117216, Россия, г. Москва, ул. Грина, д. 7
[3] Институт структуры вещества Национального исследовательского совета (ISM-CNR)
100-00133, Рим, Италия, Виа дель Фоссо дель Кавальере

E-mail: a.o.elagina@gmail.com

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Цель. С учетом действующей нормативной документации разработать состав и технологию получения шипучих таблеток на основе твердой дисперсии фуразолидона для наружного применения в виде водного раствора.

Материалы и методы. Субстанции: фуразолидон, карбонат натрия безводный (х.ч.), поливинилпирролидон-24000±2000 (х.ч.), яблочная кислота (ч.д.а.), винная кислота (х.ч.), лимонная кислота (х.ч.), бензоат натрия (х.ч.), спирт этиловый 96% (х.ч.), вода очищенная. Получение гранулятов: раздельное влажное гранулирование в псевдоожижженном слое («Mycrolab», BOSCH, Германия). Получение таблеток: прессование на ручном гидравлическом испытательном прессе («ПРГ», ВНИР, Россия). Исследовали зависимость распадаемости, истираемости и прочности на раздавливание от давления прессования. Технологические показатели гранулятов, полученные шипучие таблетки, срок годности и условия хранения исследовали согласно Государственной Фармакопее Российской Федерации XIV издания.

Результаты. Получены два состава шипучих таблеток, содержащих в качестве действующего вещества твердую дисперсию фуразолидона, образующие при растворении в 100 мл воды комнатной температуры (20°С) раствор фуразолидона с концентрацией 0,004% менее, чем за 5 мин. Осуществлена валидация методики количественного определения содержания фуразолидона в шипучих таблетках. Проведён комплекс физико-химических методов анализа таблеток. Разработаны нормы качества. Экспериментально подтверждена стабильность разработанных составов быстрорастворимых таблеток в процессе хранения в ходе ускоренных и долгосрочных испытаний. Определен предварительный срок годности и условия хранения.

Заключение. Результатом технологических и химико-фармацевтических исследований является создание и оценка качества новой быстрорастворимой лекарственной формы фуразолидона в форме шипучих таблеток.

Ключевые слова: фуразолидон; шипучие таблетки; быстрорастворимые таблетки; твердые дисперсии; растворимость; скорость растворения; поливинилпирролидон

Список сокращений: ФЗ – фуразолидон; ТД – твердая дисперсия; ДВ – действующее вещество; ЛФ – лекарственная форма; ПВП – поливинилпирролидон; ГФ РФ XIV – Государственная Фармакопея Российской Федерации XIV издания; ВВ – вспомогательные вещества; НД – нормативная документация; ГЖ – гранулирующая жидкость.

INTRODUCTION

Furazolidone (FZ) is a typical representative of the nitrofuran derivatives group, an antimicrobial and antiprotozoal drug that has been successfully used in therapy for more than 80 years to treat protozoal infections (*lambliosis, trichomoniasis*), as well as infectious diseases caused by bacteria *(Streptococcus spp., Staphylococcus spp., Escherichia coli, Salmonella spp., Shigella spp., Klebsiella spp., Enterobacter spp. Helicobacter pylori)* [1]. As a drug for the study, FZ is of particular interest because it is highly effective against a number of bacteria resistant to antibiotics and sulfonamides and, at the same time, it is characterized by a low resistance of microorganisms due to a specific mechanism of its action[1].

A separate direction in the FD application is its use for the *Helicobacter pylori* eradication in duodenal ulcer and / or stomach ulcer. FZ is used as a second-wave drug of choice in case of ineffective treatment of a patient

[1] Mashkovsky MD. Lekarstvennye sredstva [Medicines]. 16th ed.; Moscow: New Wave; 2016. – P. 1216. Russian

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with metronidazole, antibiotics and sulfonamides or, in case of intolerance, clarithromycin or amoxicillin. FZ is prescribed alone or in triple therapy with drugs based on bismuth, as well as using drugs that block H_2-receptors and inhibit the proton pump [2].

According to the clinical recommendations of the Ministry of Health dated January 23, 2019 (ICD 10: N30.0/N30.1/N30.2/N30.8), FZ is recommended for the prevention and treatment of diseases of the genitourinary system, such as urethritis and vaginitis, cystitis[2] [3]. FZ is also used topically for gargling in the complex treatment of infectious and inflammatory diseases of the oral cavity and nasopharynx. It is applied externally in the complex treatment of skin lesions, small wounds, scratches and burns prone to infection [1].

The FZ substance (Fig. 1) is a yellow or greenish-yellow fine-crystalline powder; it is odorless, non-hygroscopic[3]. FZ is recommended for an external and topical use in the form of aqueous solutions with a concentration of 0.004%[4]. However, its use is limited by physical properties, i.e.: FZ is practically insoluble in water and 95%[5] ethyl alcohol. On the domestic market, there is only one registered dosage form (DF) of FZ - 50 mg tablets for the administration *per os*[6].

The compounds characterized by a poor solubility belong to classes II and IV of the biopharmaceutical classification system (BCS) [4]. On the modern pharmaceutical market, about 40% of active ingredients (AI) have a low solubility in water, and at the development stage, the percentage of such compounds characterized by an insufficient solubility, according to various sources, reaches 75–90% [5–8].

As a substance with a poor solubility but a good permeability, FZ belongs to the BCS class II [4]. One of the priority methods to increase the solubility and dissolution rate of BCS class II AS, is the solid dispersions (SD) method. SDs are bi- or multicomponent systems consisting of an active ingredient and a carrier, which are a highly dispersed solid phase of an active agent or molecularly dispersed solid solutions with a partial formation of complexes of variable compositions with the carrier material [9–11].

In SD manufacturing, polymers of various chemical nature are used as the basic excipient (E). The introduction of a polymer supports the AS substance transition from the crystalline to the amorphous state: the polymer molecules intercalate into AS crystals, leading the ordered AS crystal to disintegration. There is a critical difference between the enthalpy of the crystal lattice and the enthalpy of solvation in favor of the process of crystal dissolution. In already the amorphous state, the bonds between the polymer and AS improve, the surface area of the interaction between the AS and biological fluid or solvent increases, resulting in the increase of the AS solubility and its dissolution rate [8, 12–14].

In the course of previous studies on increasing the solubility and dissolution rate of FZ by the SD method, several compositions with various carriers were developed and manufactured: PVP –10000; –12600; –24000 and PEG-400; –1500; –2000; –3000; –4000; –6000 at ratios with AS from 1:1 to 1:10. As a result of the experiment, PVP-24000 was chosen as the optimal carrier at the ratio of 6:1 to FZ by weight. At this ratio, the solubility of SD FZ increases by 1.56 times, and the dissolution rate – by 3 times at the point of 5 min from the start of dissolution [15]. The SD technology is used to increase the release of the AS from the DF, increase a bioavailability and a pharmacological activity by increasing the solubility and release rate of AS [16–19].

Increasing the solubility and dissolution rate of FZ due to the use of the SD method will allow the creation of the instant effervescent DF FZ, which will expand the convenience and possibilities of using this compound due to the possibility of obtaining a solution of the desired concentration in less than 5 minutes [20]. The acceleration of the AS dissolution process is achieved as a result of an acid-base reaction with the release of carbon dioxide, which acts as a super disintegrant [21, 22]. At the same time, effervescent DFs favorably differ in high stability and convenience in storage and transportation compared to liquid DFs [23]. A high dissolution rate of the AS, the speed and completeness of the manifestation of the pharmacological effect, dosing accuracy, microbiological and physicochemical stability, economic feasibility, and, most importantly, the ease of use, ensure a high adherence of patients to taking effervescent DFs [20, 24–26]. Given the numerous advantages of effervescent DFs, it is advisable to expand their range.

In accordance with Pharmaceutical development ICH Harmonised Tripartite Guideline Q8(R2)[7], to manage the development process best, it is necessary to identify the critical formulation and manufacturing parameters that affect the key characteristics of the DF. Under these conditions, the information obtained during the development process determines the requirements for quality indicators and, in the future, becomes part of the continuous quality control system for the production of a medicinal product [27].

For effervescent tablets of the developed DF, disintegration is a key characteristic. This indicator may depend on a number of factors: the solubility of AS, the amount and ratio of the acidic and basic components of the effervescent system in the composition, as well as the compacting pressure value. Thus, the critical for-

[2] Clinical recommendations of the Ministry of Health of the Russian Federation. 01/23/2019. (ICD 10: N30.0/N30.1/N30.2/N30.8). Russian
[3] State Pharmacopoeia of the Russian Federation. XIVth ed. Vol. 1–4.; M., 2018. – P. 5004. Available from: https://femb.ru/record/pharmacopea14.
[4] Mashkovsky MD. Lekarstvennye sredstva [Medicines]. 16th ed.
[5] State Pharmacopoeia of the Russian Federation. XIVth ed.
[6] Register of medicines of Russia. Furazolidone. Available from: https://www.rlsnet.ru/mnn_index_id_1371.htm. Russian
[7] Pharmaceutical development Q8(R2). ICH Harmonised Tripartite Guidline. London, 2009;28.

mulation parameters for FZ effervescent tablets are: the presence of SD as a component that increases the solubility and dissolution rate of the AS; the amount and ratio of gas-forming components of the effervescent system. The compacting pressure during the tablet production becomes a critical indicator of the manufacturing process.

Taking into account the current Product specification file, **THE AIM** of the work was to develop the composition and technology for obtaining effervescent tablets based on solid dispersions of furazolidone in the form of an aqueous solution for external use.

MATERIALS AND METHODS

Preparations and reagents

The used preparations and reagents were: Furazolidone substance (JSC Irbitsky Khimfarmzavod, Russia), anhydrous sodium carbonate (chemically pure) (Kupavnareaktiv, Russia), polyvinylpyrrolidone-24000±2000 (Sigma-Aldrich, USA), malic acid (AlbaKhim, Russia), tartaric acid (AlbaChem, Russia), citric acid (AlbaChem, Russia), sodium benzoate (Tengzhou Tenglong Chemical, China), 96% ethyl alcohol, analytical grade (LLC Constanta-Pharm M, Russia), purified water.

Devices and equipment

The devices and equipment used were as follows: laboratory balance MWP-150 (CAS, South Korea), analytical balance GH-202 (AND, Japan), UNICO 2800X SpectroQuest spectrophotometer (Unitedproducts & instruments, USA), MSH Basic magnetic stirrer (IKA, Germany), laboratory ionomer "I-160MI" (OOO Izmeritelnaya Tekhnika, Russia), moisture meter MA35M (Sartorius Weighing Technology, Germany), granulation machine Mycrolab (BOSCH, Germany), screening machine AS 200 Control (Retsch, Germany), compaction tester SVM 223 (Erweka, Germany), flowability tester GTL (Erweka, Germany), compression tester TBF 1000 (Copley Scientific, Great Britain), abrasion tester PT F30ERA (Pharma Test, Germany), a protractor, manual hydraulic "Press test" brand PRG (VNIR , Russia), climate chamber KK115 (Pol-EKO, Poland). Filtration was carried out through syringe nozzles (Minisart, Germany) with a pore diameter of 0.45 μm, the filter material was nylon.

Manufacturing technology of acid granulates

Powders of the two most widely used effervescent tablet technologies, tartaric and malic acids, weighing 1 kg, were separately loaded into the product container of the Mycrolab granulation unit, preliminarily crushed and sequentially sifted through sieves with hole diameters of 250 μ and 45 μ; then the particle fraction was granulated of more than 45 μ and less than 250 μ.

To obtain a granulating liquid (GL), PVP-24000 was dissolved in 96% ethyl alcohol when heated in a water bath (65±5°C). Granulation was carried out in a fluidized bed; the granulation parameters were standard.

Manufacturing technology of the basic granulate with SD FZ

Manufacturing of the basic granulate with SD FZ was carried out similarly to the preparation of acid granulates, with the difference in the following: the powder of anhydrous sodium carbonate and the GL – a solution of FZ and PVP-24000 in 96% ethyl alcohol – were loaded into the container of the Mycrolab granulation unit, followed by heating in a water bath at the temperature of 65±5°C).

Manufacturing technology of granulates of compositions No. 1 and No. 2

To obtain granulates of compositions No. 1 and No. 2, the basic and acid granules were mixed at the ratios of 1.0:1.3 and 1.0:1.1, respectively, and a lubricating excipient, sodium benzoate, was introduced in the amount of 2% of the powdered mass.

Tablet manufacturing technology

In the work, a manual hydraulic "Testing Press" (PRG brand, VNIR, Russia) was used. Model tablets weighing 3.800 and 3.500 g for compositions No. 1 and No. 2, respectively, were produced at different compacting pressure values on flat-cylindrical puncheons with a diameter of 25.0 mm.

Determining the authenticity of FZ

When interacting with sodium hydroxide, a FZ qualitative reaction has a brown coloration. One tablet was dissolved in 100 ml of purified water, a 10 ml sample was taken and mixed with 10 ml of a mixture of water and a 30% sodium hydroxide solution (3:2), then heated; herewith, a brown coloration was observed.

Quantitative determination of FZ

Due to the fact that FZ solutions have a clearly defined maximum in the UV spectrum, a quantitative determination is best carried out using the method of spectrometry in the UV region. In the work, a spectrophotometer and quartz cuvettes (the thickness of the absorbing layer was 10 mm) were used.

One tablet weighing 3.800 g (for composition No. 1) and another weighing 3.500 g (for composition No. 2), respectively, were placed in two 500 ml volumetric flasks, dissolved each in 100 ml of purified water, the solutions were stirred on a magnetic stirrer for 5 minutes (speed 200 rpm). The volumes of the resulting solutions were brought to the marks with purified water, mixed. The selected samples with a volume of 5 ml were filtered. The optical densities of the resulting solutions were measured at the wavelength of 367±2 nm (FZ absorption maximum); in the both cases, the reference solution was purified water. It had been preliminarily established that the excipients do not influence the maxima of the FZ absorption spectra and their intensity. The FZ concentrations were calculated using the calibration plot.

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This quantitative determination method was developed more than 20 years ago by the Departments of Analytical, Physical and Colloidal Chemistry and Pharmaceutical Technology of the Institute of Pharmacy ("First Moscow State Medical University n. a. I.M. Sechenov"). It is successfully used for a quantitative determination of poorly soluble active substances from various pharmacological groups introduced into solid dispersions with various polymers to increase bioavailability from a number of solid and soft dosage forms. A detailed description of this technique are disclosed in a number of publications, patents of the Russian Federation for the invention [15, 28–41], and in the applications for inventions deposited with Rospatent They are: No.2021105988 dated March 10, 2021 "Instantly soluble dosage form of furazolidone and its preparation method" (the authors are Krasnyuk II, Krasnyuk II(Jr), Stepanova OI, Belyatskaya AV, Elagina AO; No. 2021129748 dated October 13, 2021 "Instantly soluble dosage form of metronidazole and its preparation method" (the authors are Krasnyuk II(Jr.), Naryshkin SR, Krasnyuk II, Belyatskaya AV, Stepanova OI.

Determining shelf life of tablets

In order to study the stability and shelf life, the tablet samples were stored in accordance with GPM.1.10009.15 "Stability and shelf life of medicines"[8] in plastic tubes made of polypropylene, sealed with water absorbers lids. Long-term tests were carried out on 3 series of each composition at the temperature of 25±2°C and a relative humidity of 60±5%; accelerated tests were carried out on 3 series of each composition at the temperature of 40±2°C and a relative humidity of 75±5% using a climatic chamber. The check points for long-term testing were as follows: on the day of manufacturing and during the storage (every 3 months during the first year of storage and every 6 months during 2 years). During accelerated tests, the check points were on the day of manufacturing and during the storage (3 and 6 months). At the check points, the following indicators were investigated: description, mass uniformity, disintegration, abrasion capacity, crushing resistance, weight loss on drying, *pH* of the solution, quantitative and qualitative determination of AS.

Statistical processing of the results was carried out according to GPM 1.1.0013.15 "Validation of analytical methods"[9] (p = 95%, n = 5) using the techniques of variation statistics by means of the Microsoft Office 2010 office software package, as well as the Microsoft Excel spreadsheet processor.

RESULTS AND DISCUSSION

Preliminarily, the method for quantitative determination of the furazolidone content in effervescent tablets was validated on the drug samples and model mixtures obtained in the laboratory. The following characteristics were studied: specificity, linearity, correctness, precision (at two levels – convergence, intermediate precision), and an analytical area of the methods.

To determine the specificity of the methods on a spectrophotometer, the spectra of aqueous solutions were sequentially taken: the furazolidone substance, effervescent furazolidone tablets, and excipients. The specificity of the UV spectrophotometry method was proven by the coincidence of the spectra maxima and minima of the FZ effervescent tablet solution and the substance solution, and due to the absence of the excipients effect on the analysis results (Fig. 2).

Next, 5 samples of standard furazolidone solutions were prepared with concentrations of 0.032 mg/ml, 0.036 mg/ml, 0.04 mg/ml, 0.044 mg/ml and 0.048 mg/ml (from 80 to 120%). The optical density the five standard furazolidone solutions obtained after the dilution with concentrations of 0.0064 mg/ml, 0.0072 mg/ml, 0.0080 mg/ml, 0.0088 mg/ml and 0.0096 mg/ml was measured. A linear regression analysis of the data obtained, calculated by the least squares method, made it possible to establish that the dependence of the optical density of furazolidone on its concentration is linear and is described by the equation y = 70.000x – 0.002 (Fig. 3). The correlation coefficient (r), equal to 0.99809, meets the necessary condition $|r| \geq 0.99$[10].

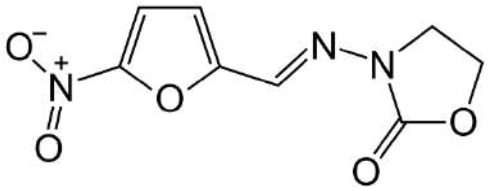
The scope of the experimental data that satisfies the linear model in the concentration range from 80 to 120% can be considered the analytical area of the technique.

The correctness of the technique was confirmed by the analysis of a series of model mixtures prepared from the excipients with the addition of a weighed batch that also corresponded to the range from 80 to 120% of the nominal FZ content in the preparation. The results of the analysis were evaluated by comparing the obtained results with the expected quantity value, e.i., the FZ content in the model mixture, mg (Table 1).

As the data in Table 1 show, the relative errors of the average result ($\bar{\varepsilon}$) are less than 2.0%; the obtained results lie in the range of the confidence interval of the analysis average result ($\overline{X} \pm \Delta\overline{X}$), which was 99.66 ± 1.27, and approach the true value. The numerical value of the Student›s coefficient ($t_{calc.}$), calculated from the results of the analysis, was 0.62. The tabular value of the Student's coefficient ($t_{tabular}$) is 2.31, i.e. $t_{calc} < t_{tabular}$. Therefore, the proposed method is characterized by a satisfactory correctness.

Precision was studied by analyzing the FZ tablets of compositions No. 1 and No. 2 in six replications in the form of parameters "Convergence" and "Intralaboratory (intermediate) precision". To assess the intralaboratory precision, the test samples were analyzed using different analysts and on different days using the same equipment. The results obtained (Tables 2, 3) testify to the satisfactory precision of the proposed method for the quantitative determination of FZ in effervescent tablets at the levels of repeatability and intralaboratory precision.

[8] State Pharmacopoeia of the Russian Federation. XIV[th] ed.
[9] Ibid.
[10] Ibid.



$C_8H_7N_3O_5$ **225,16 g/mol**

Figure 1 – Furazolidone structural chemical formula

Note: 3-{[(5-Nitrofuran-2-yl)methyliden]amino}-1,3-oxazolidin-2-on

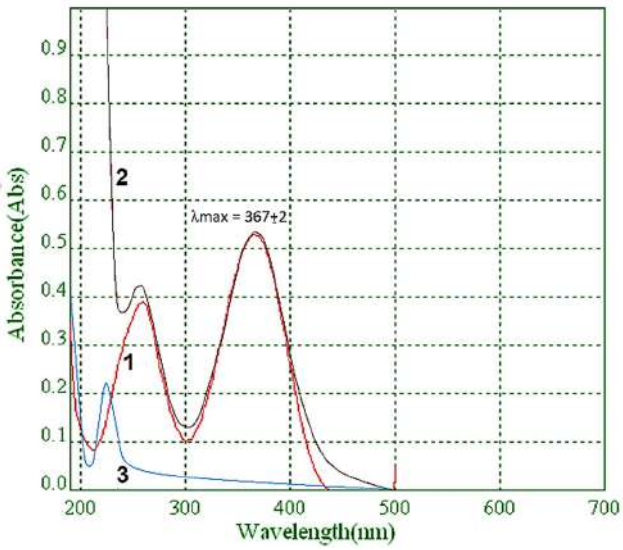


Figure 2 – Ultraviolet absorption spectra of substance furazolidone aqueous solutions (1), effervescent FZ tablets (2) and excipients (3)

Table 1 – Results of assessing correctness of methods for furazolidone quantitative determination

Added FZ, mg	Detected FZ, mg	Recovery, %	Metrological characteristics, (p = 95%, n = 9)
3.20	3.14	98.13	$\overline{X}$ = 99.66%
3.40	3.37	99.12	SD = 1.65
3.60	3.64	101.11	RSD = 1.66%
3.80	3.73	98.16	$\Delta\overline{x}$ = 1.27
4.00	4.08	102.00	Δx = 3.81
4.20	4.11	97.86	$\bar{\varepsilon}$ = 1.27%
4.40	4.46	101.36	$t_{calc.}$ 0.62
4.60	4.64	100.87	$t_{tabular.}$ = 2.31
4.80	4.72	98.33	$\overline{x} \pm \Delta\overline{x}$ = 99.66 ± 1.27

Table 2 – The results of determining the convergence of the analytical methods for the quantitative determination of the content of furazolidone in effervescent tablets

No.	Composition No.1		Composition No.2	
	Detected FZ, mg	Metrological characteristics, (n = 6)	Detected FZ, mg	Metrological characteristics, (n = 6)
1	4.06	$\overline{X}$ = 4.01	3.93	$\overline{X}$ = 3.98
2	4.02	S^2 = 0.002937	4.04	S^2 = 0.003840
3	3.94	SD = 0.054	4.00	SD = 0.062
4	4.01	RSD = 1.35%	3.97	RSD = 1.56%
5	3.95		4.04	
6	4.07		3.95	

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Table 3 – The results of the intermediate precision study of the method for the quantitative determination of the furazolidone content in effervescent tablets

No.	Explorer 1		Explorer 2		Metrological characteristics, (n = 6)			
	Composition No.1 Detected FZ, mg	Composition No.2 Detected FZ, mg	Composition No.1 Detected FZ, mg	Composition No.2 Detected FZ, mg	Explorer 1		Explorer 2	
					Composition No.1	Composition No.2	Composition No.1	Composition No.2
1	3.95	4.04	3.99	4.03	$\overline{X}$ = 3.98	$\overline{X}$ = 4.01	$\overline{X}$ = 3.93	$\overline{X}$ = 3.98
2	4.03	4.02	3.93	4.02	S^2 = 0.0024	S^2 = 0.0012	S^2 = 0.0016	S^2 = 0.0015
3	3.96	4.06	3.95	3.94	SD = 0.049	SD = 0,035	SD = 0.040	SD = 0.039
4	4.06	3.98	4.01	4.01	RSD = 1.23%	RSD = 0.87%	RSD = 1.01%	RSD = 0.98%
5	3.97	4.01	3.98	3.97	$\overline{x} \pm \Delta\overline{x}$ = 3.98±0.05	$\overline{x} \pm \Delta\overline{x}$ = 4.01 ±0.04	$\overline{x} \pm \Delta\overline{x}$ = 3.93 ± 0.04	$\overline{x} \pm \Delta\overline{x}$ = 3.98 ± 0.04
6	3.95	3.97	4.04	3.95	$t_{(95\%, 5)\ calc.}$ = 1.00	$t_{(95\%, 5)\ calc.}$ = 0.70	$t_{(95\%, 5)\ calc.}$ = 1.23	$t_{(95\%, 5)\ calc.}$ = 1.26
					$F_{calc.}$ = 1.5	$F_{calc.}$ = 1.27	$F_{calc.}$ = 1.5	$F_{calc.}$ = 1.27
					$t_{(95\%.\ 5)\ tabular.}$ = 2.57; $F_{(99\%.\ 5.\ 5)\ tabular.}$ = 10.97			

Note: $t_{(95\%, 5)\ calc.} < t_{(95\%, 5)}$; $F_{calc.} < F_{(99\%, 5, 5)\ tabular}$ – the differences between the results obtained are random, not burdened by a systematic error.

Table 4 – Compositions of the developed tablets containing furazolidone solid dispersions as an active substance

Ingredient	Composition No.1 for 1 dose (tablet)		Composition No.2 for 1 dose (tablet)	
	g	%	g	%
Furazolidone	0.004	0.105	0.004	0.114
PVP-24000 (in basic and acid granules)	0.061/ 0.006	1.605/ 0.158	0.061/ 0.005	1.743/ 0.143
Sodium carbonate anhydrous	1.558	41.000	1.574	44.971
Tartaric acid	2.095	55.132	–	–
Malic acid	–	–	1.786	51.029
Sodium benzoate	0.076	2.000	0.070	2.000
Total weight:	3.800	100.000	3.500	100.000

Table 5 – Fractional composition of granulates obtained by separate granulation methods, as well as of compositions prepared for tableting

Sample name	Size (p) of particles, mm					
	More than 2.0	2.0>p>1.25	1.25>p>710	710>p>315	315>p>0.1	Less than 0.1
	Fraction content (%), n=5; $X_{av.}\pm\Delta X$					
Granulate 1	–	0.16±0.03	9.06±0.32	65.91±2.93	24.46±1.15	0.16±0.02
Granulate 2	–	–	26.73±0.93	45.34±1.13	23.38±0.84	4.33±0.17
Granulate 3	–	–	–	25.20±1.74	72.0±1.34	2.45±0.14
Composition No.1	–	–	19.03±1.17	54.73±2.57	22.96±1.31	2.32±0.16
Composition No.2	–	0.08±0.02	4.53±0.31	45.25±2.37	48.20±2.46	1.33±0.07

Note: granulate 1 – basic granulate (FZ + anhydrous sodium carbonate + PVP-24000); granulate 2 – acid granulate (tartaric acid + PVP-24000): granulate 3 – acid granulate (malic acid + PVP-24000).

Table 6 – Quality indicators of the developed granules at the moment of manufacturing

Indicators	Granulates			Compositions	
	No.1	No.2	No.3	No.1	No.2
Appearance	Yellow granules	White granules		Mixture of white and yellow granules	
Bulk volume, (Xav±ΔX, n=3), (g/ml) before compaction	0.89±0.03	0.77±0.02	0.78±0.03	0.83±0.02	0.81±0.02
After compaction, (g/cm^3)	1.01±0.05	0.85±0.03	0.87±0.04	0.93±0.03	0.92±0.03
Flowability (Xav±ΔX, n=3), (g/s)	14.90±0.11	11.03±0.07	11.06±0.04	13.01±0.09	12.90±0.07
Angle of natural repose ($X_{cp}\pm\Delta X$, n=5), (°)	35±2	25±2	25±2	30±2	32±2
Residual moisture ($X_{cp}\pm\Delta X$, n=5), (%)	1.15±0.12	1.04±0.11	0.61±0.09	1.11±0.10	1.07±0.09

Table 7 – Quality indicators of instant tablets containing a solid furazolidone dispersion at the moment of manufacturing

Indicators	Methods (guidelines)	Composition No.1	Composition No.2
Description	SP RF XIV GPM.1.4.1.0015.15 Visual	Effervescent tablets, white, interspersed with pale yellow to bright yellow, cylindrical, flat, with a bevelled edge on both sides; tablets dissolve in water with a release of bubbles, forming a greenish-yellow, transparent, odorless solution. Roughness and marbling are allowed.	
Authenticity	SP RF XIV GPM.1.2.1.1.0003.15 GPM.2.1.0203.18 UV spectrophotometry qualitative reacion	The UV spectra of the aqueous solution from 230 to 400 nm must correspond to the characteristic peaks of the FZ standard. Qualitative reaction with sodium hydroxide, brown color appears.	
Quantification ($C_{av.}$±ΔC, n=5), (g/l)	SP RF XIV GPM.1.2.1.1.0003.15 UV spectrophotometry	0.040±0.004	0.040±0.004
Crushing resistance ($X_{av.}$±ΔX, n=10), (H)[1]	SP RF XIV GPM.1.4.2.0011.15 (not less than 50 H)	77.2±3.0	79.6±5.0
Abrasion rate (X_{av}±ΔX, n=5), (%)	SP RF XIV GPM.1.4.1.0004.15 (not more than 3%)	1.00±0.23	0.50±0.31
Weight loss on drying	SP RF XIV GPM.1.2.1.0010.15 (less than 2%)	1.5±0.5	1.3±0.5
Disintegration ($t_{av.}$±Δt, n=5), (s)	SP RF XIV GPM.1.4.1.0015.15 (less than 5 min)	135±15	125±15
pH ($X_{av.}$±ΔX, n=5)	SP RF XIV GPM.1.2.1.0004.15	6.0±0.5	6.0±0.5
Package	SP RF XIV GPM.1.1.0025.18 10 tablets in a plastic tube made of polypropylene, sealed with a lid with a desiccant		
Marking	SP RF XIV GPM.1.1.0025.18 Warning: "The tablet must be dissolved in ½ cup (100 ml) of water before use".		
Storage	SP RF XIV GPM.1.1.0010.18 In a dry place protected from light at the temperature not exceeding 25°C		
Shelf life	SP RF XIV GPM.1.1.0009.18 2 years		

Note: [1] – load on the side face, destroying the tablet.

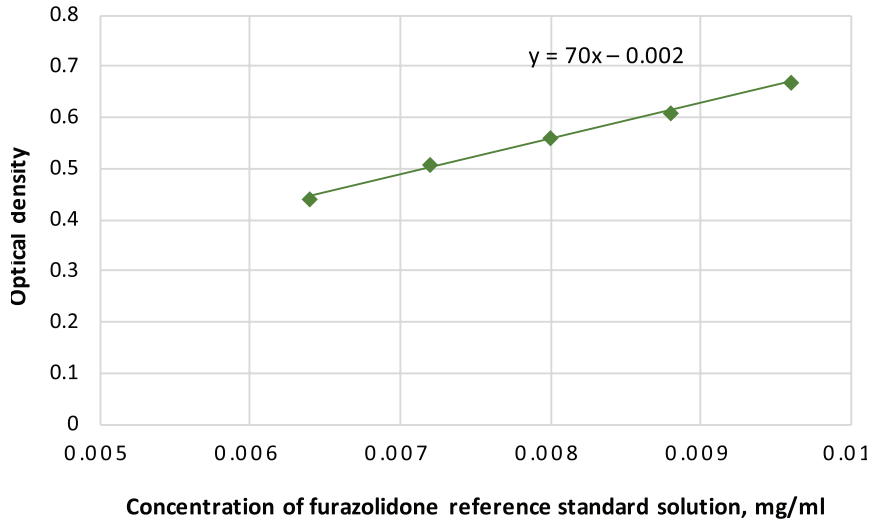

Figure 3 – Regression line for quantitative determination of furazolidone content by spectrophotometry

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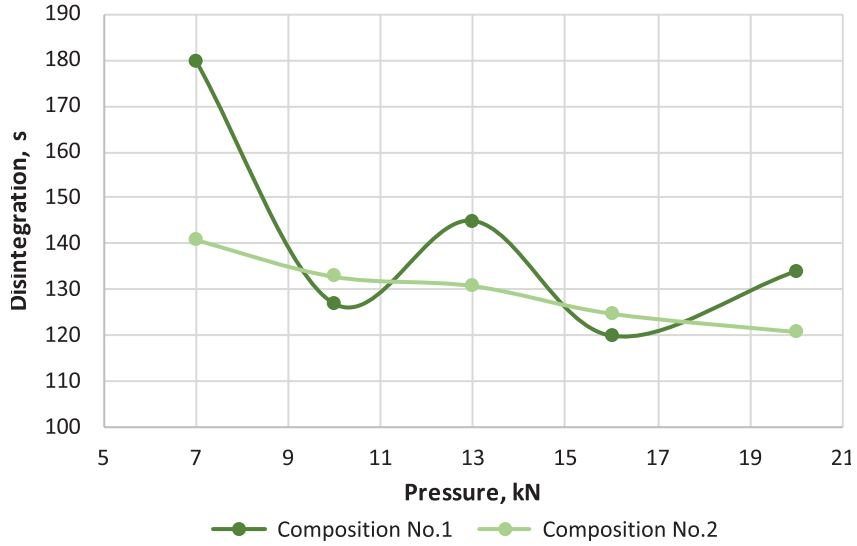

Figure 4 – Dependence of the effect of compacting pressure values on the disintegration of furazolidone effervescent tablets

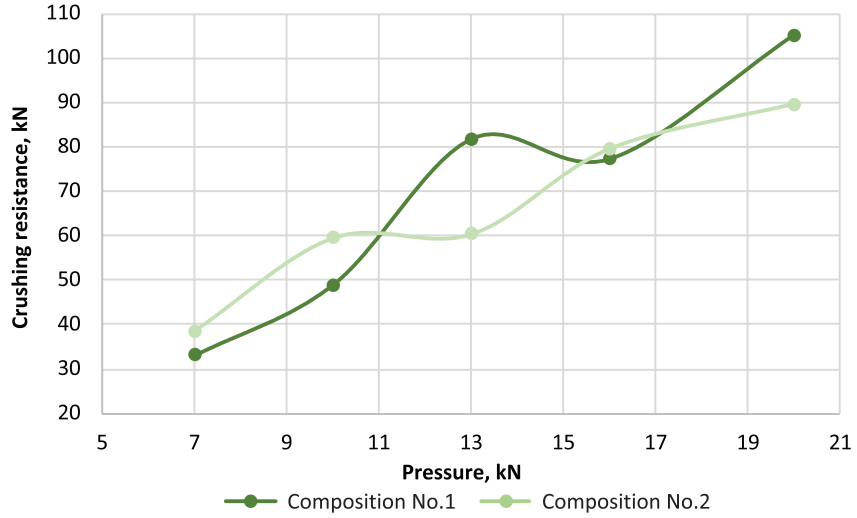

Figure 5 – Dependence of the effect of compacting pressure value on crushing resistance of furazolidone effervescent tablets

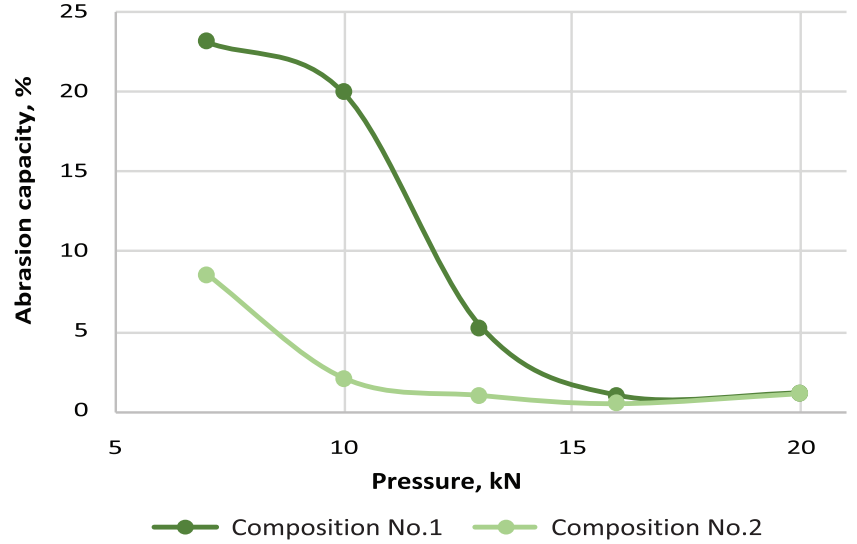

Figure 6 – Dependence of the effect of compacting pressure on the abrasion capacity of furazolidone effervescent tablets

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Figure 7 – Effervescent tablets containing a solid furazolidone dispersion as an active substance

Thus, with the help of the validation assessment, the correctness, precision, specificity and linearity in the analytical area of the developed method for the quantitative determination of FZ in effervescent tablets, have been established.

When developing the compositions of instant tablets, thermostable anhydrous components of the effervescent system were used – sodium carbonate and organic acids, which increase the stability and shelf life of the developed compositions. Bicarbonates were not used as the basic components of the effervescent system due to their instability when heated (starting from 60°C), and the presence of bound (crystallization) water, reducing the stability and shelf life of the instant DF.

In the course of choosing a gas-forming system, various combinations and mass ratios of organic acids (tartaric, malic, and citric) with sodium carbonate were studied. The basic criteria for screening were such quality indicators of DFs as disintegration, *pH* of the aqueous solution and compressibility. Compositions containing citric acid were characterized by low compressibility and disintegration, and therefore were excluded from further studies. Since the formulation of the compositions, in addition to the effervescent system, contains AS and excipients (PVP-24000 and sodium benzoate), which can also influence the pH index, the ratio of basic and acid granulates was determined experimentally.

When developing effervescent tablets, special attention was paid to the risk of a premature neutralization reaction between the basic and acid components of the gas-forming system, which could lead to such undesirable consequences as a reduced quality of finished tablets (change in color, transparency of aqueous solutions of tablets), a reduction of shelf life, an increase in the percentage of rejects, an increase in the duration of the technological process. In this regard, the granulates were obtained by separate wet granulation, using 96% ethyl alcohol as a GL solvent. The use of SD components as a GL ethanol solution is more promising, since it makes it possible to obtain SD by the "solvent removal" method[11,12] [42].

The basic and acid components of compositions No. 1 and No. 2 (Table 4) were separately granulated with an FZ alcohol solution and PVP-24000 heated to 65 ± 5°C in case of the basic granulate and with a 1% alcohol solution of PVP-24000 in case of acid granulates. Thus, the stage of obtaining SD FZ is combined into one technological stage with the stage of granulation, which greatly simplifies the technological process: it reduces the number of technological operations (the technological stage for obtaining SD, which requires a quality assessment and standardization of the intermediate product, is excluded), and reduces the load on the equipment and material costs. This solution also simplified the development process, making the empirical *pH* adjustment easy dosing. In the proposed technology, the stages of obtaining SD, mixing components, granulation and drying are carried out in one apparatus, which contributes to the creation of continuous production with high productivity [43, 44].

To obtain a tablet mass, the basic and acid granulates were mixed in ratios (by weight) of 1.0:1.3 for composition No. 1 and 1.0:1.1 for composition No. 2, respectively. These compositions make it possible to obtain an FZ solution for external use with a minimum disintegration index and a *pH* value that is comfortable for external use ≈6.0±0.5.

In the amount of 2% of the powdered mass, sodium benzoate was used as a lubricating excipient, since its good solubility in water makes it possible to obtain transparent solutions when the developed compositions are dissolved.

The fractional composition of granulates of compositions No. 1 and No. 2, as well as basic and acid granules, are presented in table 5.

[11] Tentsova AI. Biofarmacevticheskie aspekty primeneniya tvyordyh dispersiĭ [Biopharmaceutical aspects of the use of solid dispersions]. Epoch in Pharmacy. M.: Pero, 2014: 62–66. Russian

[12] Ruban EA. Sovremennye napravleniya v tekhnologii tverdyh lekarstvennyh sredstv [Modern trends in the technology of solid drugs]. Kharkov: NFAU, 2016: 88 p. Russian

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The data in Table 5 indicate that the granulates are homogeneous, they are characterized by uniform flowability and appropriate compressibility.

As Table 6 shows, analyzed granulates No. 1 and No. 2, as well as basic and acid granules, have good technological characteristics. All the samples are characterized by a high bulk density, flowability, at least twice as required values (at least 4–5 g/s), which will provide good indicators of the volumetric flow rate of the tablet mass in pressing in the future. Residual moisture is a critical indicator for the stability of instant tablets, which determines the possibility of a premature start of the neutralization reaction of the effervescent system – less than 1.5%, which is optimal for effervescent tablets [45]. The angle of natural repose also characterizes the studied compositions as well flowing, since its values for all samples are in the range of 20-35º. Such technological indicators of granulates of No. 1 and No. 2 compositions, such as flowability, a moisture content, a bulk density, meet the requirements of Product specification file, providing satisfactory compressibility.

To identify the optimal tableting mode, the dependence of disintegration, abrasion capacity and crushing resistance of tablets on compacting pressure values was investigated. In the compacting pressure range of 5–20 kN, the disintegration of the developed compositions meets the requirements of the State Pharmacopoeia of the Russian Federation, XIVth edition (Fig. 4).

A compacting pressure of more than 16 kN makes it possible to obtain tablets with a crushing resistance of more than 70 N (77.2 N and 79.6 N, respectively) (Fig. 5).

Weight loss during the test for the abrasion capacity does not exceed 3% at the compacting pressure of more than 14 and 10 kN for compositions No. 1 and No. 2, respectively (Fig. 6).

Thus, at the compacting pressure, the developed compositions of FZ effervescent tablets have satisfactory quality indicators of more than 16 kN. Therefore, tablets of compositions No. 1 and No. 2 were obtained at the optimal compacting pressure (Fig. 7).

On the basis of the conducted studies, it can be concluded that the obtained instant effervescent tablets FZ of the proposed compositions No. 1 and No. 2 at the time of manufacturing, according to the main qualitative, quantitative and technological quality indicators, meet the requirements of Product specification file (Table 7).

With the help of long-term and accelerated tests, it has been revealed that the tablets are characterized by the constancy of the main technological characteristics: description, mass uniformity, disintegration, abrasion rate, crushing resistance, weight loss on drying, *pH*, authenticity, quantitative AS content throughout the shelf life. The data obtained make it possible to recommend the shelf life of the tablets of compositions No. 1 and No. 2, packaged in polymer tubes, in a dry, light-protected place at the temperature of 25°C for 2 years.

The results obtained in the course of this study can be used for the implementation in production this highly effective antimicrobial drug for external use – fast-dissolving effervescent FZ tablets.

CONCLUSION

Two compositions of effervescent tablets including SD FZ, a gas-forming system – acidic and basic components, as well as lubricating excipients, have been developed. They make it possible to obtain an aqueous FZ solution with an AS concentration of 0.004% and *pH* 6.0 ± 0.5 in less than 5 minutes without heating and applicating mechanical efforts. The method for quantitative determination of the FZ content in effervescent tablets, has been validated. In accordance with the requirements of SP RF XIV, the quality of the developed effervescent tablets containing SD FZ has been assessed. It has been found out that the technological characteristics of the obtained compositions (description, mass uniformity, disintegration, abrasion rate, crushing resistance, weight loss on drying, *pH* of the solution, authenticity and quantitative determination of the AS) are within the standard values and meet all quality requirements. Experimentally, by methods of long-term and accelerated tests, the preliminary shelf life of effervescent FZ tablets was determined to be 2 years in a dry place protected from light at the temperature not exceeding 25°C.

Based on the results of the work, application No. 2021105988 dated 03/10/2021 “Instant dosage FZ form and method for its preparation” was deposited with Rospatent.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

Anastasia O. Elagina – text writing, production and quality assessment of effervescent tablets, analysis, processing and preparation of graphic material; Anastasiya V. Belyatskaya – general management and experiment planning, granulates production; Ivan I. (Jr) Krasnyuk – tablet pressing, tablet quality evaluation; Ivan I. Krasnyuk – general management and experiment planning; Olga I. Stepanova – literature data collecting and processing; Tatyana V. Fateeva – quality of tablets evaluation; Elena A. Smolyarchuk – graphic material analysis, processing and preparation; Sergey V. Kozin – graphic material analysis, processing and preparation; Olga N. Plakhotnaya – evaluation of tablets quality; Olga V. Rastopchina – evaluation of tablets quality; Julietta V. Rau – evaluation of tablets quality. All the authors participated in the discussion of the results and writing the article.

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AUTHORS

Anastasia O. Elagina – post-graduate student, Department of Pharmaceutical Technology, Institute of Pharmacy named after A.P. Nelyubin, Sechenov First Moscow State Medical University. ORCID ID: 0000-0001-5255-3991. E-mail: a.o.elagina@gmail.com

Anastasiya V. Belyatskaya – Candidate of Sciences (Pharmacy), Associate Professor, Associate Professor of the Department of Pharmaceutical Technology, Institute of Pharmacy named after A.P. Nelyubin, Sechenov First Moscow State Medical University. ORCID ID: 0000-0002-8214-4483. E-mail: av.beliatskaya@mail.ru

Ivan I. (Jr) Krasnyuk – Doctor of Sciences (Pharmacy), Professor, Professor of the Department of Analytical, Physical and Colloidal Chemistry, Institute of Pharmacy named after A.P. Nelyubin, Sechenov First Moscow State Medical University. ORCID ID: 0000-0001-8557-8829. E-mail: krasnyuk.79@mail.ru

Ivan I. Krasnyuk – Doctor of Sciences (Pharmacy), Professor, Professor of the Department of Pharmaceutical Technology, Institute of Pharmacy named after A.P. Nelyubin, Sechenov First Moscow State Medical University. ORCID ID: 0000-0002-7242-2988. E-mail: krasnyuki@mail.ru

Olga I. Stepanova – Candidate of Sciences (Pharmacy), Associate Professor, Associate Professor of the Department of Pharmacology, Institute of Pharmacy named after A.P. Nelyubin, Sechenov First Moscow State Medical University. ORCID ID: 0000-0002-9885-3727. E-mail: o.i.nikulina@mail.ru

Tatyana V. Fateeva – Head of the Laboratory of Microbiological Research, All-Russian Scientific-Research Institute of medicinal and aromatic plants. ORCID ID: 0000-0002-8231-0621. E-mail: fateeva2151@mail.ru

Elena A. Smolyarchuk – Candidate of Sciences (Medicine), Associate Professor, Head of the Department of Pharmacology, Institute of Pharmacy named after A.P. Nelyubin, Sechenov First Moscow State Medical University. ORCID ID: 0000-0002-2615-7167. E-mail: smolyarchuk@mail.ru

Sergey V. Kozin – Candidate of Sciences (Biology), Associate Professor, Department of Pharmacology, Institute of Pharmacy named after A.P. Nelyubin, Sechenov First Moscow State Medical University. ORCID ID: 0000-0002-4722-8315. E-mail: enfadado@yandex.ru

Olga N. Plakhotnaya – Candidate of Sciences (Chemistry), Associate Professor, Associate Professor of the Department of Analytical, Physical and Colloidal Chemistry, Institute of Pharmacy named after A.P. Nelyubin, Sechenov First Moscow State Medical University. ORCID ID: 0000-0001-7266-2933. E-mail: plahotnaya.o@mail.ru

Olga V. Rastopchina – Candidate of Sciences (Pharmacy), Associate Professor, Associate Professor of the Department of Pharmaceuticals, Institute of Pharmacy named after A.P. Nelyubin, Sechenov First Moscow State Medical University. ORCID ID: 0000-0002-5443-6980. E-mail: rastop0309@yandex.ru

Julietta V. Rau – Doctor of Sciences (Philosophy), Professor, Istituto di Struttura della Materia, Consiglio Nazionale delle Ricerche (ISM-CNR), Rome, Italy; Associate Professor, Associate Professor of the Department of Analytical, Physical and Colloidal Chemistry. Institute of Pharmacy named after A.P. Nelyubin, Sechenov First Moscow State Medical University. ORCID ID: 0000-0002-7953-1853. E-mail: giulietta.rau@ism.cnr.it

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ОРИГИНАЛЬНАЯ СТАТЬЯ

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APPLICATION OF MULTIVARIATE ANOVA AND GENERALIZED DESIRABILITY TO OPTIMIZE THE COMPOSITION AND TECHNOLOGY OF TABLETS CONTAINING N-BENZYL-N-METHYL-1-PHENYLPYRROLO [1,2-A] PYRAZINE-3-CARBOXAMIDE

S.V. Tishkov, E.V. Blynskaya, K.V. Alekseev, V.K. Alekseev, D.I. Gavrilov

Research Institute of Pharmacology named after V.V. Zakusov
8, Baltiyskaya St., Moscow, Russia, 125315

E-mail: sergey-tishkov@yandex.ru

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The creation of drugs with an anxiolytic activity, which do not have the main side effects characteristic of drugs of this group, is an important and socially significant task. For its implementation, within the framework of the development of an original drug with an anxiolytic activity, the composition and manufacturing of GML-1 tablets (N-benzyl-N-methyl-1-phenylpyrrolo [1,2-a] pyrazine-3-carboxamide) are being developed.

The aim of this article is to study, using a four-factor analysis of variance, the influence of composition factors on the manufacturing properties of GML-1 tablets and the selection of the type, the amount, stage of the disintegrant addition and the type of lubricating excipients used in the technology of wet granulation of GML-1 tablets.

Materials and methods. The materials used are: the substance – GML-1 (N-benzyl-N-methyl-1-phenylpyrrolo [1,2-a] pyrazine-3-carboxamide). Excipients: microcrystalline cellulose 101 (MCC 101); polyvinylpyrrolidone (PVP); crospovidone, croscarmellose sodium (CCS), sodium starch glycolate (SSG); magnesium stearate (MS), sodium stearyl fumarate (SSF). To obtain tablet mixtures, wet granulation and tableting with the study of their main pharmaceutical and technological properties was used.

Results. Model compositions were developed and their pharmaceutical and technological properties were studied. These results have been analyzed, the degree of these factors' influence and their interactions have been determined. In most of the cases considered, the interactions of the factors did not cause a significant change in the optimization criteria. With an increase in the amount of a disintegrant, the disintegration time decreased unevenly, so an increase in the amount of these excipients from 4 to 6 mg had a stronger effect than from 2 to 4 mg. Factor B affected the release degree non-linearly. Factor A influenced all the optimization criteria considered, especially a PS release. The best release and disintegration were observed with crospovidone, which was of a particular importance when processing the test results using a generalized desirability method.

Conclusion. In view of the conflicting variance analysis results, for particular factors, the resulting values were additionally analyzed using the generalized desirability function. The use of this method made it possible to reduce the conflicting variance analysis results to the most optimal composition.

Keywords: GML-1; tablet; analysis of variance; four-factor; influence of factors; interaction of factors; desirability function

Abbreviations: MP – medicinal product; DP – drug product; DF – dosage form; PS – pharmaceutical substance; API – active pharmaceutical ingredient; PVP – polyvinylpyrrolidone; MCC – microcrystalline cellulose; SCC – sodium croscarmellose; SSG – sodium starch glycolate; MS – magnesium stearate; SSF – sodium stearyl fumarate; GPM – General Pharmacopoeia Monograph.

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ПРИМЕНЕНИЕ МНОГОФАКТОРНОГО ДИСПЕРСИОННОГО АНАЛИЗА И ОБОБЩЁННОЙ ЖЕЛАТЕЛЬНОСТИ ДЛЯ ОПТИМИЗАЦИИ СОСТАВА И ТЕХНОЛОГИИ ТАБЛЕТОК, СОДЕРЖАЩИХ N-БЕНЗИЛ-N-МЕТИЛ-1-ФЕНИЛПИРРОЛО [1,2-А] ПИРАЗИН-3-КАРБОКСАМИД

С.В. Тишков, Е.В. Блынская, К.В. Алексеев, В.К. Алексеев, Д.И. Гаврилов

Федеральное государственное бюджетное научное учреждение
«Научно-исследовательский институт фармакологии имени В.В. Закусова»
125315, Россия, Москва, ул. Балтийская, 8

E-mail: sergey-tishkov@yandex.ru

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Создание лекарственных средств (ЛС) с анксиолитической активностью, которые не обладают основными побочными эффектами, характерными для лекарственных препаратов (ЛП) данной группы, является важной и социально значимой задачей. Для её выполнения в рамках разработки оригинального ЛС с анксиолитической активностью проводится разработка состава и технологии таблеток ГМЛ-1 (N-бензил-N-метил-1-фенилпирроло [1,2-а] пиразин-3-карбоксамид).

Цель. Изучение с помощью четырёхфакторного дисперсионного анализа влияния факторов состава на технологические свойства таблеток ГМЛ-1 и подборе типа, количества, стадии добавления дезинтегранта и смазывающего вспомогательного вещества (ВВ).

Материалы и методы. Используемые материалы: субстанция: ГМЛ-1 (N-бензил-N-метил-1-фенилпирроло[1,2-а] пиразин-3-карбоксамид). Вспомогательные вещества: микрокристаллическая целлюлоза 101 (МКЦ), поливинилпирролидон (КВП), кросповидон, натрия кроскармелоза (НКК), натрия крахмала гликолят (НКГ), магния стеарат (МС), натрия стеарил фумарат (НСФ). Применялось получение таблеточных смесей с помощью влажной грануляции и таблетирование с изучением их основных фармацевтико-технологических свойств.

Результаты. Разработаны модельные составы и изучены их фармацевтико-технологические свойства. Данные результаты проанализированы, определена степень влияния факторов и их взаимодействия. Взаимодействия факторов в большинстве рассматриваемых случаев не вызывали существенное изменение критериев оптимизации. Время распадаемости при увеличении количества дезинтегранта сокращалось неравномерно. Так, увеличение количества данных ВВ с 4 до 6 мг оказывало более сильное влияние, чем с 2 до 4 мг. На степень высвобождения фактор В воздействовал нелинейно. Фактор А влиял на все рассматриваемые критерии оптимизации, особенно на высвобождение ФС. Наилучшее высвобождение и распадаемость наблюдались при использовании кросповидона, что имело особенное значение при обработке результатов испытаний методом обобщённой желательности.

Заключение. Ввиду противоречивых результатов дисперсионного анализа, для частных факторов, получившиеся значения дополнительно проанализированы с помощью обобщённой функции желательности. Использование данного метода позволило привести противоречивые результаты дисперсионного анализа к одному наиболее оптимальному составу.

Ключевые слова: ГМЛ-1; таблетка; дисперсионный анализ; четырёхфакторный; влияние факторов; взаимодействие факторов; функция желательности

Список сокращений: ЛС – лекарственное средство; ЛП – лекарственный препарат; ЛФ – лекарственная форма; ФС – фармацевтическая субстанция; ВВ – вспомогательные вещества; ПВП – поливинилпирролидон; МКЦ – микрокристаллическая целлюлоза; НКК – натрия кроскармелоза; НКГ – натрия крахмала гликолят; МС – магния стеарат, НСФ – натрия стеарил фумарат; ОФС – общая фармакопейная статья.

INTRODUCTION

Currently, the search for new drugs for the treatment of neurotic disorders and other neuropsychiatric diseases is becoming an increasingly urgent task. For example, the global prevalence of anxiety disorders, according to various sources, ranges from 6.0 to 13.6% [1]. In addition, the use of many tranquilizers, in particular the benzodiazepine series, is limited due to the manifestation of a large number of side effects and legal restrictions. Accordingly, one of the most promising areas of psychopharmacology is the creation of drugs based on the structure of mitochondrial translocator protein ligands acting on alternative pharmacological targets without serious side effects and toxicity.

In the Research Institute of Pharmacology named after V.V. Zakusov, an original active pharmaceutical ingredient (API), which is a derivative of pyrrolopyrazine – N-benzyl-N-methyl-1-phenylpyrrolo [1,2-a] pyra-

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zine-3-carboxamide (GML-1) [2, 3], having an anxiolytic activity, was developed and synthesized (Fig. 1) [2]. API has an anxiolytic activity; pronounced antidepressant, nootropic and neuroprotective effects have also been revealed [4–6], while there are no sedative, muscle relaxant and amnestic effects characteristic of this group of drugs [7]. In addition, as a result of toxicological studies, GML-1 has shown a low acute toxicity when administered intraperitoneally to mice (LD_{50} > 1000 mg/kg) [7]. The data obtained demonstrate a high potential of this API for the creation of the drug.

For GML-1, it is planned to develop a tableted dosage form (DF), based on the carried out preclinical studies and on the characteristics of the proposed pharmacological application [9, 10].

THE AIM of this work is to study, using an analysis of variance, the effect of the type and amount of the disintegrant on the technological properties of GML-1 tablets, as well as the lubricating excipient type and the stage of incorporating the disintegrant into the tablet mass on the technological properties of GML-1 tablets.

In the presented study, using the analysis of variance and desirability function, it is necessary to select the composition and technology of tableted LF GML-1 obtained by wet granulation.

MATERIALS AND METHODS

The used materials

The substance – GML-1 (N-benzyl-N-methyl-1-phenylpyrrolo [1,2-a] pyrazine-3-carboxamide)) (Fig. 1).

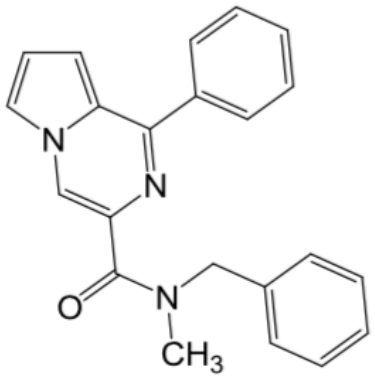

Figure 1 – Structural formula of GML-1

Excipients – microcrystalline cellulose 101 (MCC 101) (Microcel MCC 101, Blanver, Brazil); polyvinylpyrrolidone (PVP, Kollidon 25, BASF, Germany); crospovidone (Polyplasdone XL, BASF), croscarmellose sodium (CCS) (Solutab, Blanver, Brazil), sodium starch glycolate (SSG) (Solutab, Blanver, Brazil); magnesium stearate (MS) (Niticka Pharm. Specialties PVT. LTD.), sodium stearyl fumarate (SSF) (Pruv, JRS Pharma, Gerany).

Equipment and techniques used

To preparation the tablets, a manual hydraulic press PRG-50 was used. A resistance of tablets to crushing (General Pharmacopoeia Monograph (GPM). 1.4.1.0015.15, SP XIV, volume 2)[1] was tested with a resistance analyzer TBF 1000 CopleyScientific® (Great Britain).

The methods for disintegration determining (GPM.1.4.2.0011.15., SP XIV, volume 2)[2] is PTZ-S disintegration tester (Pharma Test, Germany). The test method of "dissolution" for GML-1 tablets, 1 mg, was developed by the analytical group of the Research Institute of Pharmacology named after V.V. Zakusov [11, 12] according to GPM.1.4.2.0014.15 "Dissolution for solid dosage forms"[3]. Herewith, the used device was "Paddle stirrer" type (Erweka, Germany); the dissolution medium was 900 ml of 3% sodium lauryl sulfate solution in water, the dissolution medium temperature was 37 ± 1°C, the stirrer rotation speed was 50 rpm. The samples were taken every 10 minutes. After taking each sample, the medium was replenished. The optical density of the prepared solutions was measured on a spectrophotometer at the wavelength of 256 ± 2 nm in a cuvette with a layer thickness of 10 mm, using a 3% aqueous solution of sodium lauryl sulfate as a reference solution [13].

Statistical analysis

The analysis of variance (ANOVA) is used to determine the degree of influence of factors and their interactions on the technological and physicochemical properties of tablets [14–16]. In the presented work, a cross-balanced full analysis of variance (parametric model) was used to determine the effect of: A – the type of disintegrating excipients; B – the amount of disintegrating excipients in the tablet; C – the type of lubricating excipients; D – the process of introducing a disintegrant into the tablet mass and a combination of these factors (parameters), a resistance of tablets to crushing, disintegration of tablets (c), API release (%).

The S, R and adjusted R-values reflect the correspondence in the mathematical model of the dependence of the random value on the values for the ANOVA model shown in Table 7. The S-value is measured in the units of the response variable and is the standard deviation for the data used. R (R^2) is the coefficient of the determination describing the degree of dependence of the variable explained by the factors of the process under consideration. To compare models with different numbers of variables, the value of the corrected coefficient of determination (adjusted R^2) which cannot be artificially overestimated and takes into account the number of terms in the model, is introduced. [17].

[1] State Pharmacopoeia of the Russian Federation XIV ed. T. I–IV. Available from: http://femb.ru/pharmacopea.php.
[2] Ibid.
[3] Ibid.

The factorial design of the experiment consisted of combinations of factors to describe the degree of influence of the composition, was carried out in a randomized order and to reduce the experimental error, the experiment at the center point was repeated five times on different days. The results of the average responses for the experiments are shown in Table 3. The values indicated the reproducibility of the process. A statistical evaluation of the results was performed by the analysis of variance (ANOVA) using a commercially available statistical software package (Minitab 18, PA, USA). Fisher's test was used to compare the variances of variational series, and the degree of confluence of factors was determined by its relative value of Fisher's tabular value. In addition, for the mathematical analysis of the results, the generalized desirability function was used, which makes it possible to determine the most optimal model composition. During the optimization of the composition, it is necessary to combine the partial responses of technological, physicochemical properties in order to obtain a tablet with the desired characteristics. The use of the desirability function allows this process to be carried out in one dimension and makes it possible to determine the most suitable composition for all desirability criteria.

The combination of responses in a generalized desirability function requires the computation of individual desirability functions [18, 19], which can have one-way and two-way constraints. Within the framework of this study, only one-way constraints will be considered, since the optimization parameters used have only upper and, accordingly, lower permissible values. To transform the selected partial optimization parameters into some subjective estimate or partial desirability, it is necessary to use the following equations with a one-way constraint:

$$d = \exp\left[-\exp(-y)\right], \quad (1)$$

The conversion of the values of dimensional (natural) indicators (pharmaceutical and technological characteristics) (x) into dimensionless (y) indicators, under the accepted condition of a linear relationship between them, is carried out as follows: $y = a_0 + a_1x_1$ and this expression can be calculated using the following system of equations:

$$\begin{cases} a_0 + k_1a_1 = 1,51 \\ a_0 + k_2a_2 = 0,01 \end{cases}, \quad (2)$$

where: k_1 is the best parameter value, k_2 is the worst parameter value.

The value of Harrington's generalized desirability is calculated by converting particular desirability indicators (D) into a single comprehensive assessment using the formula:

$$D = \sqrt[n]{\prod_{u=1}^{n} d_u}, \quad (3)$$

where: n is the number of used indicators of comparison parameters in this system

When recalculating according to this formula, the weight coefficients of particular indicators are not taken into account. These indicators are combined into a generalized Harrington desirability function (D) by determining the geometric mean of particular desirability (d_u). [20–23].

RESULTS

At the previous stages of the research, the properties of the API GML-1 were studied, the technology of the GML-1 tablets, wet granulation, was selected. This choice is due to the need to ensure the dosage uniformity for 1 mg of API, which has unsatisfactory physicochemical and technological properties. In addition, a filler, a binder and the optimal amounts of these excipients have been selected. The preliminary stages of optimization of the technological process have been carried out [13]. However, due to the unsatisfactory technological properties of GML-1 tablets, especially in terms of such indicators as disintegration and the API release from the tablets, it was decided to additionally introduce disintegrants.

To implement this research plan, at the next stage, the type and amount of disintegrant, as well as the stage of the introduction of disintegrating excipients and the type of lubricating excipient were selected.

To ensure the necessary technological properties, a four-factor fractional experiment was carried out and the following factors were identified as the factors affecting the quality of the tablets:

A – the type of disintegrant: A_1 – crospovidone, A_2 – sodium croscarmellose; A_3 – sodium starch glycolate;

B – the amount of disintegrant in the tablet: B_1 – 2 mg, B_2 – 4 mg, B_3 – 6 mg;

C – the type of lubricating excipient: C_1 – 8%, C_2 – 10%;

D – the process of adding disintegrant: D_1 – into the tablet mixture before moistening, D_2 – half of the amount of disintegrant into the tablet mixture and the rest at the stage of dusting.

The factors are investigated at three or two levels of change. The range of variation of the selected variable factors is shown in Table 1.

The following criteria were chosen as optimization ones: Y_1 – Resistance of tablets to crushing (N); Y_2 – Disintegration of tablets (s); Y_3 – API release (%).

The compositions of the model mixtures and the results of evaluating the indicators of the tablets are presented in Tables 2 and 3.

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Table 1 – Characteristics of variable factors affecting the technological characteristics of GML–1 tablets

Factor levels	Factors			
	A	B	C	D
	Disintegrant type	The amount of disintegrant in a tablet, mg	Type of lubricating excipient	Disintegrant addition process
1	Crospovidone	2	Magnesium stearate	Into tablet mix before moisturizing
2	Sodium crosscarmellose	4	Sodium stearate fumarate	50% before moisturizing and 50% during dusting
3	Sodium starch glycolate	6	–	–

Table 2 – Model compositions of GML–1 tablets, mg

No.	GML–1	MCC 101	PVP	Disintegrants			Lubricating excipients	
				Crospovidone	NCC	SSG	MS	SSF
1	1.0	90.0	6.0	2.0	–	–	1.0	–
2	1.0	90.0	6.0	2.0	–	–	–	1.0
3*	1.0	90.0	6.0	2.0	–	–	1.0	–
4*	1.0	90.0	6.0	2.0	–	–	–	1.0
5	1.0	88.0	6.0	4.0	–	–	1.0	–
6	1.0	88.0	6.0	4.0	–	–	–	1.0
7*	1.0	88.0	6.0	4.0	–	–	1.0	–
8*	1.0	88.0	6.0	4.0	–	–	–	1.0
9	1.0	88.0	6.0	6.0	–	–	1.0	–
10	1.0	88.0	6.0	6.0	–	–	–	1.0
11*	1.0	88.0	6.0	6.0	–	–	1.0	–
12*	1.0	88.0	6.0	6.0	–	–	–	1.0
13	1.0	90.0	6.0	–	2.0	–	1.0	–
14	1.0	90.0	6.0	–	2.0	–	–	1.0
15*	1.0	90.0	6.0	–	2.0	–	1.0	–
16*	1.0	90.0	6.0	–	2.0	–	–	1.0
17	1.0	89.0	6.0	–	4.0	–	1.0	–
18	1.0	88.0	6.0	–	4.0	–	–	1.0
19*	1.0	88.0	6.0	–	4.0	–	1.0	–
20*	1.0	88.0	6.0	–	4.0	–	–	1.0
21	1.0	88.0	6.0	–	6.0	–	1.0	–
22	1.0	88.0	6.0	–	6.0	–	–	1.0
23*	1.0	88.0	6.0	–	6.0	–	1.0	–
24*	1.0	88.0	6.0	–	6.0	–	–	1.0
25	1.0	90.0	6.0	–	–	2.0	1.0	–
26	1.0	90.0	6.0	–	–	2.0	–	1.0
27*	1.0	90.0	6.0	–	–	2.0	1.0	–
28*	1.0	90.0	6.0	–	–	2.0	–	1.0
29	1.0	88.0	6.0	–	–	4.0	1.0	–
30	1.0	88.0	6.0	–	–	4.0	–	1.0
31*	1.0	88.0	6.0	–	–	4.0	1.0	–
32*	1.0	88.0	6.0	–	–	4.0	–	1.0
33	1.0	88.0	6.0	–	–	6.0	1.0	–
34	1.0	88.0	6.0	–	–	6.0	–	1.0
35*	1.0	88.0	6.0	–	–	6.0	1.0	–
36*	1.0	88.0	6.0	–	–	6.0	–	1.0

Note: * – adding disintegrant to the tablet mixture and when dusting the granulate.

Table 3 – Research results of technological characteristics of tablet mixtures and tablets (average values)

Formulation number	Y_1	Y_2	Y_3
	Resistance to crushing (N)	Disintegration time (s)	API release (%)
1	108.1±0.03	268±0.3	78.8±1.0
2	97.4±0.02	244±0.2	79.3±0.5
3	95.8±0.02	231±0.2	77.6±0.6
4	91.4±0.03	227±0.1	78.9±0.4
5	109.3±0.05	212±0.4	89.1±0.5
6	89.9±0.04	190±0.2	87.8±0.3
7	88.7±0.04	196±0.1	81.7±0.3
8	80.1±0.02	189±0.1	83.6±0.8
9	95.4±0.03	170±0.1	83.1±1.0
10	96.1 ±0.06	165±0.2	85.3±0.5
11	75.9±0.02	157±0.5	80.2±0.4
12	78.7±0.03	159±0.2	85.1±0.2
13	117.3±0.03	249±0.5	84.6±0.3
14	114.6±0.01	243±0.4	83.1±0.2
15	106,2±0.02	239±0.6	80.4±0.1
16	107.9±0.02	238±0.5	79.6±0.4
17	105.9±0.03	351±0.6	71.5±0.2
18	104.4±0.03	349±0.3	71.3±0.3
19	93.1±0.04	230±0.5	72.7±0.4
20	90.0±0.03	224±0.4	72.8±0.5
21	99.8±0.03	210±0.2	77.2±0.2
22	102.5±0.04	213±0.2	79.4±0.3
23	88.9±0.02	201±0.1	77.6±0.6
24	85.5±0.03	200±0.2	78.1±0.5
25	127.7±0.04	378±0.5	81.4±0.3
26	115.6±0.03	367±0.2	80.9±0.2
27	100.5±0.05	360±0.6	76.4±0.3
28	99.7±0.02	355±0.5	73.2±0.6
29	101.9±0.03	351±0.4	71.5±0.3
30	101.1±0.04	349±0.4	71.3±0.3
31	99.4±0.06	233±0.2	70.7±0.5
32	98.9±0.08	232±0.6	70.9±0.6
33	115.2±0.05	212±0.6	69.7±0.4
34	108.1±0.02	224±0.5	69.5±0.4
35	89.4±0.03	215±1.0	68.3±0.3
36	85.5±0.04	214±0.9	67.8±0.4

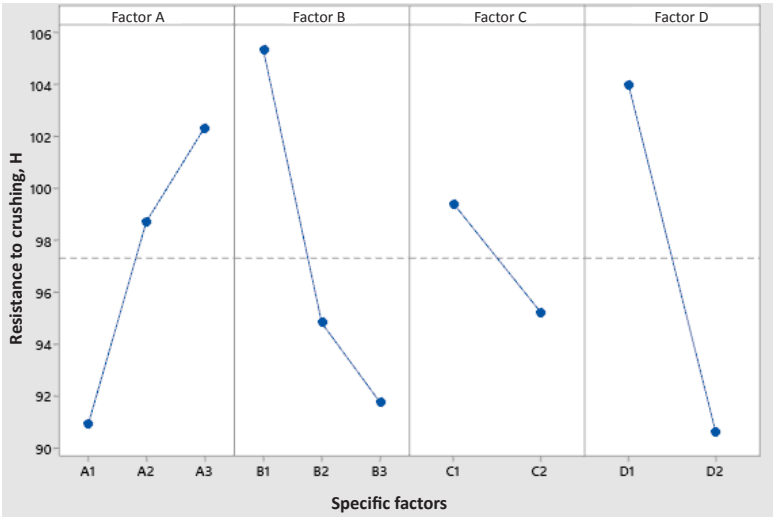

Figure 2 – Graph of the influence of the main factors effects on the average values of tablets GML-1 resistance to crushing

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Table 4 – Analysis of variance for the resistance to crushing of GML–1 tablets

Source of dispersion	Degrees of freedom, number	Sum of Squares (SS)	Average square (AS)	F_{exp}	$F_{tab.}$
Factor A	2	2465.7	1232.86	81.02	3.14
Factor B	2	3686.2	1843.11	121.12	3.14
Factor C	1	474.0	474.05	31.15	3.99
Factor D	1	4847.4	4847.39	318.55	3.99
Factor A* Factor B	4	128.3	32.07	2.11	2.52
Factor A * Factor C	2	53.2	26.60	1.75	3.14
Factor A * Factor D	2	109.6	54.81	3.60	3.14
Factor B * Factor C	2	106.3	53.13	3.49	3.14
Factor B * Factor D	2	377.7	188.85	12.41	3.14
Factor C * Factor D	1	10.9	10.89	0.72	3.99
Factor A * Factor B * Factor C	4	236.1	59.03	3.88	2.52
Factor A * Factor C * Factor D	2	79.7	39.86	2.62	3.14
Factor A * Factor B * Factor D	4	768.3	192.09	12.62	3.14
Factor B * Factor C * Factor D	2	85.0	42.49	2.79	
Within cells	76	1156.5	15.22	–	–
Total	107	14585.0	–	–	–

Table 5 – Analysis of variance for the disintegration of GML-1 tablets

Source of dispersion	Degrees of freedom, number	Sum of Squares (SS)	Average Square (AS)	F_{exp}.	$F_{tab.}$
Factor A	2	140526	70262.9	183.07	3.14
Factor B	2	159138	79568.8	207.32	3.14
Factor C	1	1836	1836.2	4.78	3.99
Factor D	1	36834	36834.4	95.97	3.99
Factor A* Factor B	4	61568	15392.0	40.10	2.52
Factor A * Factor C	2	604	302.2	0.79	3.14
Factor A * Factor D	2	6426	3212.9	8.37	3.14
Factor B * Factor C	2	175	87.5	0.23	3.14
Factor B * Factor D	2	22852	11425.9	29.77	3.14
Factor C * Factor D	1	11	10.6	0.03	3.99
Factor A * Factor B * Factor C	4	726	181.6	0.47	2.52
Factor A * Factor C * Factor D	2	970	485.0	1.26	3.14
Factor A * Factor B * Factor D	–	–	–	–	–
Factor B * Factor C * Factor D	2	814	407.2	1.06	3.14
Within cells	80	30704	383.8	–	–
Total	107	463184	–	–	–

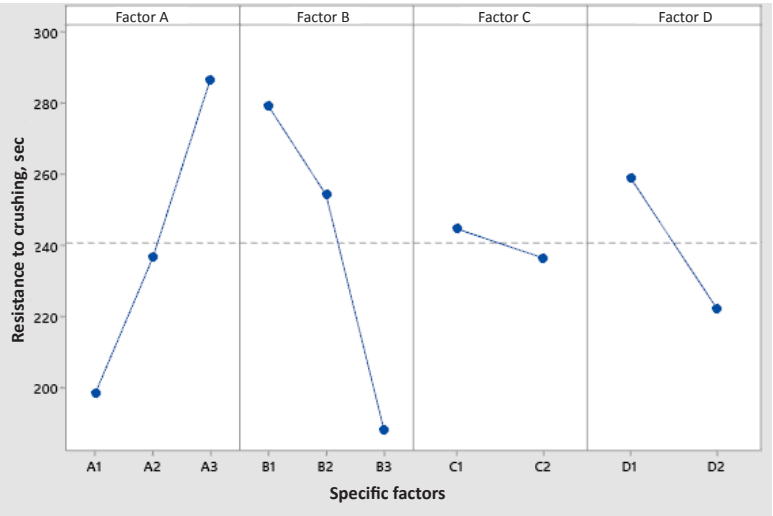

Figure 3 – Graph of the factors influence on the average values of GML-1 tablets disintegration

Table 6 – Results analysis of variance of the dissolution test of GML-1 tablets

Source of dispersion	Degrees of freedom, number	Sum of Squares (SS)	Average Square (AS)	$F_{exp.}$	$F_{tab.}$
Factor A	2	1896.08	948.041	131.44	3.14
Factor B	2	263.74	131.872	18.28	3.14
Factor C	1	0.91	0.914	0.13	3.99
Factor D	1	76.71	76.713	10.64	3.99
Factor A* Factor B	4	1145.10	286.275	39.69	2.52
Factor A * Factor C	2	14.10	7.051	0.98	3.14
Factor A * Factor D	2	25.68	12.839	1.78	3.14
Factor B * Factor C	2	21.77	10.886	1.51	3.14
Factor B * Factor D	2	18.95	9.475	1.31	3.14
Factor C * Factor D	1	1.02	1.015	0.14	3.99
Factor A * Factor B * Factor C	4	20.35	5.087	0.71	2.52
Factor A * Factor C * Factor D	–	–	–	–	–
Factor A * Factor B * Factor D	2	20.59	10.294	1.43	3.14
Factor B * Factor C * Factor D	2	1.44	0.720	0.10	3.14
Within cells	80	577.04	7.213	–	–
Total	107	4083.48	–	–	–

Table 7 – Standard deviations and coefficients of variable indicators determination in the model of GML-1tablets

Manufacturing characteristics	S	R^2	R^2 (rate)
Resistance to crushing (N)	3.90089	92.07%	88.84%
Disintegration time (s)	13.3661	97.07%	95.87%
API release (%)	2.49791	88.39%	83.65%

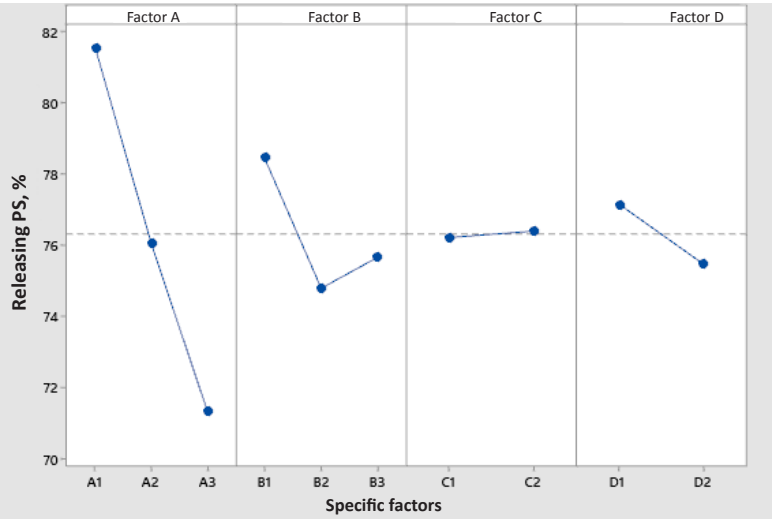

Figure 4 – Graph of the main influence effects of particular factors on the average kinetics values of the GML-1 tablets dissolution

The test results were subjected to the analysis-of-variance method to obtain Fisher's F-test for each term in the model. The experimental values of Fisher's F-test were compared with the tabular value of the F-test, which is described for the significance level α = 0.05, the degrees of freedom for each factor. The shown comparison reveals the degree of influence of each factor on the optimization criteria for model tablets GML-1 (α = 0.05; $F_{exp} > F_{tab.}$), as well as the interactions of factors (Tables 4–8) [24]. The obtained data were additionally compared with the average values of particular factors to explain the obtained regularities.

When processing the analysis of variance results on the resistance to crushing values of GML-1 tablets (Table 4), a significant exceedance of the experimental F-criterion values above the theoretical $F_{80,2,0,95}$ in factors A and B, $F_{80,1,0,95}$, in factors C and D, as well as a relative exceedance in the interaction of factors B and D was observed.

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Table 8 – Values of particular and generalized desirability parameters

Sequential number	Y_1	Y_2	Y_3	d_1	d_2	d_3	D
1	108.1±0.03	268±0.3	78.8±1.0	0.677	0.625	0.634	0.645
2	97.4±0.02	244±0.2	79.3±0.5	0.588	0.671	0.644	0.633
3	95.8±0.02	231±0.2	77.6±0.6	0.573	0.694	0.609	0.623
4	91.4±0.03	227±0.1	78.9±0.4	0.532	0.701	0.636	0.619
5	**109.3±0.05**	**212±0.4**	**89.1±0.5**	**0.686**	**0.726**	**0.802**	**0.736**
6	89.9±0.04	190±0.2	87.8±0.3	0.517	0.759	0.785	0.675
7	88.7±0.04	196±0.1	81.7±0.3	0.505	0.750	0.689	0.639
8	80.1±0.02	189±0.1	83.6±0.8	0.416	0.760	0.722	0.611
9	95.4±0.03	170±0.1	83.1±1.0	0.570	0.786	0.714	0.684
10	96.1 ±0.06	165±0.2	85.3±0.5	0.576	0.792	0.749	0.699
11	75.9±0.02	157±0.5	80.2±0.4	0.372	0.802	0.661	0.582
12	78.7±0.03	159±0.2	85.1±0.2	0.401	0.799	0.746	0.621
13	117.3±0.03	249±0.5	84.6±0.3	0.742	0.662	0.738	0.713
14	114.6±0.01	243±0.4	83.1±0.2	0.724	0.673	0.714	0.703
15	106.2±0.02	239±0.6	80.4±0.1	0.663	0.680	0.665	0.669
16	107.9±0.02	238±0.5	79.6±0.4	0.676	0.682	0.650	0.669
17	105.9±0.03	351±0.6	71.5±0.2	0.660	0.439	0.466	0.513
18	104.4±0.03	349±0.3	71.3±0.3	0.648	0.443	0.461	0.510
19	93.1±0.04	230±0.5	72.7±0.4	0.548	0.696	0.496	0.574
20	90.0±0.03	224±0.4	72.8±0.5	0.518	0.706	0.498	0.567
21	99.8±0.03	210±0.2	77.2±0.2	0.609	0.729	0.600	0.643
22	102.5±0.04	213±0.2	79.4±0.3	0.632	0.724	0.646	0.666
23	88.9±0.02	201±0.1	77.6±0.6	0.507	0.742	0.609	0.612
24	85.5±0.03	200±0.2	78.1±0.5	0.472	0.744	0.619	0.602
25	127.7±0.04	378±0.5	81.4±0.3	0.802	0.372	0.684	0.588
26	115.6±0.03	367±0.2	80.9±0.2	0.731	0.399	0.675	0.582
27	100.5±0.05	360±0.6	76.4±0.3	0.615	0.416	0.583	0.530
28	99.7±0.02	355±0.5	73.2±0.6	0.608	0.429	0.508	0.510
29	101.9±0.03	351±0.4	71.5±0.3	0.627	0.439	0.466	0.504
30	101.1±0.04	349±0.4	71.3±0.3	0.620	0.443	0.461	0.503
31	99.4±0.06	233±0.2	70.7±0.5	0.606	0.691	0.446	0.571
32	98.9±0.08	232±0.6	70.9±0.6	0.601	0.692	0.451	0.573
33	115.2±0.05	212±0.6	69.7±0.4	0.728	0.726	0.421	0.606
34	108.1±0.02	224±0.5	69.5±0.4	0.677	0.706	0.415	0.584
35	89.4±0.03	215±1.0	68.3±0.3	0.512	0.721	0.385	0.522
36	85.5±0.04	214±0.9	67.8±0.4	0.472	0.722	0.372	0.502

Table 9 – GML-1 tablets composition, 1 mg, according to the results of research and the mathematical analysis methods

Composition	Quantity, g
GML-1	0.001
MCC 101	0.088
Kollidon 25	0.006
Crospovidone	0.004
Magnesium stearate	0.001
Tablet weight	0.100

Accordingly, all factors of the presented analysis of variance and the interaction of factors B and D influenced the resistance to crushing of the GML-1 tablets.

The stage of adding disintegrant to the tablet mass had the greatest influence on the resistance to crushing index. Fig. 2 can explain this phenomenon by a decrease in the binding capacity for the tablet mass during the compression when the disintegrant is between the granules. The second largest impact was the amount of disintegrant, as well as its type, which is explained by a change in the processes of brittle and plastic deformation with changes in A and B factors. The least effect was exerted by the type of a lubricating excipient, due to its low amount in the tablet mass. Among the interactions of the factors, the interaction between the amount and the stage of adding a disintegrant stands out, since these factors are indirectly interrelated, but their influence is relatively insignificant. The distribution of the average

values of the resistance to crushing of the GML-1 tablets by particular factors is shown in Fig. 2.

The graphs in Fig. 2 make it possible for us to conclude that the lowest resistance of tablets to crushing is when the disintegrant crospovidone is used, and the highest resistance is for the compositions with sodium starch glycolate. There was also an uneven decrease in resistance with an increase in the amount of a disintegrant, as well as a lower resistance takes place for formulations containing sodium stearate fumarate and a disintegrant in the granule dust.

Factors A and B, as well as factor D, had a significant effect on the disintegration rate, as it was expected. The most significant effect was produced by the amount of a disintegrant, and the next was the type of disintegrant and the process of introducing this disintegrant into the tablet mass.

These effects can be explained by the functional purpose of this group of substances. The disintegration time was also affected by interactions between the type, amount and process of adding disintegrant at the stage of dusting, since the total amount of disintegrant affects the amount of disintegrant inside the granules and in the dusting, respectively, exacerbating the influence of this factor. Factor C had the least effect on the disintegration time due to relatively low amounts of lubricating excipients in GML-1 tablets.

Perhaps, partially due to the decrease in the tablet resistance, formulations with crospovidone (Fig. 3) showed shorter disintegration times, and formulations with sodium starch glycolate – longer. As expected, with an increase in the amount of a disintegrant, the disintegration time decreased (Fig. 3), the difference between the compositions with 4 and 6 mg of a disintegrant is much greater than the difference between 2 and 4 mg. The separation of the disintegrant and its addition at different stages of the technological process, on average, can reduce the disintegration time by 40 s. Despite a small effect of the type of lubricating excipients, the inclusion of stearate fumarate in the composition of sodium makes it possible to reduce the disintegration time due to the hydrophilic groups in the composition of the excipients.

The study of the factors determining the degree of the GML-1 release in the dissolution test showed (Table 6) that a type of disintegrant affects the optimization parameter much stronger than other factors due to the different nature of the polymers, which, in addition to the disintegrating effect, may have a solubilizing effect. The effect on the GML-1 release manifested by the interaction of the type and amount of desitegrants, is twice as weak. The next factors influencing the GML-1 release, are exerted by factor B (the amount of disintegrant) and factor D (the process of introducing a disintegrant into the composition of the tablet).

The influence of particular factors on the resistance of GML-1 tablets to crushing is reflected in the graphs of average release values (Fig. 4) from which it can be concluded that the API release from GML-1 tablets is the best when crospovidone is used. The worst results were observed with the use of sodium starch glycolate, with the quantitative content of disintegrant 4 mg and the addition of half of the disintegrant at the stage of dusting.

Table 7 shows the values of the determination coefficients adjusted coefficients of determination, which illustrate the relationship between the factors considered in this model and the parameters responses of the analysis of variance optimization [23].

Based on the coefficients of determination for the mathematical model shown in Table 9, a conclusion can be made about the applicability of the presented model and a high degree of connectivity of the considered factors with the optimization criteria. This conclusion is based on the high values of the determination (R^2) coefficient from 88.39 to 97.07% and the adjusted coefficient of determination (rate R^2) from 83.65 to 95.87% for all considered manufacturing characteristics. The lowest R^2 values among other indicators were observed in the analysis of the API release, since the demonstrated indicator was influenced, to a greater extent, by random factors that were not included in this ANOVA model, e.g., the conditions of the dissolution test, the influence of other excipients, etc.

Due to the multidirectionality of the influence of particular factors of variance analysis and the varying degree of these factors' influence, the generalized desirability method was used to select one of the most rational model composition. To determine the value of the generalized desirability in accordance with paragraph 2.2.5. "Materials and Methods" were transformed into dimensionless quantities considered in Table 2, response values (Table 3): resistance of tablets to crushing (N), disintegration (s), the API release (%), The obtained response values (Y) according to these parameters were converted into partial desirability (d), the values of which were distributed on the desirability curve (Fig. 5) from 0 to 1, where 1 is the best value of the parameter, and 0 manifests absolutely unsatisfactory results. Then the particular desirability was transformed into a generalized one (D) by finding the geometric mean. The values of the optimization parameters, as well as the calculated partial and generalized desirability, are shown in Table 8.

Analyzing the data obtained and the of the particular and generalized desirability functions, the authors conclude that there are no absolutely unsatisfactory model compositions with D values less than 0.2 among the considered ones.

Model composition No. 5 has the value of the generalized desirability function (0.736) closest to 1 and, accordingly, is suitable for the totality of the studied parameters. In addition, the presented composition has the highest values of the API GML-1 release, which is a key optimization parameter under the conditions of a

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sparingly soluble substance. Based on the obtained results of the generalized desirability and analysis of variance, the following composition of model GML-1 tablets, 1 mg, was selected (Table 9).

DISCUSSION

As a result of the analysis of variance, a conclusion was made about the absence of one factor that most intensively affects all manufacturing characteristics. However, due to the low content of lubricating excipients in the tablets, their appearance had the least effect on the studied manufacturing characteristics, or in the case of the API release, it did not have a statistically significant result. The resistance of tablets to crushing largely determines the process of adding a disintegrant to the GML-1 tablet mass. The duration of disintegration is largely determined by the amount of a disintegrant, and the degree of the API release by the type of disintegrant. Among the particular factors of the dispersion analysis, crospovidone should be distinguished, which most intensively reduces the resistance of tablets to crushing, disintegration time and increases the degree of the API release. At the same time, the amount of disintegrant had a non-linear effect on the degree of release, for example, 4 mg slowed down the API release, and with 2 mg, the release was the most intensive. Besides, the addition of half of the disintegrant during the dusting step decreased the resistance of tablets to crushing, disintegration time, and the API release rate. In most cases, the interaction of factors did not have a statistically significant effect; however, there was a mutual influence of B and D factors on the resistance of tablets to crushing and on disintegration. The interaction of factors A and B had a statistically significant effect on the process of the API release. The use of the analysis of variance in this development did not allow us to identify the most optimal composition, however, a statistically significant relationship was established between the results obtained and the variable factors. In addition, the available data on the predominant influence of factors and the peculiarities of their interaction with pharmaceutical and manufacturing characteristics allows us to draw long-term conclusions for further developments. The selection of the most optimal factor is most conveniently carried out by other methods, for example, using the function of the generalized desirability based on the expert assessments of researchers. In this method, each model composition, regardless of the optimization factors, is considered separately and the combination of its pharmaceutical and technological characteristics determines its position on the desirability curve.

CONCLUSION

The methods of mathematical planning used in this work have shown their effectiveness in optimizing the composition and manufacturing process of adding a disintegrant to the composition of model tablets. The analysis of variance made it possible to identify the factors affecting the resistance of tablets to crushing, disintegration and the API release from GML-1 tablets. It is shown that the main number of interactions of factors did not cause a significant change in the considered optimization criteria. In addition, the consideration of the influence of each factor led to conflicting results and did not allow us to identify the most optimal composition.

The use of the generalized desirability method made it possible to reduce the conflicting results of the analysis of variance to one, the most optimal composition. As a result of using the methods of mathematical analysis, composition No. 5 was selected: it has the most optimal composition and technology for preparing GML-1 tablets and meets all manufacturing requirements.

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CONFLICT OF INTEREST

The autors declare no conflict of interest/

AUTHORS CONTRIBUTION

Sergey V. Tishkov – obtaining the research material. writing the text of the manuscript; Evgeniya V. Blynskaya – development of the research design. generalization of the research material; Konstantin V. Alekseev – development of the research design. analysis of the data obtained; Viktor K. Alekseev – the review of publications on the topic. the material analysis; Dmitry I. Gavrilov – review of publications on the topic, analysis of the material, conducting an experiment.

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AUTHORS

Sergey V. Tishkov – Candidate of Sciences (Pharmacy), Senior Researcher of the Laboratory of Finished Dosage Forms, Research Institute of Pharmacology n.a. V.V. Zakusov. ORCID ID: 0000-0002-8321-6952. E-mail: sergey-tishkov@yandex.ru

Evgeniya V. Blynskaya – Doctor of Sciences (Pharmacy), Head of the Laboratory of Finished Dosage Forms, Research Institute of Pharmacology n.a. V.V. Zakusov. ORCID ID: 0000-0002-9494-1332. E-mail: mrsaureussnape@yandex.ru

Konstantin V. Alekseev – Doctor of Sciences (Pharmacy), Professor, Chief Researcher of the Laboratory of Finished Dosage Forms, Research Institute of Pharmacology n.a. V.V. Zakusov. ORCID ID: 0000-0003-3506-9051. E-mail: convieck@yandex.ru

Viktor K. Alekseev – Junior Researcher of the Laboratory of Finished Dosage Forms, Research Institute of Pharmacology n.a. V.V. Zakusov. ORCID ID: 0000-0003-3542-0024. E-mail: conwieck@yandex.ru

Dmitry I. Gavrilov – Junior Researcher of the Laboratory of Finished Dosage Forms, Research Institute of Pharmacology n.a. V.V. Zakusov. ORCID ID: 0000-0001-8821-4174. E-mail: dimagavrilov@list.ru

RESEARCH ARTICLE

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PHARMACOLOGICAL ACTIVITY OF EXTRACTS FROM PLANTS OF *COSMOS BIPINNATUS CAV.* SPECIES

E.O. Kulichenko, O.A. Andreeva, E.O. Sergeeva, S.S. Sigareva, A.Yu. Terekhov, E.T. Oganesyan, S.Yu. Sidorskaya

Pyatigorsk Medical and Pharmaceutical Institute – a branch of Volgograd State Medical University
11, Kalinin Ave., Pyatigorsk, Russia, 357532

E-mail: evgenia.kuli4encko@yandex.ru

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The aim of the study is to determine antioxidant, anti-inflammatory and hypolipidemic activities of the extracts from *Cosmos bipinnatus Cav.* three varieties, obtained by the extraction with 70% ethyl alcohol.

Materials and methods. The antioxidant effect was studied *in vitro* using a model of iron-induced lipid peroxidation in the lecithin liposome system. The study of the anti-inflammatory activity was carried out on 30 male rats of the "Wistar" line. Diclofenac was used as a reference drug (p. o., the dose of 13 mg/kg in terms of the weight of an adult). The substances were administered to animals in the form of an aqueous suspension stabilized with Tween-80. To simulate the inflammatory process, a model of "cotton granuloma" was created. A hypolipidemic activity of the extracts was studied on 36 white male rats of the "Wistar" line. To study the hypolipidemic activity of the studied extracts, a tween model was used to create a hyperlipidemic state in rats, the concentrations of total cholesterol and triglycerides was determined in the serum of the experimental animals.

Results. The conducted model experiments made it possible to conclude that the alcohol extracts obtained from the dried inflorescences of the *"Dazzler", "Rosea"* and "*Purity"* varieties of *Cosmos bipinnatus Cav.* have antioxidant, anti-inflammatory and hypolipidemic kinds of activities. It has been established that the extracts from the *"Dazzler"* and *"Rosea"* varieties (*Cosmos bipinnatus Cav.)* contribute to a better reduction in the accumulation of peroxide compounds, compared to the extract obtained from the *"Purity"* variety. The data analysis on the anti-inflammatory activity shows that all the studied objects significantly (p = 0.05) reduce the stage of exudation compared with the control group animals by 50% (the «*Purity»* variety), by 52% (the «*Rosea»* variety) and by 40% (the *"Dazzler*" variety).

An experiment on the study of a hypolipidemic activity in the control group of the animals revealed a significant, in relation to the values of the intact group, increase in the cholesterol level of the blood serum by 78%, and in the level of triglycerides (TGCs) – by 64%.

The administration of the extracts obtained from *Cosmos bipinnatus Cav. "Purity"*, *"Rosea"*, *"Dazzler"* varieties to the animals, led to a decrease in cholesterol in blood serum by 44%, 47%, 50%, and triglycerides by 52%, 52% and 57%, respectively. Both indicators reached the normal level and did not differ significantly from the values in healthy (intact) animals.

Conclusion. According to the conducted studies, it can be concluded that alcohol extracts obtained from *Cosmos bipinnatus Cav*., have pronounced antioxidant, anti-inflammatory and hypolipidemic kinds of effect.

Keywords: *Cosmos bipinnatus Cav*.; hypolipidemic activity; cholesterol; triacylglycerides; anti-inflammatory activity; antioxidant activity, TBA-active products

Abbreviations: DNA – deoxyribonucleic acid; DMSO – dimethyl sulfoxide; TGCs – triglycerides; TBA – thiobarbituric acid; LPO – lipid peroxidation.

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ФАРМАКОЛОГИЧЕСКАЯ АКТИВНОСТЬ ИЗВЛЕЧЕНИЙ РАСТЕНИЙ ВИДА *COSMOS BIPINNATUS CAV.*

Е.О. Куличенко, О.А. Андреева, Е.О. Сергеева, С.С. Сигарева, А.Ю. Терехов, Э.Т. Оганесян, С.Ю. Сидорская

Пятигорский медико-фармацевтический институт – филиал федерального государственного бюджетного образовательного учреждения высшего образования «Волгоградский государственный медицинский университет» Министерства здравоохранения Российской Федерации
375532, Россия, г. Пятигорск, пр-кт Калинина, 11

E-mail: evgenia.kuli4encko@yandex.ru

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Цель. Определить антиоксидантную, противовоспалительную и гиполипидемическую активности извлечений, полученных экстракцией спиртом этиловым 70% трех видов Космеи дваждыперистой.

Материалы и методы. Антиоксидантное действие изучалось *in vitro* с применением модели железо-индуцированного перекисного окисления липидов в системе лецитиновых липосом. Исследование противовоспалительной активности проводилось на 30-ти крысах-самцах линии «Wistar». В качестве препарата сравнения использовали диклофенак (перорально, доза 13 мг/кг в пересчете на массу взрослого человека). Вещества животным вводили в виде водной суспензии, стабилизированной Твин-80. Для моделирования воспалительного процесса создавали модель «ватной гранулемы». Гиполипидемическую активность исследуемых экстрактов исследовали на 36-ти белых крысах-самцах линии «Wistar». Для изучения гиполипидемической активности исследуемых извлечений применяли твиновую модель для создания гиперлипидемического состояния у крыс, в сыворотке подопытных животных определяли концентрацию общего холестерина и триглицеридов.

Результаты. Проведенные модельные эксперименты позволили сделать вывод о том, что спиртовые извлечения, полученные из высушенных соцветий космеи дваждыперистой сортов *«Dazzler»*, *«Rosea»* и *«Purity»* обладают антиоксидантной, противовоспалительной и гиполипидемической видами активности. Установлено, что извлечения из космеи дваждыперистой сортов *«Dazzler»* и *«Rosea»* способствуют лучшему снижению накопления перекисных соединений по сравнению с извлечением, полученным из сорта *«Purity»*. Анализ данных по противовоспалительной активности показывает, что все исследуемые объекты достоверно (p=0,05) снижают стадию экссудации по сравнению с контрольной группой животных на 50% (сорт «*Purity*»), 52% (сорт «*Rosea*») и 40% (сорт «*Dazzler*»).

Эксперимент по изучению гиполипидемической активности у контрольной группы животных выявил достоверное, по отношению к значениям интактной группы, увеличение уровня холестерола в сыворотке крови на 78%, а уровня триглицеридов (ТРГ) на 64%. Введение животным извлечений, полученных из космеи дваждыперистой сортов *«Purity», «Rosea», «Dazzler»*, привел к снижению содержания холестерола в сыворотке крови на 44%, 47%, 50%, а триглицеридов на 52%, 52% и 57%, соответственно. Оба показателя достигли уровня нормы и достоверно не отличались от значений у здоровых (интактных) животных.

Заключение. Согласно проведенным исследованиям можно сделать вывод, что спиртовые извлечения, полученные из космеи дваждыперистой, обладают выраженным антиоксидантным, противовоспалительным и гиполипидемическим действиями.

Ключевые слова: *Cosmos bipinnatus Cav.*; Космея дваждыперистая; гиполипидемическая активность; холестерин; триацилглицериды; противовоспалительная активность; антиоксидантная активность; ТБК-активные продукты

Список сокращений: ДНК – дезоксирибонуклеиновая кислота; ДМСО – диметилсульфоксид; ТРГ – триглицериды; ТБК – тиобарбитуровая кислота; ПОЛ – перекисное окисление липидов.

INTRODUCTION

The search for natural biologically active compounds is an urgent and time-consuming task. Synthetic drugs are pharmacologically effective, but being broad-spectrum drugs, they often have many serious side effects. Preparations based on natural compounds exhibit fewer undesirable properties and are more effective when taken prophylactically. The use of plants as a source of drugs is very promising from the point of view of creating total substances characterized by a low toxicity and intended for a long-term use in the treatment of various diseases [1–5].

The native range of *Cosmos bipinnatus Cav.* is Mexico; in Russia it is used as an ornamental crop (a plant up to 1.5 meters high). Depending on the variety, it can have white (“*Purity*” variety), pink (“*Rosea*” variety) or purple (“*Dazzler*” variety) flowers, blooms from late May till late autumn and is very resistant to sudden chang-

es in temperature, not susceptible to various infections (Fig. 1) [6].

In the folk medicine of South America, the plants of the genus *Cosmos Cav.* are used as an antimalarial agent [7]. Africans use them to treat headaches and indigestion, as well as against bed bugs and lice, which indicates *Cosmos Cav.* insecticidal properties. In oriental traditional medicine (Japan, China), *Cosmos bipinnatus Cav.* has been used as a substitute for lotus, as a tonic and invigorating agent [8].

In China and Mexico, in traditional medicine, this plant is used as a hepatoprotector and a remedy for headaches, in the treatment of jaundice, intermittent malarial fever, splenomegaly, and flatulence [9]. The use of plants of the genus *Cosmos* as anti-inflammatory and antifungal agents has also been noted; its effectiveness in the treatment of arthritis, stomach ulcers and diabetes mellitus has been described [10]. There is evidence of the ability of *Cosmos bipinnatus Cav.* hydroalcoholic extracts to act as an antioxidant and protect DNA from damage and oxidation. Various extracts from inflorescences of the genus *Cosmos* plants have cytotoxic effects against cell lines of gastric and colorectal adenocarcinoma, and have an antidiabetic activity *in vitro* [9].

The chemical composition of all parts of the plant is mainly represented by polyphenols and essential oil components. Thus, the presence of chalcones (butein, ocanine, lanceoletin) [11], phenolic acids (chlorogenic and caffeic acids), anthocyanins (cosmocyanin), flavonoids (cosmosiin, luteolinglucuronide, trifolin, isoquercitrin, nelyumboside) [12], tannins [13], essential oils: monoterpenes and sesquiterpenes (E)-β-Ocimene, germacrene D, sabinine, α-cadinol, α-farnesin and terpinen-4-ol, β-elemen, β-caryophyllene, germacrene D and bicyclogermacrene [8, 11] has been established.

The following triterpene alcohols have been found out in the tubular flowers of *Cosmos bipinnatus Cav.*: helianol, taraxerol, β-amirin, cycloartenol, α-amirin, lupeol, 24-methylenecycloartanol. The triterpene composition of the reed flowers of the plant is slightly different: helianol, dammaradienol, β-amirin, tirucalla-7,24-dienol, α-amirin, lupeol, 24-methylenecycloartanol, ψ-taraxasterol, taraxasterol [15] (Fig. 2).

Unfortunately, there is very limited scientific information in the literature confirming the medicinal potential of *Cosmos bipinnatus Cav.* The present study is devoted to the study of the biological activity of extracts from three varieties of *Cosmos bipinnatus Cav*., obtained by the extraction with 70% ethanol.

THE AIM of this study was to determine the antioxidant, anti-inflammatory and hypolipidemic activity of alcoholic extracts of three varieties of *Cosmos bipinnatus Cav.*

MATERIALS AND METHODS

Objects of study

For the study of a biological activity, the extracts obtained from the inflorescences of three varieties *of Cosmos bipinnatus Cav. – "Purity", "Rosea", "Dazzler"* – were selected.

Obtaining active substances

Raw materials were collected in September 2018 in the Botanical Garden of the Pyatigorsk Medical and Pharmaceutical Institute, a branch of the Federal State Budgetary Educational Institution of Higher Education "Volgograd State Medical University" of the Ministry of Health of the Russian Federation. The raw material was subjected to shade drying. The active substances were obtained by an exhaustive threefold extraction of 300 grams of raw material with 70% ethyl alcohol in a flask heated at reflux in a boiling water bath, each time for 30 minutes. The extracts were combined and filtered, and after short-term boiling, the filtrate was thickened in a vacuum rotary evaporator to the state of a thick extract, after that the residue was dried in an oven at 40–50°C to the constant weight (Fig. 3) [16, 17].

Experimental animals

Anti-inflammatory and hypolipidemic kinds of activity were determined on Wistar male rats obtained from the Rappolovo nursery (St. Petersburg) and kept in the PMFI vivarium, a branch of the Volgograd State Medical University. At the time of the experiment, the weight of the experimental animals was 250–280 g. All manipulations performed on the animals complied with the "Rules of the European Convention for the Protection of Vertebrate Animals" (Strasbourg, 1986).

During the experiment, optimal conditions were maintained in the vivarium: the air temperature of 22±2°C, a relative air humidity of 65±5%. The animals were housed in macrolon cages (T3) equipped with a food cavity and steel bars. For bedding, sawdust of any non-coniferous species was poured out into the cages. The animals were fed according to the standard diet and provided with a free access to food. The tap water was supplied in standard drinking bowls[1].

Reference drugs

When testing for an antioxidant activity, the reference drug was quercetin, a high antioxidant activity of which is known. Quercetin (Merck, Germany) was introduced into the reference sample at the concentration of 10 μg/ml, previously dissolved in dimethyl sulfoxide ("Vekton", Russia).

As a reference drug in the study of an anti-inflammatory activity, diclofenac was used (Enteric-coated tablets, 50 mg each, the manufacturer Hemofarm LLC, Obninsk, Russia, series 0291017). The reference drug was taken in the amount of 13 mg/kg in terms of the weight

[1] Directive 2010/63 / EU of the European Parliament and of the Council on the protection of animals used for scientific purposes, September 22, 2010.

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of an adult, the dose was calculated taking into account the interspecies dose conversion factor[2]. The substance was administered as an aqueous suspension stabilized with Tween-80 (Ferak Berlin, Germany).

In tests for a hypolipidemic activity, simvastatin was used as a reference drug, the drug was administered at the dose of 1.7 mg/kg (film-coated tablets, 20 mg each, the manufacturer – Ozon LLC, Zhigulevsk, Samara region, Russia, series 090618), and the dose was calculated taking into account the interspecies dose conversion factor[3].

Statistical processing

Student's t-test was used to assess the reliability of the study's results. The critical level of significance (p) when testing statistical hypotheses in this case was taken equal to 0.05. The sample size of the animals in each group was n=6[4].

When conducting the experiment on the study of the anti-inflammatory activity, the obtained data were statistically processed and presented as: *M±m,* where: *M* is the sample mean, *m* is the error of the mean.

Experiment design

The study design is shown in Figure 4.

Antioxidant activity

This type of activity was studied *in vitro* using the model of iron-induced lipid peroxidation (LPO) in the system of lecithin (phosphatidylcholine) liposomes (Fig. 4). Liposomes were obtained from lecithin (BAS MosLecithin, manufacturer of the Research Institute of Biomedical Chemistry, Russian Academy of Medical Sciences (Russia) at the lipid concentration of 40 mg/ml according to the methods described in the works by I.P. Kodonidi et al. [20]; M. Atas et al. [21]. To determine the effectiveness of the antioxidant action, the degree of inhibition of the lipid peroxidation intensity of lecithin liposomes in the test samples in relation to the control samples was studied. The absorption intensity of TBA-active products was measured 15 min before the incubation. The reaction was carried out in a water bath at 37°C with a continuous barbotage. The studied extracts were added to the test samples in the form of solutions in DMSO. Only the solvent was added to the control samples. The intensity of TBA-active products absorption was measured on SF-102 (SPA Akvilon, Russia) at 532 nm. The percentage of LPO inhibition was calculated in relation to the control sample using the formula:

$$AOA = \frac{\Delta D_k - \Delta D_{op.}}{\Delta D_k} \cdot 100\%,$$

$$\Delta D_k = D_k - D_k^0;$$

$$\Delta D_{op.} = D_{op.} - D_{op.}^0, \quad (1)$$

where: AOA – antioxidant activity, %; D_k^0 and $D_{op.}^0$ – are optical densities before incubation; D_k and $D_{op.}$ – optical density after 15 min of incubation.

Anti-inflammatory activity

To set up the experiment, aqueous suspensions of *Cosmos bipinnatus Cav.* alcoholic extracts of different varieties were used. To increase the stability of the suspension, the dried extracts were preliminarily triturated with a drop of Tween-80 (Fig. 4).

The study of the anti-inflammatory activity was carried out on 30 male rats of the Wistar line. It is known that the mechanism of the diclofenac action is associated with a decrease in the rate of prostaglandins synthesis, and prostaglandins play one of the leading roles in the development of inflammatory processes [22].

White rats were anesthetized with chloral hydrate at the dose of 350 mg/kg, then the hair was cut off in the back area and the supposed site of the subsequent incision was shaved. On a prepared site, in compliance with the rules of asepsis, the skin and subcutaneous tissue incision about 1 cm long was made with scissors. Then, a cavity was formed in the subcutaneous tissue through the incision with tweezers; there a pre-sterilized cotton ball weighing up to 15 mg was placed. After the manipulations, two or three sutures were applied to the wound. The animals were awakened and left under the standard conditions for a week. After 7 days, the balls were removed along with the granular tissue that had grown around them. The rats had been euthanized with chloral hydrate in advance. Then the balls were weighed and dried to constant weight at 60°C. The difference in the mass of the ball before and after drying indicated the size of the exudative phase of inflammation, the difference between the mass of the dried ball and its initial weight (up to 15 mg) was telling of the size of the proliferative phase.

The exudation was calculated by the formula:

$$m_3 = m_1 - m_2, \quad (2)$$

where: m_3 – exudation, g; m_1 – weight of the cotton ball immediately after the removal from the animal, g; m_2 – weight of the cotton ball after drying, g

Proliferation was calculated by the formula:

$$m_4 = m_2 - 0{,}015, \quad (3)$$

where: m_4 – proliferation, g; m_2 – weight of the cotton ball after drying, g; 0,015 – initial weight of the cotton ball, g.

The suspension of the studied extracts was administered at the dose of 300 mg/kg. Both test extracts and the reference drug were administered in equal volumes by gavage into the stomach for 7 days. The control group of the animals was injected with saline in the same way[5].

[2] Mironov AN. Guidelines for conducting preclinical studies of drugs. Part one. M.: Grif and K, 2013: 944 p. Russian
[3] Ibid.
[4] Glantz S. Medical and biological statistics. M.: Practice, 1998: 459 p. Russian
[5] Menshikov VV. Laboratory research methods in the clinic. M.: Medicine. 1987. – P. 365. Russian

Variety "*Dazzler*" **Variety "*Rosea*"** **Variety "*Purity*"**

Figure 1 – Appearance of *Cosmos bipinnatus Cav.* different varieties
Note: these photos are subject to copyright.

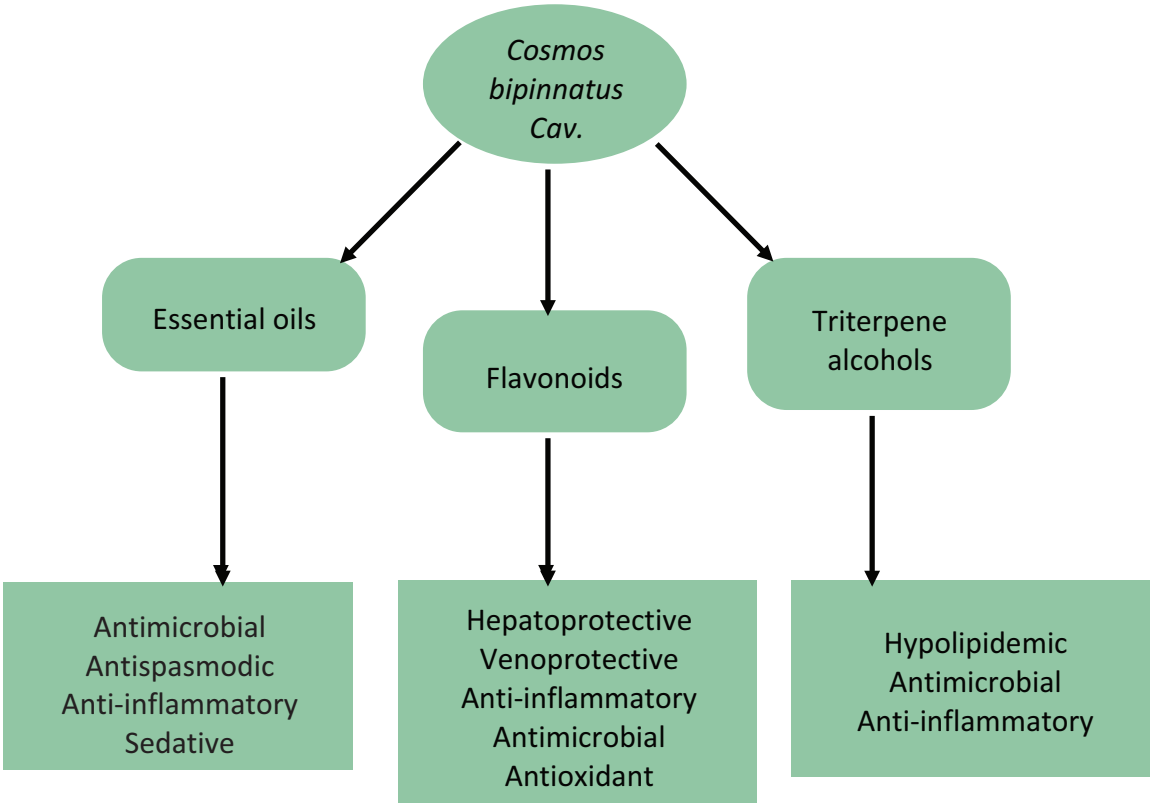

Figure 2 – Chemical composition of *Cosmos bipinnatus Cav.* and predicted pharmacological activity of the main classes chemicals

Table 1 – Effect of the sum of biologically active substances of alcoholic extracts from the *Cosmos bipinnatus Cav.* different varieties on the antioxidant activity

Final concentration of the studied extracts, mcg/ml	% in LPO decrease, n=3			
	Variety «*Purity*», n=3	Variety «*Rosea*», n=3	Variety «*Dazzler*», n=3	Quercetin, n=3 (concentration 10 µg/ml)
200	–24.3±1.29	–52.4 ±2.36	–54.3±3.23	–71 ±4.24

Note: n – is the number of the samples for each concentration

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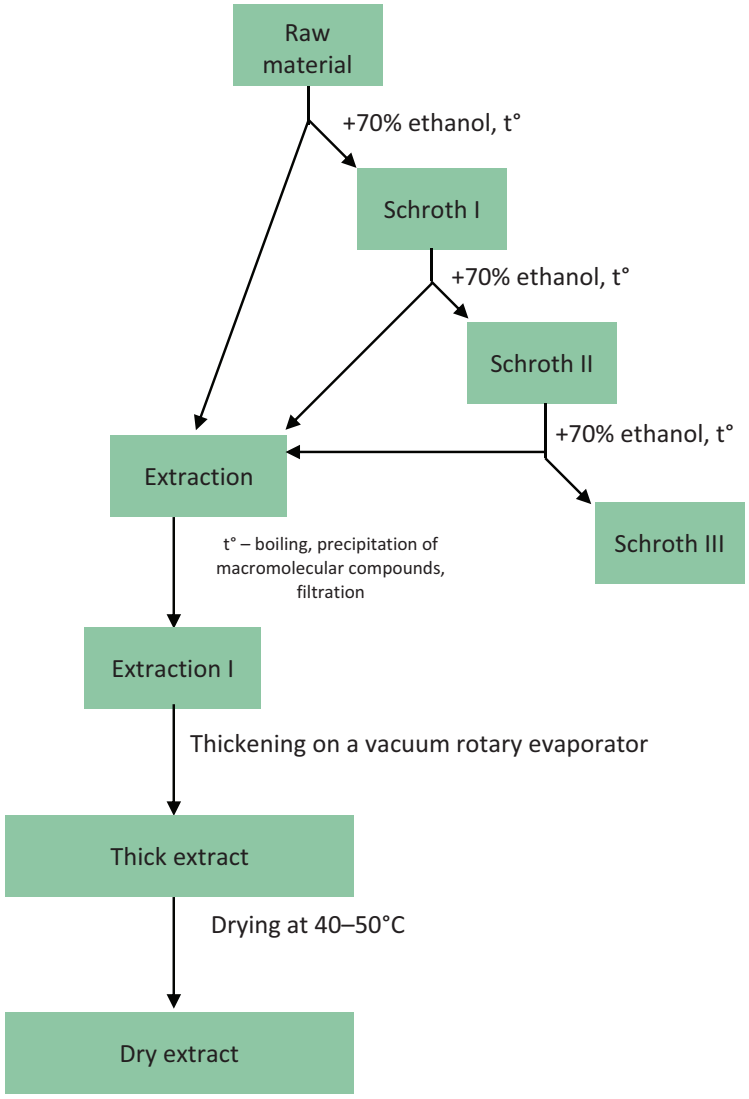

Figure 3 – Scheme for obtaining active substances from *Cosmos bipinnatus Cav.* raw materials

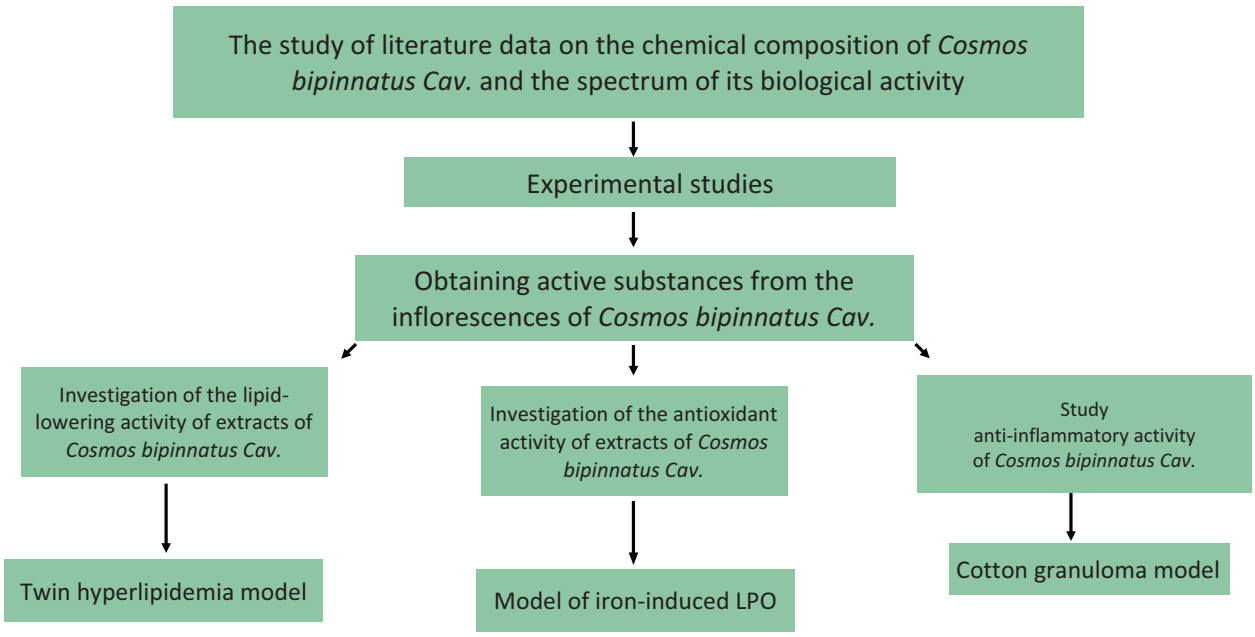

Figure 4 – Experiment design

Table 2 – Results of determining the anti-inflammatory activity of the studied *Cosmos bipinnatus Cav.* extracts in comparison with diclofenac

Object	Exudation, mg	Proliferation, mg
Control	210.0±7.1	33.0±2.9
Diclofenac, 13 mg/kg	117.7±8.9* P_C<0.005 – 44%	30.0 ± 1.7* P_C >0.1 P_D >0.1
70% alcohol extract from *Cosmos bipinnatus Cav.* of *"Purity»* variety", dose of 300 mg/kg	105.3±26.1* P_C <0.001 – 50% P_D >0.1	65.0±9.0*^ P_C <0.001 + 97% P_D <0.001 + 117%
70% alcohol extract from *Cosmos bipinnatus Cav.* of *«Rosea»* variety, dose of 300 mg/kg	101.8±28.3* P_C <0.001 – 52% P_D >0.1	59.0±4.1 *^ P_C <0.001 + 79% P_D <0.001 + 97%
70% alcohol extract from *Cosmos bipinnatus Cav.* of *«Dazzler»* variety, dose of 300 mg/kg	125.7±4.7* P_C <0.001 – 40% P_D >0.1	69.0±4.1 *^ P_C <0.001 + 109% P_D <0.001 + 130%

Note: P_C – the level of significant difference in relation to the control values; P_D – the level of significant difference in relation to the diclofenac values * – significant in relation to control; ^ – significant in relation to diclofenac.

Table 3 – Effect of alcohol extracts from *Cosmos bipinnatus Cav.* of different varieties on the parameters of lipid metabolism in blood against the background of Tween hyperlipidemia

Animal groups and their quantities	Indicators	
n=6	Serum cholesterol, mmol/l	Serum triglycerides, mmol/l
Intact group of animals	1.8±0.35	1.4±0.28
Control group of animals	3.2±0.28 P_I <0.001;+78%	2.3±0.06 P_I <0.001; +64%
Experimental group that received a 70% alcohol extract of *Cosmos bipinnatus Cav.* of *"Purity"* variety	1.8±0.25 P_C <0.001; –44% P_I >0.01	1.1±0.34 P_C <0.001; –52% P_I >0.01
Experimental group that received a 70% alcohol extract of *Cosmos bipinnatus Cav.* of *"Rosea"* variety	1.7±0.19 P_C <0.001; –47% P_I >0.01	1.1±0.30 P_C <0.001; –52% P_I >0.01
An experimental group that received a 70% alcohol extract of *Cosmos bipinnatus Cav.* of *"Dazzler"* variety	1.6±0.35 P_C <0.001; –50% P_I >0.01	1.0±0.36 P_C <0.001; –57% P_I >0.01
Comparison group treated with simvastatin	2.0±0.50 P_C <0.001; –38% P_I >0.01	1.2±0.22 P_C <0.001; –48% P_I >0.01

Note: P_I – the level of significant difference in relation to intact values; P_C – the level of significant difference in relation to the control values; n – number of animals in the group.

Hypolipidemic activity

The hypolipidemic activity of the extracts under investigation was studied on 36 white male rats of the Wistar line. The animals weighing 250–180 grams were taken. The experimental animals were in the same conditions as in the previous experiment. They were divided into 6 groups: 1 – intact; 2 – control; 3, 4, 5 – experimental; 6 – comparison groups. The intact animals were kept under the same conditions as the rest of the groups. The control group animals received distilled water in the equivalent volume of the studied suspensions. The animals from the experimental groups were injected with the extracts obtained from different varieties of *Cosmos bipinnatus Cav*. Group 3 received the extract from the inflorescences of *Cosmos bipinnatus Cav*. of the *"Purity"* variety; group 4 received the extract from the inflorescences of *Cosmos bipinnatus Cav.* of the *"Rosea"* variety; the 5th group received the extract from the inflorescences *of Cosmos bipinnatus Cav.* of the *"Dazzler"* variety. The sixth group of the animals was the control and received the reference drug (Fig. 4).

To study the hypolipidemic activity of the studied extracts, a Tween model was used to create a hyperlipidemic state in the rats: a single intraperitoneal injection of Tween-80 in the amount of 250 mg per 100 grams of the animal body weight [8]. The animals were administered with the dose of 300 mg/kg, p. o., daily (for a week) by means of probes into the stomach of the animals. The

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purpose of the suspension administration during a week was to saturate the organs and tissues of the animals with biologically active compounds, which are associated with lipid metabolism. On the 7th day after the start of soldering, the animals were intraperitoneally injected with Tween-80, and 12 hours after the injection, the animals were slaughtered by decapitation. 12 hours before the slaughter, the animals had been deprived of food

The indicators of cholesterol and triacylglycerides were determined on an automatic biochemical analyzer BS-380 (Mindray, China).

RESULTS AND DISCUSSION

Antioxidant activity

The antioxidant activity investigation of the plant extracts in the models on integral multicellular microorganisms, makes it possible to evaluate only the intensity of the influence of the substances on free radical processes and the activity of the body antioxidant system, but it is not always possible to elucidate the mechanism of their antioxidant action. *In vitro* experiments often provide a clear understanding of the antioxidant effect of the studied substances or their combinations [10, 26].

The method of iron-induced peroxidation is based on the ability of oxidizing agents comprised by the studied extracts, to inhibit the formation of LPO products. Their content is determined by the ability to form colored complex compounds with thiobarbituric acid.

In this experiment, the degree of lipid peroxidation inhibition of liposomes by extracts introduced into the reaction was taken as the value of the antioxidant activity. The results obtained were compared with the results of the control samples. The following extracts were added to the test tubes – 70% extracts obtained from *Cosmos bipinnatus Cav.* three varieties: "*Purity", "Rosea",* and "*Dazzler"* in the concentration of 200 μg/ml. Only dimethyl sulfoxide as the solvent was added to the control samples. Based on the results obtained, the percentage of lipid peroxidation reduction in relation to the control sample was calculated.

Table 1 presents the data on the effect of biologically active compounds of the studied extracts on the accumulation of lipid peroxidation products complexes with thiobarbituric acid (TBA-active products) in the final concentration of 200 μg/ml. This concentration was chosen as the most effective.

It follows from Table 1 that alcohol extracts obtained from the *Cosmos bipinnatus Cav.* varieties *"Dazzler"* and *"Rosea*" contribute to a better reduction in the accumulation of peroxide compounds compared to those obtained from the *Cosmos bipinnatus Cav.* of the*"Purity"* variety. Thus, at the concentration of 200 μg/ml, the alcohol extracts obtained from the *Cosmos bipinnatus Cav.* varieties *"Dazzler"* and *"Rosea",* reduced the content of malondialdehyde by 54% and 52%, respectively, and the alcohol extract obtained from the *Cosmos bipinnatus Cav.* varieties *"Purity"* – only by 24%. When increasing the dose of the studied objects to 500 μg/ml, there was no significant decrease in lipid peroxidation. Quercetin at the concentration of 10 μg/ml contributed to the suppression of lipid peroxidation by 71% compared with the control – DMSO.

Therefore, the conducted model experiments showed that the studied extracts have an antioxidant effect. The mechanism of the antioxidants action directly depends on the environment in which the oxidation substrate and the antioxidant itself are located. In this experiment, the mechanism of the antioxidant action may be implemented by binding the resulting free lipid radicals with the antioxidants or the chelate complexes formation with ferrous ions [10, 26].

The results of the antioxidant activity study show that the extracts obtained from the three varieties of *Cosmos bipinnatus Cav.* – "*Purity", "Rosea", "Dazzler"* – show a clear antioxidant effect. The activity against free radicals and the ability to form chelate complexes with iron, underlies blocking of the inducing ability of iron against free radicals. Alcoholic extracts from the *Cosmos bipinnatus Cav.* varieties *"Rosea"* and *"Dazzler"* have the highest antiradical and antioxidant activities. Probably, this feature is due to the richer polyphenolic composition of the extracts. The corollas of these plants' varieties are colored in bright colors (pink and purple); this fact indicates a possible high content of anthocyanins, known for their high antioxidant activity.

Anti-inflammatory activity

From the experimental data obtained in the study of the anti-inflammatory activity it follows that alcohol extracts from the *Cosmos bipinnatus Cav.* varieties "*Purity", "Rosea", "Dazzler"* reduced the stage of exudation by 50%; 52% and 40%, respectively, significantly in relation to the control group of the animals. These indicators were compared with the experimental groups and the control group receiving diclofenac, no significant differences were found out between them. In terms of limiting exudation, the alcohol extract from the *Cosmos bipinnatus Cav.* "*Purity"* variety does not significantly differ in the effect of the alcohol extract from the *Cosmos bipinnatus Cav.* "*Rosea"* variety, the extraction from the *Cosmos bipinnatus Cav.* "*Dazzler"* variety is slightly inferior in the reduction of this indicator. The results of the experiment are presented in Table 2.

In the course of the results analysis, it was found out that all the studied substances significantly increased the proliferative phase of inflammation in comparison with the control group of the animals. The leader in terms of this indicator was the alcohol extract from *Cosmos bipinnatus Cav.* of the "*Dazzler"* variety.

It was established that against the background of chronic proliferative inflammation (a cotton granuloma model), the test substances in therapeutic and effective doses of 300 mg/kg have significant proliferative and anti-exudative activities, which can be compared with the reference drug.

In the experiment on the anti-inflammatory research of the extracts under study, it was found out that alcohol extracts at the dose of 200 μg/ml exhibit a distinct anti-inflammatory activity, cause a decrease in exudation in the inflammatory focus, which is caused by phlogogenic agents. The leader in terms of the anti-inflammatory activity is the alcohol extract from *Cosmos bipinnatus Cav.* of the *Dazzler* variety. It is possible that the anti-inflammatory effect of the studied extracts is due to their ability to reduce the degree of release of the body's natural inflammatory mediators from mast cells and basophils.

Hypolipidemic activity

According to the WHO, cardiovascular diseases are currently among the top three causes of death in the world. One of the causes and complications of cardiovascular diseases is a violation of lipid metabolism, accompanied by atherosclerosis. Worldwide, there is a trend towards the rejuvenation of cardiovascular diseases[6].

Current protocols for the treatment of lipid disorders are usually based on drug treatment and prevention of atherosclerosis. Usually, drugs for the correction of lipid metabolism are synthetic compounds. Currently, there are three main groups of hypolipidemic drugs: statins, fibrates, and fatty acid sequestrants. The use of these groups of drugs usually leads to a number of side effects: myopathy, asthenia, anorexia, etc. [26].

In the works of recent years, a hypolipidemic effect of plants and various herbal total preparations is often highlighted. A rather high efficiency of such drugs coupled with low toxicity, has been proved [24].

To characterize the influence degree of compounds on the blood lipid profile of animals, it is customary to determine the concentration of total cholesterol, triacylglycerides, low density lipoproteins and high density lipoproteins. In the described study, the content of total cholesterol and triglycerides (TGC) was determined in blood serum. The indicators of cholesterol and triacylglycerides were determined on an automatic biochemical analyzer BS-380 (TRG).

The administration of Tween-80 in experimental animals once intraperitoneally, is accompanied by severe hyperlipidemia. An increase in total serum cholesterol by 78%, and triglycerides by 64% was established. The concentration of total cholesterol and triglycerides in the blood serum of the intact animals corresponded to the norm (Table 3).

The suspension was administered to the animals at the dose of 300 mg/kg. The administration of a suspension in such a dose led to a decrease in cholesterol in the blood serum by 44%, 47%, 50%, when the animals received the extracts obtained from the *Cosmos bipinnatus Cav.* varieties *"Purity", "Rosea", "Dazzler"*, respectively. From the study, it can also be concluded that the concentration of cholesterol in the blood is completely normalized in comparison with the values in healthy (intact) animals. The level of blood triglycerides also decreased by 52%, 52% and 57% in the case of the use of the corresponding extracts obtained from the *Cosmos bipinnatus Cav.* varieties "*Purity", "Rosea", "Dazzler"*, respectively, and reached the normal level. The values of these indicators were comparable with the values of the reference drug.

When conducting the experiment to study the hypolipidemic activity of the extracts, it was found out that the oral administration of the extracts to the animals for a week led to a significant decrease in the concentration of free cholesterol in the blood and the level of triglycerides. The degree of reduction in the concentration of cholesterol and triglycerides in the blood serum of all extracts is approximately at the same level, and exceeds the hypolipidemic effect of the reference drug. The concentration of total cholesterol in the blood serum was most intensively reduced by a 70% alcohol extract of the *Cosmos bipinnatus Cav.* variety *"Dazzler"*, and triglycerides by a 70% alcohol extract of *Cosmos bipinnatus Cav.* varieties *"Purity"* and *"Rosea"*.

It is known that some triterpene alcohols (e.g., gelianol), comprised by the plants of the genus *Cosmos*, have an anti-inflammatory activity. [16].

Butein is chalcone, which is a part of plants of the genus *Cosmos*; it is a powerful antioxidant against lipids and low density lipoproteins (LDL), and also has an anti-inflammatory activity, is able to inhibit aromatase and cyclooxygenase [19, 20].

CONCLUSION

According to the conducted studies, it can be concluded that alcohol extracts obtained from *Cosmos bipinnatus Cav.* have pronounced antioxidant, anti-inflammatory and hypolipidemic effects.

The manifestation of the kinds of the biological activity described above, can be associated with the chemical composition of *Cosmos bipinnatus Cav.* The literature sources and the authors' previous studies have shown that the chemical composition of *Cosmos bipinnatus Cav* includes polyphenols (catechins, anthocyanins, flavonoids), organic acids, amino acids and polysaccharides. The manifestation of antioxidant, anti-inflammatory and hypolipidemic properties of 70% alcohol extracts from *Cosmos bipinnatus Cav.,* can be associated with the presence of the described classes of biologically active compounds in them.

The study and the data obtained in the course of it makes it possible to recommend three varieties of *Cosmos bipinnatus Cav*.: "*Purity", "Rosea", "Dazzler"* as of biologically active compounds with a wide range of a biological activity.

[6] World Health Organization (WHO). The top 10 causes of death. Available from: https://www.who.int/news-room/fact-sheets/detail/the-top-10-causes-of-death.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest. All illustrations, drawings and photographs are made by the team of the authors, are original and do not violate anyone's copyrights.

AUTHORS' CONTRIBUTION

Evgenia O. Kulichenko – collection of plant materials for the experiment, conducting the experiment and collecting the data, analyzing and interpreting the data obtained, statistical processing of the results obtained, analyzing the literature, writing the manuscript; Olga A. Andreeva – collection of plant materials for the experiment; Elena O. Sergeeva – participation in the experiment and data collection, participation in writing the manuscript; Svetlana S. Sigareva – participation in the experiment; Alexander Yu. Terekhov – participation in the planning of the study and the development of the concept and design of the study; Eduard T. Oganesyan – study planning, participation in the development of the concept and design of the study, verification of critical intellectual content, final approval for publication of the manuscript; Svetlana Yu. Sidorskaya – statistical processing of the obtained results.

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AUTHORS

Evgenia O. Kulichenko – Senior Lecturer of the Department of Microbiology and Immunology with a course of Biological Chemistry, postgraduate student of the Department of Organic Chemistry, Pyatigorsk Medical and Pharmaceutical Institute – a branch of Volgograd State Medical University. ORCID ID: 0000-0002-0727-6689. E-mail: evgenia.kuli4encko@yandex.ru

Olga A. Andreeva – Candidate of Sciences (Chemistry), Associate Professor of the Department of Organic Chemistry, Pyatigorsk Medical and Pharmaceutical Institute – a branch of Volgograd State Medical University. ORCID ID: 0000-0002-7865-0762. E-mail: oa-51934@yandex.ru

Elena O. Sergeeva – Candidate of Sciences (Pharmacy), Associate Professor, Associate Professor of the Department of Pathology of Pyatigorsk Medical and Pharmaceutical Institute – a branch of Volgograd State Medical University. ORCID ID: 0000-0001-7496-3967. E-mail: maklea@yandex.ru

Svetlana S. Sigareva – Senior Lecturer of the Department of Microbiology and Immunology with a Course of Biological Chemistry, Pyatigorsk Medical and Pharmaceutical Institute – a branch of Volgograd State Medical University. ORCID ID: 0000-0002-6510-6602. E-mail: svgritchina@yandex.ru

Alexander Yu. Terekhov – Candidate of Sciences (Pharmacy), Associate Professor, Head of the Department of Pathology, Pyatigorsk Medical and Pharmaceutical Institute – a branch of Volgograd State Medical University. ORCID ID: 0000-0002-7781-362X. E-mail: tau200@yandex.ru

Eduard T. Oganesyan – Doctor of Sciences (Pharmacy), Professor, Head of the Department of Organic Chemistry, Pyatigorsk Medical and Pharmaceutical Institute – a branch of Volgograd State Medical University. ORCID ID: 0000-0002-2756-9382. E-mail: edwardov@mail.ru

Svetlana Yu. Sidorskaya – Candidate of Sciences (Pharmacy), Associate Professor, Department of Pathology, Pyatigorsk Medical and Pharmaceutical Institute – a branch of Volgograd State Medical University. ORCID ID: 0000-0001-8218-412X. E-mail: sidorskayasvet@yandex.ru

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PHARMACOTHERAPY POSSIBILITIES OF CARDIOVASCULAR AUTONOMOUS NEUROPATHY IN CHILDREN WITH TYPE 1 DIABETES MELLITUS AT THE PRECLINICAL STAGE

N.V. Malyuzhinskaya[1], I.N. Shishimorov[1], O.V. Magnitskaya[1], K.V. Stepanenko[1], O.V. Polyakova[1], G.V. Klitochenko[1], I.V. Petrova[1], S.A. Emelyanova[2], A.P. Skiba[1]

[1] Volgograd State Medical University,
1, Pavshikh Bortsov Sq., Volgograd, Russia, 400131
[2] Volgograd Regional Children's Clinical Hospital
76, Zemlyachki St., Volgograd, Russia, 400076

E-mail kozhevnikova.kv@yandex.ru

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The aim of the article is to evaluate the effectiveness of the thioctic acid preparation in the complex therapy of type 1 diabetes mellitus (T1DM) in children with cardiovascular autonomic neuropathy at the preclinical stage.

Materials and methods. A design is a prospective randomized study. A clinical and instrumental examination of 64 children with preclinical stage signs of diabetic cardiovascular autonomic neuropathy (DCAN) was carried out. The cohort was divided into 2 groups: in the main and control groups, glycemic control was normalized by adjusting a dose of insulin therapy; in the main group, the children additionally received thioctic acid at the dose of 600 mg/day for 3 months. To control the effectiveness of the therapy, the technique of laser Doppler flowmetry was used.

Results. After the pharmacological intervention, there was an improvement in the disease course, normalization of carbohydrate and lipid metabolism, increased vasomotor mechanisms of the regulation of the tissue blood flow due to an increase in endothelial and neurogenic kinds of activity in combination with a decrease in the intravascular tone and an increase in the effective perfusion in tissues. An increase in the heart rate variability was detected, positive dynamics of cardiovascular tests indicators according to D. Ewing, temporal (pNN50%, SDNN) and spectral indicators (VLF) were diagnosed. Achievement and maintenance of the target values of glycemic control indicators, as well as the absence of glycemic variability, turned out to be clinically significant for reducing the manifestations of neuropathy. The non-invasive technique of laser Doppler flowmetry is informative for the early diagnosis of DCAN in T1DM children.

Conclusion. The carried out studies have demonstrated the effectiveness of the lipoic acid use at the dose of 600 mg/day for 3 months in the children with DCAN signs at the preclinical stage. The method of laser Doppler flowmetry for determining indications and monitoring the effectiveness of therapy makes it possible to implement a personalized approach to prescribing preventive treatment in T1DM children.

Keywords: diabetic cardiovascular autonomic neuropathy; α-lipoic acid; preventive treatment of complications in T1DM children; laser Doppler flowmetry

Abbreviations: GCP – good clinical practice; HbA1c – glicated hemoglobin; LADA – Latent Autoimmune Diabetes of Adults; MODY – maturity onset diabetes in youth; pNN50% – the mean number of times an hour in which the change in successive normal sinus (NN) intervals exceeds 50 ms; SDNN – standard deviation normal to normal; VLF – very low frequencies; AVA – arteriolo-venular anastomoses; ALA – Alphalipoic acid/α-lipoic acid; DCAN – diabetic cardiovascular autonomic neuropathy; VVR – vegetative-vascular regulation; ESPALIPON II – an octanoic acid bridged with *two* sulfurs; NATHAN I – Neurological Assessment of Thioctic Acid in Diabetic Neuropathy; LDF – Laser Doppler Flowmetry; HDLP- High-Density Lipoprotein; LDLP – Low-Density Lipoprotein; HR – heart rate; T1DM – type 1 diabetes mellitus; TG – triglycerids; ECG – electrocardiography; BMI – body mass index.

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ВОЗМОЖНОСТИ ФАРМАКОТЕРАПИИ НА ПРЕКЛИНИЧЕСКОЙ СТАДИИ КАРДИОВАСКУЛЯРНОЙ АВТОНОМНОЙ НЕЙРОПАТИИ У ДЕТЕЙ С САХАРНЫМ ДИАБЕТОМ 1 ТИПА

Н.В. Малюжинская[1], И.Н. Шишиморов[1], О.В. Магницкая[1], К.В. Степаненко[1], О.В. Полякова[1], Г.В. Клиточенко[1], И.В. Петрова[1], С.А. Емельянова[2], А.П. Скиба[1]

[1] Федеральное государственное бюджетное образовательное учреждение высшего образования «Волгоградский государственный медицинский университет» Министерства здравоохранения Российской Федерации
400131, Россия, г. Волгоград, пл. Павших борцов, д. 1
[2] Государственное бюджетное учреждение здравоохранения «Волгоградская областная детская клиническая больница»
400076, Россия, г. Волгоград, ул. Землячки, д. 76

E-mail: kozhevnikova.kv@yandex.ru

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Цель. Оценить эффективность препарата тиоктовой кислоты в комплексной терапии сахарного диабета 1 (СД 1) типа у детей с кардиоваскулярной автономной нейропатией на доклинической стадии.
Материалы и методы. Дизайн – проспективное рандомизированное исследование. Проведено клинико-инструментальное обследование 64 детей с признаками доклинической стадии диабетической кардиоваскулярной автономной нейропатии (ДКАН). Когорта ранжирована на 2 группы: в основной и контрольной группах проводилась нормализация гликемического контроля путём коррекции дозы инсулинотерапии; в основной группе дети дополнительно получали препарат тиоктовой кислоты в дозе 600 мг/сутки в течение 3-х месяцев. Для контроля эффективности терапии использовалась методика лазерной допплеровской флоуметрии.
Результаты. После фармакологической интервенции наблюдалось улучшение течения заболевания, нормализация показателей углеводного и липидного обмена, усиление вазомоторных механизмов регуляции тканевого кровотока за счёт увеличения эндотелиальной и нейрогенной активности в сочетании со снижением внутрисосудистого тонуса и увеличением эффективной перфузии в тканях, выявлено увеличение вариабельности ритма сердца, диагностирована положительная динамика показателей кардиоваскулярных тестов по D. Ewing, временных (pNN50%, SDNN) и спектральных показателей (VLF). Клинически значимым для уменьшения проявлений нейропатии оказалось достижение и поддержание целевых значений показателей гликемического контроля, а также отсутствие вариабельности гликемии. Неинвазивная методика лазерной допплеровской флоуметрии является информативной для ранней диагностики ДКАН у детей с СД типа 1.
Заключение. Проведенные исследования продемонстрировали эффективность применения липоевой кислоты в дозе 600 мг/сутки в течение 3-х месяцев у детей с признаками ДКАН на доклинической стадии. Метод лазерной допплеровской флоуметрии для определения показаний и контроля эффективности терапии позволяет реализовать персонализированный подход для назначения превентивного лечения у детей с сахарным диабетом 1 типа.
Ключевые слова: диабетическая кардиоваскулярная автономная нейропатия; α-липоевая кислота; превентивное лечение осложнений у детей с СД типа 1; лазерная допплеровская флоуметрия
Список сокращений: GCP – надлежащая клиническая практика; HbA1c – гликированный гемоглобин; LADA – латентный аутоиммунный диабет взрослых; MODY – диабет зрелого типа у молодых; pNN50% – процент (доля) последовательных интервалов, различие между которыми превышает 50 мс; SDNN – стандартное отклонение величин нормальных интервалов; VLF – очень низкочастотные; АВА – артериоло-венулярные анастомозы; АЛК – альфа-липоевая кислота; DECAN – Германское исследование кардиальной автономной нейропатии; ВСР – вегето-сосудистая регуляция; ДКАН – диабетическая кардиоваскулярная автономная нейропатия; ESPALIPON II – эффективность препаратов тиоктовой кислоты; NATHAN I – Неврологическая оценка эффекта тиоктовой кислоты при диабетической нейропатии; ЛДФ – лазерная допплеровская флоуметрия; ЛПВП – липопротеины высокой плотности; ЛПНП – липопротеины низкой плотности; ЧСС – частота сердечных сокращений; СД 1 – сахарный диабет типа 1; ТГ – триглицериды; ЭКГ – электрокардиография; ИМТ – индекс массы тела.

INTRODUCTION

Type 1 diabetes mellitus (T1DM) remains one of the most urgent problems of modern pediatrics due to the high level of disability and mortality [1, 2]. Alongside with the development of diabetology, an early detection and treatment of cardiovascular complications in children remain unresolved [2]. One of the most formidable complications of type 1 diabetes mellitus in children is diabetic cardiovascular autonomic neuropathy, which is characterized by denervation of the autonomic nervous system that regulates a vascular tone and a cardiac activity [1, 3]. Due to the complexity of diagnosing dia-

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betic cardiovascular autonomic neuropathy (DCAN), the data on the occurrence frequency are contradictory. The analysis of the literature shows that this index ranges from 20 to 60% [4, 5]. The main mechanism for the development of DCAN is microcirculatory disorders, which are accompanied by a decrease in the formation of nitric oxide, endoneural hypoxia, leading to the ischemic damage to nerve fibers [1, 6, 7]. Due to the development of glucose toxicity, metabolic disorders also play an important pathogenetic role [7–9]. There is a relationship between an increase in blood glucose levels and the severity of an oxidative stress. With hyperglycemia, there is an increase in lipid peroxidation, a violation of the nitric oxide formation by the vascular endothelium, an increase in the synthesis of pro-inflammatory adhesion molecules, and an increase in the sensitivity of smooth muscle cells of the vascular wall to vasoconstrictive stimuli [10–12]. The oxidative stress accompanies metabolic T1DM disorders in, which result in contributing to the development of late vascular complications.

Promising drugs for the treatment of DCAN are α-lipoic acid preparations, which have antioxidant, neurotrophic and hypoglycemic effects [8, 9, 13–15]. In diabetes mellitus in adult patients, the neuroprotective effect of alpha-lipoic acid (ALA) has been proven due to a decrease in the formation of advanced glycation endproducts of proteins in nerve cells, endoneural hypoxia and ischemia, as well as an increase in the concentration of the antioxidant glutathione [16–20]. In the DCAN study carried out by the German Cardiac Autonomic Neuropathy Research, the treatment of T1DM patients with DCAN by thioctic acid resulted in a significant improvement in the function of the nerve fibers of the autonomic nervous system, which was manifested by an increase in the heart rate variability [21]. The effectiveness of therapy in clinical DCAN is 30%, while the regression of disorders during the pharmacological intervention at the preclinical stage of DCAN is observed in 70% of patients [22, 23]. However, the studies on the pediatric population are singular, the indications and regimens for prescribing the thioctic acid preparation have not been determined, and the effectiveness of its use at the preclinical stage of DCAN has not been proven. One of the obstacles to the development of pathogenetic therapy for DCAN is the lack of "a gold standard" of preclinical diagnostics, which makes it possible to determine the indications for prescribing in this category of patients. It has been proven that the earliest changes in T1DM occur in the microvasculature. Therefore, an early functional diagnosis of cardiovascular complications, their dynamic control and the possibility of the early pathogenetic treatment are very important [1, 3, 10].

Currently, to assess the functional state of the microvasculature, a modern non-invasive technique of laser Doppler flowmetry (LDF) is used, which determines the tissue perfusion by measuring the Doppler frequency shift during probing and emitting a helium-neon laser at the wavelength of 632.8 nm and registering this radiation [9]. During the study, fluctuations in the blood flow in the microvasculature are recorded [24–27]. The use of LDF for the early diagnosis of DCAN opens up new prospects for personalized approaches to the preventive treatment of T1DM children.

THE AIM of the article is to evaluate the effectiveness of the thioctic acid preparation in the complex therapy of T1DM children with cardiovascular autonomic neuropathy at the preclinical stage.

MATERIALS AND METHODS

Study design

The principles of World Medical Association Declaration of Helsinki (WMA)[1] and the Rules of Good Clinical Practice (GCP)[2] of the Eurasian Economic Union served as the basis for a prospective, randomized, simple comparative study in parallel groups.

The Ethics Committee of the Federal State Budgetary Educational Institution of Volgograd State Medical University of the Ministry of Health of Russia (protocol No. 17 dated September 16, 2019) approved of the study. Written informed consent (IC) to participate in it was signed by all patients or their legal representatives prior to the inclusion in the research.

94 children aged 10 to 17 years with a verified diagnosis of T1DM, were examined. The exclusion criteria were: the age under 10 or over 17; T2DM, latent autoimmune diabetes in adults (LADA) and maturity onset diabetes in youth (MODY); the presence of primary arterial hypertension and other cardiovascular pathology not associated with T1DM; concurrent participation in another clinical trial; lack of a signed informed consent (IC) to participate in the study.

The withdrawal criteria were as follows: a refusal to participate in the study at any stage; decompensation of carbohydrate metabolism with ketoacidosis; somatic diseases at the acute stage in combination with T1DM.

Monitored parameters

The study was conducted on the basis of the endocrinology department of the Volgograd Regional Children's Clinical Hospital. All patients underwent clinical, anamnestic and laboratory examinations, including the determination of fasting plasma glucose, HbA1c, total cholesterol, TG, LDLP, HDLP. The instrumental examination included the following: 24-hour ECG monitoring using the Cardiotekhnika-04-3 hardware-software complex (Inkart, Russia) under the conditions of free activity of

[1] Declaration of Helsinki by the World Medical Association (WMA). Ethical principles for conducting medical research involving a person as a subject. Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964, amended by the 64th WMA General Assembly, Fortaleza, Brazil, October 2013. Available from: https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/

[2] Decision No. 79 "On Approval of the Rules of Good Clinical Practice of the Eurasian Economic Union" dated November 03, 2016. Available from: https://docs.cntd.ru/document/456026110

the subject, 24-hour blood pressure monitoring (ABRM-04 model, Meditech, Hungary); D. Ewing cardiovascular tests were performed: a slow breathing test (6 per minute), Valsalva test, Shelong test (orthostatic test), 30:15 test, an isometric load test. The assessment of the state of the microvasculature was carried out using the LDF method based on a two-channel laser Doppler flowmeter LAKK-OP (NPP LAZMA, Russia, Moscow) according to the attached methods for studying skin areas with arteriolo-venular anastomoses and without arteriolo-venular anastomoses. The basic state of microcirculation was assessed; the amplitude-frequency spectrum of perfusion fluctuations, the endothelial and neurogenic sympathetic activity, passive frequency ranges (respiratory and cardio rhythms) were analyzed [25].

After the examination, 64 children were diagnosed with DCAN signs of varying severity.

After the inclusion in the study, at the preclinical stage, the DCAN patients were divided into 2 groups by a simple randomization method. The main group included 32 children (15 girls and 17 boys), the mean age was 13.53±2.54 years; in the control group there were 32 children (16 girls and 16 boys), the average age was 13.15±2.03 years. The groups were comparable in terms of the age, sex, duration of the disease, the level of compensation for carbohydrate and lipid metabolism (Table 1). All the patients were on bolus insulin therapy, and the insulin therapy was adjusted if necessary. According to the ESPALIPON II study (the effectiveness of thioctic acid preparations), the same efficacy and safety of oral and infusion forms of thioctic acid at the dosage of 600 mg were proved. Therefore, the patients of the main group received a thioctic acid preparation (Lipoic acid, Marbiopharm, Russia) at the dose of 600 mg/day within 3 months. After 3 months, a re-examination was carried out.

Statistical processing

The Shapiro-Wilk test was used to assess the distribution of quantitative indicators. In the form of mean values (M ± SD), the results of the normal distribution of features are presented, in the non-normal distribution, the median (Me) and quartiles (25^{th} and 75^{th} percentiles) are presented. The non-parametric Wilcoxon test was used to assess the statistically significant difference in related quantitative traits. Contingency tables with the two-tailed Fisher's exact test were used to assess the significance of the relationship between two variables. At $p<0.05$, the difference was statistically significant. The statistical package STATISTICA 10.0 (StatSoft, Tulsa, USA) was used to process the results.

RESULTS AND DISCUSSION

For 3 months, the dose of insulin was adjusted to the patients under control by a pediatric endocrinologist. The indicators of carbohydrate metabolism are presented in Table. 2. During the observation period, the indicators of carbohydrate metabolism compensation improved in both groups. After 3 months, a decrease in the level of glycated hemoglobin was observed in most patients. However, in the group of the children treated with lipoic acid, there was a more significant decrease in the studied indicator than in the control group (7.45 [6.4; 8.2] and 7.9 [6.84; 9.1], respectively (p=0.0378). The presence of positive dynamics in the two study groups at once can be primarily explained by an increase in the level of patients' compliance during the participation in the study.

A more pronounced decrease in the level of glycated hemoglobin in the main group may be associated with the hypoglycemic effect of alphalipoic acid (ALA) due to improving the utilization of glucose by peripheral tissues and increasing insulin sensitivity (a decrease in the glucose-insulin index).

The improved glucose utilization by ALC tissues seems to be associated with phosphorylation of tyrosine residues of insulin receptors, activation of glucose transporters, and a number of other effects in insulin-dependent tissues. These factors increase glucose uptake by adipocytes and increase the activity of tyrosine kinase and serine/threonine kinase [28].

In 1999, in a placebo-controlled study, there was an increase in the insulin sensitivity in T2DM patients after a month of using ALA at the dose of 600 mg/day [29]. Ansar H. et al. [17] recorded a decrease in plasma glucose levels on an empty stomach and 2 hours after a meal, insulin resistance in T2DM patients while taking ALA at the dose of 300 mg/day. In another randomized study, in T2DM patients, taking ALA at the dose of 300 to 1200 mg/day for six months improved the glycemic profile and reduced indicators of an oxidative stress [30]. The multicenter, double-blind, placebo-controlled study NATHAN I (Neurological Assessment of Thioctic Acid in Diabetic Neuropathy) showed a decrease in HbA1c levels [31].

The systematic review and meta-analysis [13] of 20 randomized clinical trials investigating the effect of ALA on glycemic profile in the patients with metabolic disorders showed that its administration at the dose of 200–1800 mg/day from 2 weeks to 1 year, led to a decrease in glucose levels and fasting plasma insulin, HbA1c concentration and insulin resistance.

It is known that lipid profile indicators in T1DM children and adolescents depend on glycemic control [3, 8, 9]. In addition, dyslipidemia and hyperglycemia are the main pathogenetic factors of diabetic neuropathy, which lead to neurodegeneration through metabolic and inflammatory mechanisms. In T1DM children with lipid metabolism disorders, changes that indicate the initial manifestations of autonomic heart rhythm dysregulation in the form of hypersympathicotonia and electrical myocardial instability, have been identified [27].

In this study, all the groups were initially comparable in terms of lipid metabolism (Table 3). Lipid metabolism

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Table 1 – Clinical and laboratory characteristics of the groups under study

Indicator	Main group (n = 32)	Control group (n = 32)	p
Disease duration	5.12±3.1	4.96±4.35	p=0.87
Debut	7.86±3.12	8.2±4.28	p=0.72
Glucose variability	7.93±3.41	7.61±4.07	p=0.73
HbA1c level	8.2 [7.5; 9.6]	8.6 [7.3; 9.8]	p=0.85
Average daily insulin dose	0.97 [0.7; 1.01]	0.91 [0.67; 1.14]	p=0.82
Total cholesterol	4.34±0.86	4.22±0.94	p=0.60
Triglycerides	0.82 [0.48; 1.26]	0.79 [0.59; 1.21]	p=0.71
BMI	19.89±2.68	19.26±2.96	p=0.83

Table 2 – Indicators of carbohydrate metabolism in the study groups at baseline and after 3 months of therapy with thioctic acid

Indicator	Main group (n = 32)		Control group (n = 32)	
	Baseline	After 3 months of therapy	Baseline	After 3 months of therapy
Glucose variability	7.93±3.41	6.34±3.15	7.61±4.07	6.45±2.19
HbA1c level	8.2 [7.5; 9.6]	7.45*[1] [6.4; 8.2]	8.4 [7.3; 9.8]	7.9*[2]*[3] [6.84; 9.1]
Average daily insulin dose	0.97 [0.7; 1.01]	0.99 [0.81; 1.18]	0.91 [0.67; 1.14]	0.96 [0.75; 1.26]

Note: *[1]p=0.013 compared to baseline; *[2]p=0.019 compared to baseline; *[3]p=0.033 compared to group 1.

Table 3 – Indicators of lipid metabolism in the study groups at baseline and after 3 months of therapy with thioctic acid

Indicator	Main group (n = 32)		Control group (n = 32)	
	Initially	After 3 months of therapy	Initially	After 3 months of therapy
Total cholesterol	4.34 [3.91; 5.22]	4.07*[1] [3.69; 4.51]	4.28 [3.79; 5.31]	4.22*[2]*[3] [3.72; 4.98]
Triglycerides	0.82 [0.48; 1.26]	0.78 [0.46; 1.08]	0.79 [0.59; 1.21]	0.80 [0.58; 1.24]
LDLP	2.83 [2.33; 3.04]	2.25*[4] [2.07; 2.88]	2.96 [2.30; 3.11]	2.90 [2.28; 3.12]
HDLP	1.75 [1.26; 2.06]	1.89*[5] [1.34; 2.27]	1.78 [1.22; 2.14]	1.81 [1.20; 2.17]
Atherogenic index	1.89 ± 0.82	1.78± 0.71	1.87 ± 0.78	1.89±0.97

Note: *[1]p=0.028 compared to baseline; *[2]p=0.039 compared to baseline; *[3]p=0.031 compared to group 1; *[4]p=0.015 compared to baseline; *[5]p=0.044 compared to baseline.

disorders in 14 children in the study group (43.7%) and in 12 children in the control group (15.6%) were manifested as an increase in total cholesterol above the target values (>4.5 mmol/l). In 10 of them in the first group and in 9 in the second, an increase in LDLP was determined (>2.5 mmol/l). At the same time, the atherogenic index was within the normal range. In most children in the study group, the level of TG was within the reference values, however, in 5 children of the intervention group (15.6%) and in 7 children of the control group (21.8%) it was increased.

By the end of the study, the lipid levels had reached significant differences between the groups. The analysis of lipid spectrum indicators in the main and control groups revealed a statistically significant decrease in the level of total cholesterol (p=0.028 and p=0.039, respectively). It should be notified that by the end of the observation, a decrease in total cholesterol had been notified only in the children of the control group with a significant decrease in the level of glycated hemoglobin and glycemia (21.8%; 7/32). However, in the children of the intervention group who had been receiving lipoic acid, this indicator was significantly lower at the end of the study (p = 0.033).

The TG level had a pronounced downward trend in almost all patients in the main group (25/32), but it was not statistically significant (p = 0.22), and did not change in the control group, while there was no significant difference between the groups (p =0.29). However, it turned out that the children who had been receiving lipoic acid were significantly more likely to have a decrease in this indicator relative to outcomes (71.8% vs 28.1%, p = 0.024). The presence of positive changes in blood lipids in the comparison group can be explained by the improved control of hyperglycemia, as well as increased adherence of patients to medical prescrip-

Table 4 – Indicators of basic microcirculation and the amplitude-frequency spectrum of blood flow fluctuations in T1DM children in the area without arteriolo-venular anastomoses before therapy

Indicator	Main group (n = 32)	Control group (n = 32)	
	Initially	Initially	p
M	6.64 [4.37; 8.71]	6.28 [4.28; 7.57]	p=0.324
σ	0.845 [0.7; 0.96]	0.86 [0.7; 0.97]	p=0.76
Cv	12.89 [9.81; 16.34]	14.29 [10.01; 19.39]	p=0.042
P_{eff}	1.43 [1.14; 2.91]	1.51 [1.33; 2.06]	p=0.31
Ae	0.16 [0.12; 0.21]	0.19 [0.16; 0.27]	p=0.68
ET	4.67 [3.38; 6.08]	4.28 [3.21; 5.89]	p=0.46
Ae/3σ	7.16 [5.52; 9.95]	7.27 [5.32; 10.05]	p=0.37
Ae/M	2.61 [1.6; 4.18]	3.41 [1.4; 5.11]	p=0.56
An	0.21 [0.16; 0.3]	0.26 [0.21; 0.4]	p=0.34
NT	3.87 [2.87; 5.29]	3.89 [2.6; 5.84]	p=0.047
An/3σ	8.62 [6.81; 11.67]	8.44 [6.74; 10.61]	p=0.63
An/M	3.62 [1.84; 6.88]	3.04 [1.68; 7.34]	p=0.52
Am	0.23 [0.17; 0.34]	0.32 [0.21; 0.38]	p=0.08
MT	3.43 [2.22; 5.09]	3.2 [2.69; 4.58]	p=0.54
Am/3σ	7.84 [5.97; 13.63]	8.01 [6.03; 14.28]	p=0.28
Am/M	2.66 [1.95; 6.5]	3.02 [2.09; 7.86]	p=0.65
Ar	0.22 [0.17; 0.38]	0.28 [0,14; 0,42]	p=0.39
Ar/3σ	6.72 [5.16; 10.43]	6.84 [6.02; 9.89]	p=0.27
Ar/M	4.89 [3.14; 6.07]	4.62 [3.23; 5.54]	p=0.57
Ac	0.26 [0.24; 0.51]	0.24 [0.21; 0.49]	p=0.84
Ac/3σ	14.02 [11.03; 18.29]	12.82 [12.43; 17.49]	p=0.047
Ac/M	5.62 [3.25; 8.94]	5.17 [3.07; 7.36]	p=0.778
Ac/Ar	1.7 [1.22; 2.35]	1.57 [1.17; 2.67]	p=0.28
SI	0.94 [0.88; 1.09]	0.93 [0.75; 1.12]	p=0.08
IVT	0.66 [0.59; 0.85]	0.57 [0.56; 0.64]	p=0.37

Note: M – mean perfusion; σ – standard amplitude deviation of blood flow fluctuations; Cv – coefficient of variation; P_{eff} – effective perfusion; Ae – fluctuations of neurogenic nature; ET – endothelial tone; An – neurogenic fluctuations; NT – neurogenic tone; Am – fluctuations of myogenic nature; MT – myogenic tone; Ar – fluctuations of respiratory nature; Ac – fluctuations of cardiac nature; A/M – amplitude of fluctuations relative to mean perfusion; A/3σ – amplitude of fluctuations relative to mean modulation of blood flow; SI – shunt index; IVT – intravascular tone.

tions, strict adherence to dietary recommendations and lifestyle modifications during their participation in the study.

It has been established that ALA increases the synthesis of coenzyme A, promotes the transfer of fatty acids and acetate into the mitochondrial matrix, and also has a positive lipotropic effect [3, 9, 28]. Recent studies have shown that ALA shifts the spectrum of blood lipids towards unsaturated fatty acids, reduces the content of total cholesterol, and increases the HDLP fraction.

In the process of observation, in the main group of the children treated with lipoic acid, there was a statistically significant increase in HDLP (p=0.044). There were no significant changes in the control group, while the indicator, in general, tended to increase (p=0.19). The positive dynamics in LDLP was observed in the main group. By the end of the study, the level of this indicator had decreased (p=0.015), in the control group it had not changed significantly in most patients, while, in general, it had also tended to decrease in the group (p=0.24). These studies are consistent with the results obtained by other authors for T1DM and T2DM patients and indicate the ability of ALA to have a direct effect on lipid metabolism. So, Wollin S. et al. reported an increase in HDLP levels with the use of ALA [32] . In 2009, Gianturco V. et al. established that taking ALA at the dose of 400 mg/day reduces the indicators of an oxidative stress and the antiatherogenic fraction of cholesterol in T2DM patients [16]. In another study, Zhang Y. et al. also found out a decrease in cholesterol, LDLP and TG (p <0.01) with the use of ALA [33].

Cardiovascular autonomic neuropathy is detected at the early stages of the disease in DM patients and can be subclinical, i.e., it is manifested only in special tests [1, 4, 5, 22, 32]. The study of cardiovascular reflexes, which have a high sensitivity and a good reproducibility, is “a gold standard” for the clinical DCAN detection [1, 3, 5, 9]. Resting tachycardia is often an early clinical sign of developing neuropathy. In DCAN, the vagus nerve is the first to be affected, which leads to an increase in sympathetic influences on the heart and the appearance of resting tachycardia. In this study, resting tachycardia was initially detected in 11 children in the main group (34.4%) and in 10 children in the

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Table 5 – Indicators of basic microcirculation and amplitude-frequency spectrum of blood flow fluctuations in T1DM children in the area without arteriolo-venular anastomoses after 3 months of therapy with thioctic acid

Indicator	Main group (n = 32)			Control group (n = 32)		
	Initially	After 3 months	p	Initially	After 3 months	p
M	6.64 [4.37; 8.71]	6.21 [4.26; 7.68]	p=0.061	6.28 [4.28; 7.57]	6.01 [4.18; 8.11]*	p=0.234
s	0.845 [0.7; 0.96]	1.09 [0.82; 1.56]	p=0.035	0.86 [0.7; 0.97]	0.84 [0.66; 1.07]*	p=0.67
Cv	12.89 [9.81; 16.34]	27.06 [14.97; 28.71]	p=0.0004	14.29 [10.01; 19.39]	19.63 [9.72; 22.12]*	p=0.048
P_{eff}	1.43 [1.14; 2.91]	2.2 [1.58; 4.28]	p=0.034	1.51 [1.33; 2.06]	1.64 [1.18; 2.94]*	p=0.12
Ae	0.16 [0.12; 0.21]	0.3 [0.18; 0.42]	p=0.008	0.19 [0.16; 0.27]	0.21 [0.19; 0.37]*	p=0.07
ET	4.67 [3.38; 6.08]	4.22 [3.03; 5.1]	p=0.004	4.28 [3.21; 5.89]	4.18 [3.09; 4.99]*	p=0.37
Ae/3s	7.16 [5.52; 9.95]	7.91 [6.52; 10.99]	p=0.33	7.27 [5.32; 10.0]	7.65 [6.13; 9.97]*	p=0.28
Ae/M	2.61 [1.6; 4.18]	5.54 [2.31; 9.93]	p=0.019	3.41 [1.4; 5.11]	3.34 [1.82; 7.63]*	p=0.45
An	0.21 [0.16; 0.3]	0.28 [0.19; 0.6]	p=0.054	0.26 [0.21; 0.4]	0.24 [0.18; 0.34]*	p=0.23
NT	3.87 [2.87; 5.29]	3.19 [2.26; 4.37]	p=0.03	3.89 [2.6; 5.84]	4.01 [2.73; 6.09]*	p=0.048
An/3s	8.62 [6.81; 11.67]	8.55 [5.5; 12.82]	p=0.71	8.44 [6.74; 10.6]	8.69 [5.8; 11.67]*	p=0.54
An/M	3.62 [1.84; 6.88]	5.07 [3.8; 10.72]	p=0.07	3.04 [1.68; 7.34]	3.17 [1.75; 6.99]*	p=0.43
Am	0.23 [0.17; 0.34]	0.4 [0.24; 0.6]	p=0.09	0.32 [0.21; 0.38]	0.34 [0.17; 0.34]*	p=0.09
MT	3.43 [2.22; 5.09]	3.29 [2.19; 4.88]	p=0.234	3.2 [2.69; 4.58]	3.18 [2.39; 4.46]*	p=0.44
Am/3s	7.84 [5.97; 13.63]	9.19 [5.5; 14.96]	p=0.63	8.0 [6.03; 14.28]	8.14 [6.3; 13.44]*	p=0.37
Am/M	2.66 [1.95; 6.5]	7.45 [4.43; 11.58]	p=0.02	3.02 [2.09; 7.86]	3.24 [1.91; 8.03]*	p=0.56
Ar	0.22 [0.17; 0.38]	0.185 [0.13; 0.26]	p=0.04	0.28 [0.14; 0.42]	0.26 [0.15; 0.36]*	p=0.28
Ar/3s	6.72 [5.16; 10.43]	7.44 [6.57; 10.04]	p=0.12	6.84 [6.02; 9.89]	7.24 [5.75; 10.0]*	p=0.38
Ar/M	4.89 [3.14; 6.07]	3.3 [2.98; 3.52]	p=0.04	4.62 [3.23; 5.54]	4.97 [3.01; 5.87]*	p=0.65
Ac	0.26 [0.24; 0.51]	0.26 [0.22; 0.5]	p=0.89	0.24 [0.21; 0.49]	0.26 [0.22; 0.43]*	p=0.73
Ac/3s	14.02 [11.03; 18.29]	8.5 [6.72; 11.75]	p=0.0003	12.82 [12.43; 17.49]	10.42 [8.73; 15.27]**	p=0.02
Ar/M	5.62 [3.25; 8.94]	6.07 [3.56; 8.64]	p=0.36	5.17 [3.07; 7.36]	5.92 [3.34; 8.04]*	p=0.67
Ar/Ac	1.7 [1.22; 2.35]	1.26 [1.0; 1.64]	p=0.021	1.57 [1.17; 2.67]	1.63 [1.34; 2.12]*	p=0.19
SI	0.94 [0.88; 1.09]	0.92 [0.65; 1.17]	p=0.33	0.93 [0.75; 1.12]	0.96 [0.95; 1.28]*	p=0.07
IVT	0.66 [0.59; 0.85]	0.46 [0.38; 0.63]	p=0.003	0.57 [0.56; 0.64]	0.54 [0.48; 0.91]*	p=0.28

Note: M – mean perfusion; σ – standard amplitude deviation of blood flow fluctuations; Cv – coefficient of variation; P_{eff} – effective perfusion; Ae – fluctuations of neurogenic nature; ET – endothelial tone; An – neurogenic fluctuations; NT – neurogenic tone; Am – fluctuations of myogenic nature; MT – myogenic tone; Ar – fluctuations of respiratory nature; Ac – fluctuations of cardiac nature; A/M – amplitude of fluctuations relative to mean perfusion; A/3σ – amplitude of fluctuations relative to mean modulation of blood flow; SI – shunt index; IVT – intravascular tone; * – p<0.05 compared to the main group; ** – p>0.05 compared to the main group.

control group (31.25%). At the end of the study, resting tachycardia was observed only in 5 children in the main group (15.63%; p=0.08), and in 7 children in the control group (21.88%; p=0.4).

When performing a slow breathing test in the main group, there was an increase in the difference between the minimum and maximum heart rates (HRs) after a course of drug therapy with lipoic acid (initially it was 12.04±5.41, after 3 months – 17.18±2.14; p<0.001). In the control group, the increase was not statistically significant (initially – 13.14±4.75, after 3 months – 14.36±3.98; p=0.27).

During the Schelong test, there was a decrease in the blood pressure fall in the children treated with lipoic acid (initially it was 18.0±8.4, after 3 months – 11.45±7.8; p=0.002). According to D. Ewing, in the control group, there were no significant changes in the results of cardiovascular tests.

Since the largest number of parasympathetic and sympathetic fibers are located in the sinus and atrioventricular nodes of the heart conduction system, changes in the vegetative status significantly affect functioning of the cardiac conduction system, contribute to the development of atrioventricular tachycardia and life-threatening ventricular arrhythmia. The cause of a sudden cardiac death in diabetic patients may be a neuropathic prolongation of the QT interval, which is associated with changes in sympathetic and parasympathetic functions [1, 3, 4, 10, 22, 23, 33]. Therefore, the study of the heart rate variability, the measurement of the corrected QT interval and the dispersion of the QT interval are necessary methods in the diagnosis of autonomic neuropathy. In this study, a statistically significant decrease in the corrected QT interval in the main group was not obtained, however, a tendency to its normalization was revealed (initially, it was 0.452 [0.431; 0.467], after 3 months – 0.446 [0.425; 0.455], p = 0.063). During the initial examination, the heart rate variability was reduced in every third child in both groups (in the main group n=10, 31.25%; in the control group n=11, 34.4%). After 3 months, according to the Holter ECG monitoring, the heart rate variability was observed only in 6 children in

the main group (18.75%; p=0.09), in the control group – in 10 children (31.2%). After the treatment course in the main group, there was a trend towards normalization of temporary heart rate indicators (initially, pNN50% was 16 [8; 23], after 3 months it was 21 [19; 36], p=0.03; initially, SDNN was 128 [109; 166], after a month it was 164.5 [115; 172], p=0.048). In the control group, no statistically significant change in the indicators of time analysis was received. When assessing the spectral analysis of the heart rate variability in the children treated with lipoic acid, an increase in slow waves was revealed (initially, VLF were 2403 [1698; 3132], after 3 months – 3417 [2443; 4621], p = 0.01), in the control group there was a tendency to a decrease in waves in the slow range (initially, VLF were 2632 [1758; 3956], after 3 months – 2412 [1703; 3423], p=0.07).

Thus, the administration of lipoic acid improves a heart parasympathetic regulation in T1DM children. This is consistent with the results of a randomized study of vegetative-vascular regulation (VVR) in Korea, in which the authors found a positive trend in some parameters of VVR in DM patients who had been taking ALA 600 mg/day p. o. for the first 12 weeks and 1200 mg/day for the next 12 weeks. The DCAN study showed an improvement in the heart rate variability compared to placebo (p<0.05) in T2DM patients with impaired VVR who had been on ALC therapy at the dose of 800 mg/day [21].

In the groups under study, no statistically significant changes in the sympatho-parasympathetic balance index and daily blood pressure profile were found out.

Violation of microcirculation is the cause for nerve hypoxia, which is involved in the pathogenesis of diabetic neuropathy. It has been established that in DCAN patients, blood oxygen saturation and blood flow velocity in the vessels supplying the nerves, are reduced. These data indicate the importance of vascular factors along with hyperglycemia in the pathogenesis of neuropathy [3, 4, 7–9, 33, 35]. The prospective results of this microcirculation study are presented in Tables 4 and 5. Initially, the groups were comparable to each other (Table 4).

In the analysis of the basic microcirculation in the children of the studied groups, no pronounced change in the mean perfusion was detected, while a statistically significant increase in the coefficient of variation was observed, and in the main group, there was also an increase in the standard deviation of the amplitude of blood flow fluctuations (Table 5). These changes may be associated with an improved glycemic control, accompanied by a decrease in the glycemic variability during a day, which contributed to the functioning improvement of microvasculature regulatory systems. This assumption proves a significant increase in the effective perfusion index during treatment with lipoic acid in the main group (p=0.034).

When assessing fluctuations in the active tonus-forming range, in the children of the main group, a decrease in the endothelial-dependent component of the vascular tone (p=0.0004) was revealed. It was combined with an increase in the activity of fluctuations in the endothelial tonus-forming range (p=0.008) and an increase in the normalized amplitudes of the endothelial range relative to the mean perfusion (p=0.019). In the control group, no statistically significant difference in the amplitudes of fluctuations in the endothelial range was obtained, however, there was a tendency to increase them after improving glycemic control (p=0.07). In the main group, at the end of the treatment course, a decrease in the neurogenic component of vascular tone was observed (p=0.03), a tendency to an increase in the amplitudes of fluctuations in the active neurogenic range (p=0.54) was revealed. In the control group, an increase in the neurogenic component of the vascular tone was diagnosed (p=0.048), which indicates a gradual progression of neuropathy against the background of stable compensation of carbohydrate metabolism. The results obtained are confirmed by the DCCT study (The Diabetes Control and Complications Trial), which proved that after achieving a stable compensation of carbohydrate metabolism, the regression of diabetic neuropathy is doubtful [12].

When assessing the amplitude spectrum in the passive range against the background of therapy in the main group, a decrease in the amplitude of fluctuations in the respiratory range was observed (p=0.04).

The total indicator of intravascular tone on the background of the therapy in the main group significantly decreased (p=0.003). These changes may indicate the restoration of compensatory mechanisms due to an increase in the influence of active tone-forming factors and a decrease in intravascular tone. These trends indicate an improvement in microcirculation due to a decrease in the tone of metarteriols and precapillary sphincters in the microvasculature and, as a result, an improvement in the nutritional blood flow. Against the background of the therapy, the total indicator of the intravascular tone in the main group significantly decreased (p=0.003). These changes may indicate the restoration of compensatory mechanisms due to an increase in the influence of active tone-forming factors and a decrease in the intravascular tone. These trends indicate an improvement in microcirculation due to the decrease in the tone of metarteriols and precapillary sphincters in the microvasculature and, as a result, an improvement in the nutritional blood flow.

CONCLUSION

Thus, the inclusion of lipoic acid in the complex therapy of T1DM children, leads to an improvement in the course of the disease, contributes to the normalization of carbohydrate and lipid metabolism. The results obtained indicate the need to achieve and maintain optimal glycemic control with the absence of high glycemic variability, especially in the children with the first DCAN

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signs but this does not guarantee a complete regression of neuropathy manifestations.

To diagnose the initial manifestations of DCAN by studying the functional state of the microvasculature and its reserve capabilities, it is rational to operate with modern non-invasive LDF methods, which can be also used to control the ongoing therapy. The use of lipoic acid preparations at the daily dose of 600 mg for 3 months in the complex therapy in T1DM children with DCAN improves glycemic control, blood lipids, and also leads to an increase in vasomotor mechanisms of a tissue blood flow regulation due to an increase in endothelial and neurogenic activities accompanied by a decrease in intravascular tone and an increase in effective perfusion in tissues. The observed positive dynamics of D. Ewing cardiovascular tests, time (pNN50%, SDNN) and spectral parameters (VLF) of the heart rate variability during lipoic acid therapy can be explained, among other things, by the antioxidant and neuroprotective effects of the drug. The carried out study makes it possible to suggest that the use of thioctic acid at the dose of 600 mg/day for 3 months in the complex therapy of T1DM children at the preclinical stage of DCAN, leads to a regression of nerve fiber damage. The use of the LDF method for an early DCAN diagnosis and monitoring the effectiveness of therapy makes it possible to implement a personalized approach to the implementation of preventive treatment of T1DM children.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

Natalya V. Malyuzhinskaya, Ivan N. Shishimorov, Olga V. Magnitskaya –concept and design of the study, literature analysis, interpretation and visualization of results, text writing; Olga V. Polyakova, Grigory V. Klitochenko, Irina V. Petrova, Svetlana A. Emelyanova – research methodology, interpretation and visualization of results, text writing; Ksenia V. Stepanenko, Anna P. Skiba – conducting functional tests and other studies, statistical processing of results, interpretation of results, text writing.

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AUTHORS

Natalya V. Malyuzhinskaya – Doctor of Sciences (Medicine), Professor, Head of the Department of Children's Diseases, Pediatric Faculty, Volgograd State Medical University. ORCID ID: 0000-0003-4624-8813. E-mail: maluzginskaia@yandex.ru

Ivan N. Shishimorov – Doctor of Sciences (Medicine), Associate Professor, Head of the Department of Pediatrics and Neonatology, the Institute of Continuing Medical and Pharmaceutical Education, Volgograd State Medical University. ORCID ID: 0000-0001-6098-7028. E-mail: drshishimorov@gmail.com

Olga V. Magnitskaya – Doctor of Sciences (Medicine), Associate Professor, Professor of the Department of Pediatrics and Neonatology, the Institute of Continuing Medical and Pharmaceutical Education, Volgograd State Medical University. ORCID ID: 0000-0001-6670-9029. E-mail: magol73@yandex.ru

Ksenia V. Stepanenko – Assistant of the Department of Children's Diseases of the Pediatric Faculty, Volgograd State Medical University. ORCID ID: 0000-0001-8167-0003. E-mail: kozhevnikova.kv@yandex.ru

Olga V. Polyakova – Candidate of Sciences (Medicine), Associate Professor, Associate Professor of the Department of Children's Diseases of the Pediatric Faculty, Volgograd State Medical University. ORCID ID: 0000-0001-6006-8136. E-mail: olvlpolyakova@gmail.com

Grigory V. Klitochenko – Doctor of Sciences (Medicine), Associate Professor, Professor of the Department of Children's Diseases of the Pediatric Faculty, Volgograd State Medical University. ORCID ID: 0000-0001-9341-2298. E-mail: klitoch@mail.ru

Irina V. Petrova – Candidate of Sciences (Medicine), Associate Professor, Associate Professor of the Department of Children's Diseases, Faculty of Pediatrics, Volgograd State Medical University. ORCID ID: 0000-0003-0091-4980. E-mail: irina-petrova_09@mail.ru

Svetlana A. Emelyanova – Candidate of Sciences (Medicine), Associate Professor, Volgograd Regional Children's Clinical Hospital. ORCID ID: 0000-0001-5049-4078. E-mail: vodkb@volganet.ru

Anna P. Skiba – post-graduate student of the Department of Children's Diseases, Faculty of Pediatrics, Volgograd State Medical University. ORCID ID: 0000-0002-4901-3043. E-mail: anna-patashova@rambler.ru

RESEARCH ARTICLE

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EVALUATION OF ABCB1-PROTEIN INHIBITION PROSPECTIVENESS IN HEMATOENCEPHALIC BARRIER AS METHOD FOR INCREASING EFFICIENCY OF PHARMACOTHERAPY IN CEREBRAL ISCHEMIA

I.V. Chernykh, A.V. Shchulkin, M.V. Gatsanoga, E.N. Yakusheva

Ryazan State Medical University named after academician I.P. Pavlov
9, Vysokovoltnaya St., Ryazan, Russia, 390026

E-mail: ivchernykh88@mail.ru

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The aim of the article is to evaluate the ABCB1 protein inhibition in the blood-brain barrier to increase the effectiveness of neuroprotective therapy for focal and global cerebral ischemia.

Materials and methods. The work was performed on 103 male Wistar rats. In the 1st group (n=33), the neuroprotective activity of the ABCB1 protein substrate, nimodipine (0.4 mg/kg), was analyzed in terms of reducing the area of the brain necrosis after a 1-hour occlusion of the middle cerebral artery with a 24-hour reperfusion (focal ischemia). In the 2nd group (n=60), the effectiveness of nimodipine was analyzed by reducing the lethality of rats and the neurological deficit (ND) level against the background of the bilateral occlusion of the common carotid arteries (global ischemia). In both groups, nimodipine was used alone or in the combination with omeprazole, the ABCB1 protein inhibitor (17.6 mg/kg). The drugs were administered intravenously.

Results. The nimodipine administration to the 1st group led to the reduction of the necrosis focus by 28% compared with the control series. Omeprazole did not cause a change in the area of the necrosis. The combination of drugs caused a decrease in the area of the necrosis in relation to the control by 29%; there were no differences in comparison with the nimodipine series. Nimodipine reduced the rats' lethality in the 2nd group against the background of the pathology (a tendency). Omeprazole alone did not change the mortality. The drug combination reduced the mortality compared to the control and nimodipine series. The administration of omeprazole alone did not reduce the neurological deficit score relative to the control. In the nimodipine series, ND was 88% lower than the control, after 24 hours. With the administration of the drugs combination, this indicator decreased in relation to the control by 88%, 80%, 88%, 87% and 86% after 4, 12, 24, 48 and 72 hours, respectively, and in relation to the nimodipine series it decreased by 60% and 67% after 4 and 48 hours.

Conclusion. The ABCB1 protein inhibition is promising for increasing the effectiveness of neuroprotective therapy for global ischemia, but not for focal cerebral ischemia.

Keywords: ABCB1 protein; occlusion-reperfusion of the middle cerebral artery; bilateral occlusion of the common carotid arteries; nimodipine; omeprazole

Abbreviations: BBB – blood-brain barrier; TTC – triphenyltetrazolium chloride; ATP – adenosine triphosphate; creb – cAMP response element-binding; Akt – protein kinase B (intracellular protein kinase B enzyme); IC_{50} – half-maximal (50%) inhibitory concentration.

ОЦЕНКА ПЕРСПЕКТИВНОСТИ ИНГИБИРОВАНИЯ ABCB1-БЕЛКА В ГЕМАТОЭНЦЕФАЛИЧЕСКОМ БАРЬЕРЕ КАК МЕТОДА ПОВЫШЕНИЯ ЭФФЕКТИВНОСТИ ФАРМАКОТЕРАПИИ ЦЕРЕБРАЛЬНОЙ ИШЕМИИ

И.В. Черных, А.В. Щулькин, М.В. Гацанога, Е.Н. Якушева

Федеральное государственное бюджетное образовательное учреждение высшего образования
«Рязанский государственный медицинский университет имени академика И.П. Павлова»
Министерства здравоохранения Российской Федерации
390026, Россия, г. Рязань, ул. Высоковольтная, д. 9

E-mail: ivchernykh88@mail.ru

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Цель. Оценка ингибирования ABCB1-белка в гематоэнцефалическом барьере с целью повышения эффективности нейропротекторной терапии фокальной и глобальной церебральной ишемии.

Материалы и методы. Работа выполнена на 103 крысах-самцах линии Вистар. На 1-й группе (n=33) анализировали нейропротекторную активность субстрата ABCB1-белка – нимодипина (0,4 мг/кг) по снижению площади некроза головного мозга после 1-часовой окклюзии средней мозговой артерии с 24-часовой реперфузией (фокальная ишемия). На 2-й группе (n=60) эффективность нимодипина анализировали по снижению летальности крыс и уровня неврологического дефицита (НД) на фоне билатеральной окклюзии общих сонных артерий (глобальная ишемия). В обеих группах нимодипин использовался отдельно или в сочетании с ингибитором ABCB1-белка – омепразолом (17,6 мг/кг). Препараты вводились внутривенно.

Результаты. Введение нимодипина 1-й группе привело к сокращению очага некроза на 28% по сравнению с серией контроля. Омепразол не вызвал изменения площади некроза. Комбинация препаратов вызвала снижение площади некроза по отношению к контролю на 29%, в сравнении с серией нимодипина различий не было. Нимодипин сокращал летальность крыс 2-й группы на фоне патологии (тенденция). Омепразол не изменял летальность. Комбинация препаратов снижала летальность по сравнению с сериями контроля и нимодипина. Введение омепразола не сокращало балл неврологического дефицита относительно контроля. В серии нимодипина НД был ниже контроля через 24 ч. на 88%. При введении комбинации препаратов данный показатель снижался по отношению к контролю через 4, 12, 24, 48 и 72 ч. на 88%, 80%, 88%, 87% и 86%, а по отношению к серии нимодипина через 4 и 48 ч – на 60% и 67%.

Заключение. Ингибирование ABCB1-белка является перспективным для повышения эффективности нейропротекторной терапии глобальной ишемии, но не фокальной ишемии мозга.

Ключевые слова: ABCB1-белок; окклюзия-реперфузия средней мозговой артерии; билатеральная окклюзия общих сонных артерий; нимодипин; омепразол

Список сокращений: ГЭБ – гематоэнцефалический барьер; ТТХ – трифенилтетразолия хлорид; АТФ – аденозинтрифосфат; CREB – белок, связывающийся с цАМФ-зависимым элементом; АКТ – внутриклеточный фермент протеинкиназы B; IC_{50} – концентрация полумаксимального ингибирования.

INTRODUCTION

P-glycoprotein (ABCB1-protein, Pgp) is a product of the MDR1 gene expression, as well as a membrane efflux ATP-dependent transporter protein with a wide range of substrates different in their chemical structure. In significant amounts, it is localized on the biliary surface of hepatocytes, in the proximal tubules of the renal nephrons, in the enterocytes of the small intestine and in the endotheliocytes of the histohematic barriers. One of the leading functions of the ABCB1 protein in the blood-brain barrier (BBB) is to prevent the penetration of lipophilic endogenous and exogenous substrate substances from the blood into the brain. It should be notified that the functional activity of the ABCB1 protein can vary significantly against the background of various influences, i.e., the inflammatory process, oxygen deficiency, oxidative stress, food intake, and a lot of drugs [1].

By far, a stroke is the leading cause of disability in the adult population and the second leading cause of death in the world. In 85% of cases, an ischemic type of stroke occurs. At the same time, the use of a tissue plasminogen activator, which is one of the few approved drugs for restoring a blood flow, is limited by a narrow “therapeutic window” (4.5 hours). Thus, the preferred therapeutic strategy for a stroke is neuroprotection, e. i. maintaining the viability of neurons in the penumbra zone [2].

Among the ABCB1 protein substrates, there is a number of drugs with a proven or potential neuroprotective activity: nimodipine, acetylcholinesterase inhibitors rivastigmine, donepezil, galanthamine, the antipsychotic drug paliperidone, and a number of others [3–5]. The substances inefficiency of this pharmacological group in clinical trials, demonstrated by foreign researchers [6], may be due to their insufficient transport through the BBB [7], for example, due to ABCB1-protein efflux. The indirect evidence of such a theory is the activity induction and expression of the transporter against the background of oxygen deficiency, the main pathogenetic link in cerebral ischemia, which was revealed in *in vitro* and *in vivo* experiments [8, 9]. Due to the wide spread of ischemic stroke and chronic cerebral ischemia in the world and, in particular, in Russia [10], the study of such pathogenetic mechanisms and an attempt to overcome them is beyond doubt.

The analysis of the available scientific data suggested the prospect of pharmacological inhibition of the ABCB1 protein in the BBB against the background of cerebral ischemia in order to intensify the delivery of neuroprotectors to the brain and enhance their central effects. The selectivity of reducing the local ABCB1 protein activity in the barrier will minimize the number of pharmacokinetic and pharmacodynamic complications associated with the important functions of the transporter in controlling not only penetration into the brain, but also the enteral absorption and excretion of the drug substrates [1].

THE AIM of the article is to evaluate the ABCB1 protein inhibition in the blood-brain barrier, to increase the effectiveness of pharmacotherapy for the consequences of focal and global kinds of cerebral ischemia in the *in vivo* experiment.

MATERIALS AND METHODS

Laboratory animals

The work was performed on 103 male Wistar rats weighing 200–280 g. The animal manipulations were carried out in accordance with the rules of good laboratory practice (Order of the Ministry of Health of the Russian Federation dated April 1, 2016, No. 199n, and also with international standards (Guide for the Care and Use of Laboratory Animals: VIII ed.) under the operating conditions of a conventional vivarium, and approved by the Commission for the Control of the Maintenance and Use of Laboratory Animals (Protocol No. 7 dated April 3, 2018) of Ryazan State Medical University. The surgical interventions were carried out against the background of the intraperitoneal administration of Zoletil® 50 (INN – tiletamine, zolazepam; Virbac, France) to the rats at the dose of 10 mg/kg. The intravenous administration of the drugs was carried out in the rats' tail vein.

Experiment design

The experimental animals were divided into 2 groups.

In the first group (n=33), the possibility of the ABCB1 protein pharmacological inhibition was analyzed in order to increase the treatment effectiveness of the focal cerebral ischemia transporter with neuroprotective drugs – substrates. That kind of ischemia was an experimental analogue of an ischemic stroke in humans [11]. A neuroprotective activity was assessed by a decrease in the area of the necrosis focus against the background of the brain ischemia-reperfusion. It had been modeled by a 60-minute endovascular occlusion of the middle cerebral artery with a polypropylene thread with a diameter of 4–0 (0.15–0.199 mm) together with a permanent ligation of the common and external carotid arteries followed by recanalization. During the surgical procedures and for 2 hours after them, the animals were warmed up with lamps to maintain the rectal temperature at 37°C. The animals were withdrawn from the experiment 24 hours after the reperfusion by the overdose of Zoletil (30 mg/kg). A similar model is widely used in scientific studies [12, 13]. The rats with a lethal outcome were not included in the study and not reflected in the total number.

The animals of the first group were divided into 4 series, each of 7 animals: Series 1 – the animals with ischemia-reperfusion with a saline intravenous administration (1 ml/kg) at the time of the middle cerebral artery reperfusion. The rats of the 2nd series were subjected to the ischemia-reperfusion with an intravenous injection of the ABCB1 protein substrate, a cerebral vasodilator with a neuroprotective activity, nimodipine (Nimotop, Russia), at the dose of 0.4 mg/kg of the animal body weight (the solution 0.4 mg/ml – 1 ml/kg) [14] at the time of reperfusion. The neuroprotective activity of nimodipine, regardless of the cell type, had been shown in numerous studies both *in vitro* and *in vivo*. According to the latest data, the main role is assigned to the prevention of a stress-induced apoptosis by reducing the activity of caspase-3 and 7, as well as by activating the transcription factor, the protein that binds to the cAMP-responsible element (creb) and the intracellular enzyme protein kinase B (Akt) of signaling pathways [15]. The animals of the 3rd experimental series were the rats with ischemia-reperfusion with an intravenous injection of the ABCB1 protein inhibitor in the BBB, omeprazole (Omez, Russia) at the dose of 17.6 mg/kg of body weight (the solution 17.6 mg/ml – 1 ml/kg) [16, 17] at the time of reperfusion. The 4th series were the animals with ischemia-reperfusion with the introduction of nimodipine in combination with an intravenous injection of omeprazole at the time of recanalization (the drugs were administered sequentially without any interruption).

The dose and scheme of the neuroprotective substrate ABCB1 protein (nimodipine) administration used in this work, is explained by the revealed efficiency in reducing the volume of necrosis in the experiment [14]. Typical neuroprotective drugs (piracetam, etc.) were not used in the work due to the absence of the drugs – substrates of the ABCB1 protein – among them.

The proton pump blocker omeprazole was chosen as a transporter inhibitor. The drugs of this pharmacological group, such as omeprazole, pantoprazole and lansoprazole, demonstrated an inhibitory activity against the ABCB1 protein on Caco-2 and L-MDRI cell cultures with a half-maximal inhibition concentration (IC_{50}) of 17.7, 17.9 and 62.8, respectively. μM, which was revealed by the degree of translocation of the transporter substrate, digoxin [17]. When omeprazole is administered to rats intravenously at the dose of 3.45 mg/kg, its maximum plasma concentration is about 3.5 μM [16]. To obtain 17.7 μM (IC_{50} in relation to the ABCB1 protein for this substance), it must be administered by 5.1 times more, i.e. 17.6 mg/kg. This is confirmed by the linearity of the pharmacokinetics of omeprazole with its single administration [18].

Despite the fact that omeprazole slightly reduces the activity of microsomal liver enzymes *in vitro* [19], its administration to the rats at the indicated dose cannot affect the intensity of nimodipine biotransformation, due to the fact that only the CYP3A isoform is involved in the metabolism of the latter [20].

In addition, 5 animals underwent "a sham operation" with opening the skin and soft tissues of the neck without any direct occlusion-reperfusion of the arteries to confirm the absence of the influence of experimental manipulations on the results of the study.

To assess the brain necrosis zone in the animals of all the series, the brain was removed, washed in an isotonic sodium chloride solution, followed by freezing and cutting in the frontal plane into sections 2 mm thick. Next, the sections were placed in a Petri dish, 10 ml of phosphate buffer with pH=7.4 was added to them, then

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2 ml of a 2% solution of triphenyltetrazolium chloride (TTC) was added and then 2 ml of a 2% solution of sodium succinate was added. The sections were kept in the indicated mixture in the thermostat at 38°C for 1 h, then at room temperature in 10% neutral formalin for also 1 h. Subsequently, the sections were photographed with a Canon Power Shot G5 digital camera. The percentage ratio of the sum of the TTX-negative zones areas to the sum of the total brain area was estimated [21].

Additionally, to explain the results obtained in the first group of the animals, the integrity of the BBB was studied against the background of the occlusion-reperfusion of the middle cerebral artery. Herewith, the degree of the Evans blue dye accumulation (Evans blue, Sigma, USA) in the brain tissue by the intravenous administration of a 2% isotonic solution in the volume dose of 0.4 ml at 100 g in the tail vein at the time of reperfusion was analyzed. Under the deep anesthesia, immediately before decapitation, the rats underwent transcardial perfusion with 100 ml of an isotonic solution to remove the intravascular dye. Then the animals were withdrawn from the experiment with the extraction of the cerebral cortex (the side supplied by the occluded artery) and its grinding with scissors. The dye was extracted by homogenizing the tissue in dimethylformamide (1 ml at 100 mg of the tissue) at 26,000 rpm for 1 min and followed by incubating at 60°C for a day in a thermostat. The resulting suspension was centrifuged for 5 min at 1750 g.

The Evans blue concentration was determined spectrophotometrically (Bio-Rad spectrophotometer, USA) at 620 nm. The quantitative determination was carried out by the method of an external standard in a similar solvent [22]. The experiment was repeated on 5 rats, 5 rats were used as control, which reproduced "the sham operation".

In the second group of rats (n=60), the prospects of the ABCB1 protein pharmacological inhibition were evaluated in order to increase the effectiveness of treatment with neuroprotective drugs that are substrates of the global cerebral ischemia transporter, an experimental pathology similar in pathogenesis to cerebral hypoperfusion in humans [23]. The neuroprotective potential was analyzed by the decrease in the percentage of the animals' deaths and the severity of a neurological deficit according to the McGrow Stroke-index scale modified by I.V. Gannushkina against the background of a bilateral ligation of the common carotid arteries lasting 4, 12, 24, 48, and 72 hours. In each series, a number of rats with mild (0.5–2.5 points), moderate (2.5–5.5 points), and severe (5.5–10 points) neurological deficits was assessed. This scale is recommended for assessing the neurological status of animals in preclinical studies[1].

The animals of the second group were divided into 5 series: the 1st (n=6) were sham-operated animals (a control series), the 2nd (n=14) were the rats that were simulated cerebral ischemia by the bilateral occlusion of the common carotid arteries intravenously administrated with physiological saline (1 ml/kg) 30 minutes before pathology (the pathology control). Group 3 (n=13) were the animals that were intravenously injected with the neuroprotector nimodipine, a substrate of the ABCB1 protein, at the dose of 0.4 mg/kg 30 min before the arterial ligation. The 4th group (n=11) were the rats that were intravenously injected with a transporter inhibitor omeprazole at the dose of 17.6 mg/kg body weight 30 min before the pathology. The 5th group (n=16) were the animals with cerebral ischemia, intravenously injected with a combination of nimodipine and omeprazole in the similar doses 30 min before pathology modeling.

Statistical processing of results

The results of the study were processed using the Statistica 13.0 program. The nature of the data distribution was assessed by the Shapiro-Wilk test. To compare the size of the necrosis zone, the ANOVA analysis of variance and the Newman-Keuls test for a pairwise comparison were used. The comparison of the animals' neurological deficit level was performed using the Kruskal-Wallis test (an analogue of the analysis of variance for the data distributed in a non-normal way), pairwise comparisons – the Mann-Whitney test with the Bonferroni correction, which allows analyzing samples of different sizes. The animals' survival in the postoperative period was assessed by constructing Kaplan-Meier survival curves. The survival comparison was assessed by the Cox F-test. The differences were considered significant at the confidence level more than 95%.

RESULTS

In the first group, the lethality of the animals after modeling the occlusion-reperfusion of the middle cerebral artery was 22.7±1.1% and did not differ in the groups ($p>0.05$). There was no mortality in the group of "sham-operated" animals.

Fig. 1 shows the brain sections of the experimental animals' samples in various series.

In all the experimental series, in percent, the sizes of the necrosis foci are shown in Table 1 (arithmetic mean ± standard deviation).

A 60-minute occlusion of the middle cerebral artery followed by recanalization for 24 hours, led to the formation of a necrosis focus in the control rats' brain with a relative area of 32.2±7.1%. The similar results correspond to the literature data [24].

An isolated administration of the L-type calcium channel blocker neuroprotector nimodipine to the animals at the time of the middle cerebral artery reperfusion led to a significant decrease in the size of the necrotic lesion by 27.7% compared with the pathology control group ($p<0.05$).

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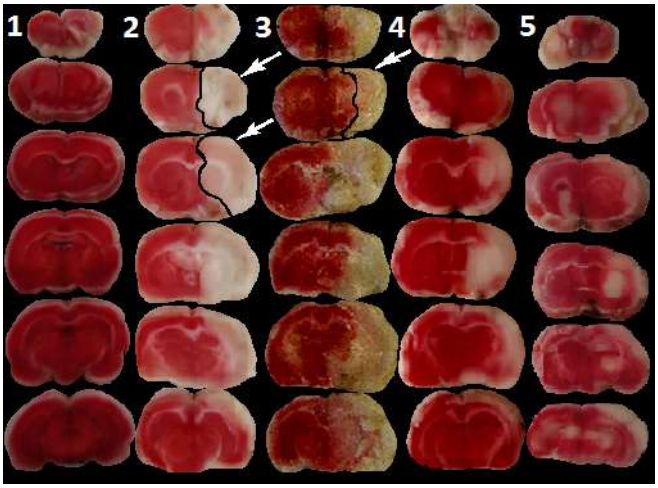

Figure 1 – Samples of rats' brain sections after the occlusion-reperfusion of the middle cerebral artery
Note: 1 – sham operation; 2 – pathology control; 3 – omeprazole; 4 – nimodipine; 5 – combination of nimodipine and omeprazole; arrows indicate the area of brain necrosis.

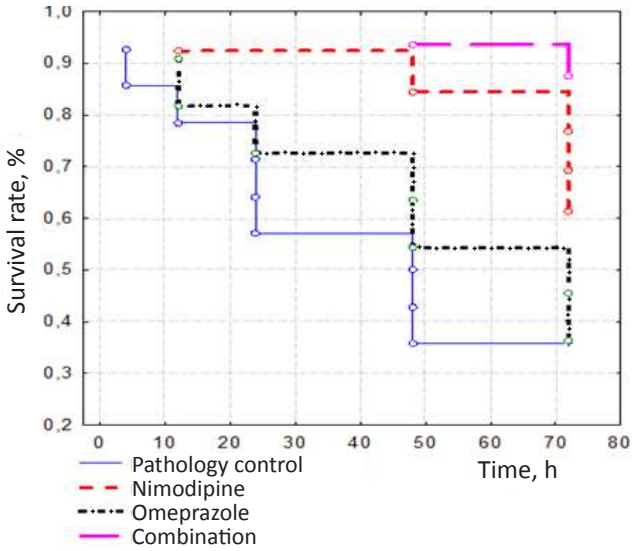

Figure 2 – Graph of cumulative proportion of survived animals according to Kaplan-Meier in different experimental series

Table 1 – Relative area of the rats' brain necrosis zone against the background of occlusion-reperfusion of the middle cerebral artery

Experiment series	Relative area of necrosis, %
Sham operation	0
Control	32.2±7.1
Omeprazole	34.4±7.6
Nimodipine	23.3±4.7*#
Nimodipine + omeprazole	23.0±2.8*#

Note: * – significant differences with the parameter of the pathology control series; # – significant differences with the omeprazole series.

Table 2 – The content of Evans blue dye in the rats' brain against the background of occlusion-reperfusion of the middle cerebral artery

Experiment Series	Dye level, μg/g brain
Sham operation, n=5	2.39±0.85
Occlusion-reperfusion of the middle cerebral artery, n=5	11.55±8.40

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Table 3 – The degree of neurological deficit according to McGrow in the modification by I.V. Gannushkina against the background of bilateral occlusion of the common carotid arteries

Series	Degree of neurological deficit, points				
	4 hr	12 hr	24 hr	48 hr	72 hr
Sham operation,	0.5±0.11	0	0	0	0
Pathology control	4.5 (2.0; 5.5)	5.0 (2.0; 10.0)	8.5 (1.5; 10.0)	7.5 (1.5; 10.0)	7.0 (2.0; 10.0)
Nimodipine	2.5 (2.5; 2.5)	3.0 (1.0; 4.0)	1.0 (1.0; 2.5)*#	3.0 (2.0; 3.0)#	1.5 (1.0; 2.0)#
Omeprazole	2.5 (2.5; 3.5)	3.5 (2.0; 5.0)	3.5 (3.0; 10.0)"	10.0 (3.5; 10.0)"	10.0 (4.5; 10.0)"
Combination	1.0 (0.0; 1.0)*#"	2.0 (1.0; 5.0)*	1.0 (1.0; 3.0)*#	1.0 (0.0; 3.0)*#"	1.0 (1.0; 3.0)*#

Note: * – significant differences with the pathology control group (p<0.05); # – significant differences with the omeprazole group (p<0.05); " – significant differences with the nimodipine group (p<0.05). Data are presented as median, lower and upper quartiles.

An isolated administration of the ABCB1 protein inhibitor omeprazole to the animals did not cause a statistically significant change in the area of the necrosis focus (p>0.05).

A combined administration of nimodipine and omeprazole to the rats caused a decrease in the area of necrosis compared to the pathology control by 28.8% (p<0.05), and in relation to the omeprazole group – by 33.2% (p<0.05). However, no statistically significant changes between the groups of the drugs combination and an isolated administration of nimodipine have been revealed (p>0.05).

The occlusion-reperfusion of the rats' middle cerebral artery led to the impaired BBB permeability, which was confirmed by the accumulation of Evans blue diazo dye in the ischemic brain tissue of the animals. Normally, it does not penetrate into the brain due to the strong bonds with blood plasma albumins [22]. Against the background of the ischemia-reperfusion, the level of the dye in the brain exceeded that of the sham-operated animals by 4.83 times (p<0.05) (Table 2).

The appearance of the dye in the non-ischemic brain of sham-operated rats is probably due to its adsorption on the vessel wall after the perfusion procedure.

Fig. 2 shows a graph of the survived animals' cumulative proportion in the second group according to Kaplan-Meier. None of the sham-operated animals died during the entire observation period. The animals' deaths in the pathology control group was observed 4 hours after the operation; by the end of the first day, 57.1% of the rats died.

The injection of nimodipine before cerebral ischemia led to a decrease in the number of dead animals compared with the pathology control series at the tendency level (0.05<p<0.1). The administration of omeprazole before ischemia did not cause significant changes in the lethality of the animals compared with ischemia control. The combination of nimodipine and omeprazole resulted in the lower mortality in the both pathology control series and in the nimodipine administration series (p<0.05).

The severity and manifestations of ischemia were clinically analyzed by assessing neurological abnormalities according to the McGrow scale modified by I.V. Gannushkina (Table 3). The sham-operated animals showed no signs of neurological deficit (with the exception of minor changes after 4 hours, probably associated with the recovery from anesthesia). After the bilateral occlusion of the common carotid arteries, the survived of animals showed such symptoms as lethargy, unilateral and bilateral blepharoptosis, general tremor, circling behavior, paresis of the extremities. The score of neurological deficit in the pathology control group increased until the end of the first day after the surgery, then decreased, but at all the periods, except 4 hours, it was classified as severe.

Thus, the permanent occlusion of the common carotid arteries of the rats was accompanied by a high mortality of the animals and the development of a severe neuropsychiatric deficit.

The omeprazole injection to the rats 30 min before pathology did not lead to a significant reduction in the neurological deficit score compared to the pathology control at any of the observed periods (p>0.05). In the animals treated with nimodipine, the score of neurological deficit after the surgery was lower than in the control animals: after 24 hours – by 88.2% (p<0.05); after 4 and 72 hours – by 44.4% and 16.0%, respectively, at the trend level (0.05<p<0.1). The administration of nimodipine also led to a decrease in the level of neurological deficit compared to the omeprazole group: after 24 hours – by 71.4% (p<0.05), after 48 hours – by 70.0% (p<0.05) and after 72 hours – by 85.0% (p<0.05).

The administration of a substrate and the ABCB1 protein inhibitor combination to the rats resulted in a decrease in the neurological deficit score compared to all the experimental series. In relation to the pathology control, the level of neurological deficit was lower after 4, 12, 24, 48 and 72 hours by 87.5% (p<0.05), 80.0% (at the level of a pronounced trend, p=0.05), 88.2% (p<0.05), 86.7% (p<0.05) and 85.7% (p<0.05), respectively. In comparison with the group of the omeprazole administration, after 4, 24, 48 and 72 hours it was lower by 60.0% (p<0.05), 71.4% (p<0.05), 90.0% (p<0 .05) and 90.5% (p<0.05), respectively; and compared with the nimodipine series, after 4 hours it was lower by 60.0% (p<0.05), after 48 hours – by 66.7% (p<0.05).

DISCUSSION

The study analyzed the feasibility of the local pharmacological inhibition of the ABCB1 protein in the BBB as a way to increase the effectiveness of neuroprotectors in cerebral ischemia. Moreover, two pathological models were chosen: global ischemia, which causes damage to the white matter of the brain, similar to that in chronic cerebral hypoperfusion in humans [23], and focal ischemia, an experimental analogue of an ischemic stroke [11]. Rats were chosen as a test system for analyzing the ABCB1 protein functioning against the background of cerebral ischemia, due to the demonstrated 93% similarity of the substrates and the modulators spectrum of the transporter activity in these animals and humans [25].

To date, the attempts to reduce the activity of the ABCB1 protein transporter to intensify the delivery of cytotoxic agents to tumor cells and overcome the phenomenon of multidrug resistance (in the formation of which the hyperfunction of the ABCB1 protein plays an important role) have not been successful due to a significant number of pharmacokinetic and pharmacodynamic limitations. On the other hand, the strategy of the ABCB1 protein inhibition in the BBB in the experiments to increase the delivery of drugs, in particular neuroprotective drugs, to the brain, is being successfully developed. Thus, in nonhuman primates, the efficiency of reducing the activity of the transporter in the BBB by infusion of the specific inhibitor elacridar in order to increase the cerebral penetration of the ABCB1 protein substrate, erlotinib was found [26]. It was also established by positron emission tomography that the permeability of the baboon barrier for the labeled radioactive substrate ABCB1-protein [(11)C]-N-desmethyl-loperamide against the background of an intravenous administration of a therapeutic dose (15 mg/kg/h) of cyclosporine (a transporter inhibitor), was significantly increased [27].

The results of the carried out work indicate that a local decrease in the activity of the ABCB1 protein in the BBB during focal cerebral ischemia is not justified. A decrease in the activity of the transporter at the system level, which is more easily achievable in practice, is dangerous due to the changes in the pharmacokinetics of its substrates and the possibility of their relative overdose. A probable reason for the obtained results is an increase in the permeability of the BBB against the background of the occlusion-reperfusion of the middle cerebral artery, which is a typical consequence of cerebral ischemia. In addition, in a previous work, the authors even found a decrease in the functional activity of the ABCB1 protein in the BBB when modeling the occlusion-reperfusion of the middle cerebral artery. That had been revealed by the accumulation degree of the transporter marker substrate, fexofenadine, in the brain [28], despite an increase in the amount of ABCB1-protein against the background of focal cerebral ischemia. In this regard, the inefficiency of a combined use of the neuroprotective substrate ABCB1 protein with the inhibitor of the transporter functional activity, is probably a consequence of a large area of the brain damage in which the structure of the BBB is disturbed, and its permeability is already maximum. This assumption was confirmed by the authors' analysis of the Evans blue dye accumulation in the brain against the background of the applied experimental pathology. At the same time, the ABCB1 protein, most likely, is no longer an obstacle to the penetration of nimodipine into the brain tissue, so the drug has the same maximum therapeutic effect as when administered in isolation.

Other dynamics was obtained by the authors using the model of global cerebral ischemia. In this kind of ischemia, such a pronounced violation of the BBB integrity probably does not occur. The ABCB1 protein pharmacological inhibition in the barrier led to a more significant cerebroprotective effect of nimodipine, manifested by a decrease in mortality and the severity of neurological deficit in the animals against the background of the bilateral occlusion of their common carotid arteries. This is probably due to the penetration intensification of the neuroprotector into the brain due to a decrease in the efflux activity of the transporter. Thus, the authors believe, they can speak about the expediency of reducing the functional activity of the ABCB1 protein in neurological diseases, the pathogenesis of which includes global cerebral ischemia.

CONCLUSION

The combination of the inhibitor and the ABCB1 protein substrate, omeprazole and nimodipine, respectively, does not increase the neuroprotective potential of the latter (the necrosis zone does not significantly decrease), and the ABCB1 protein inhibition cannot be considered as a promising aim to increase the effectiveness of pharmacotherapy in a focal cerebrovascular accident. This phenomenon is probably associated with a violation of the BBB integrity, in which the barrier functions of the transporter no longer play a significant role in the control of its substrates cerebral accumulation. However, such a tactic is justified in global cerebral ischemia, which is confirmed by a significant decrease in mortality and the level of neurological deficit in the animals with this pathology against the background of the combined use of these agents.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

Ivan V. Chernykh – performing experiments on animals (modeling pathologies), article writing; Aleksey V. Shchulkin – statistical processing of the results obtained; Maria V. Gatsanoga – work with laboratory animals (drugs administration, brain sections staining, neurological deficit and mortality assessment); Elena N. Yakusheva – research design planning, article writing.

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AUTHORS

Ivan V. Chernykh – Candidate of Sciences (Biology), Associate Professor, Head of the Department of Pharmaceutical Chemistry, Ryazan State Medical University. ORCID ID: 0000-0002-5618-7607. E-mail: ivchernykh88@mail.ru

Aleksey V. Shchulkin – Doctor of Sciences (Medicine), Associate Professor, Professor of the Department of Pharmacology with the Course of Pharmacy, Ryazan State Medical University. ORCID ID: 0000-0003-1688-0017. E-mail: alekseyshulkin@rambler.ru

Maria V. Gatsanoga – Candidate of Sciences (Medicine), Assistant of the Department of Pharmacology with the Course of Pharmacy, Ryazan State Medical University. ORCID ID: 0000-0002-1116-6271. E-mail: mvgatsanoga@mail.ru

Elena N. Yakusheva – Doctor of Sciences (Medicine), Professor, Head of the Department of Pharmacology with a Course in Pharmacy, Ryazan State Medical University. ORCID ID: 0000-0001-6887-4888. E-mail: e.yakusheva@rzgmu.ru

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EFFECTIVENESS AND SAFETY OF FAVIPIRAVIR INFUSION IN PATIENTS HOSPITALIZED WITH COVID-19

L.A. Balykova[1], K.Ya. Zaslavskaya[2], V.F. Pavelkina[1], N.A. Pyataev[1], N.M. Selezneva[1], N.V. Kirichenko[3], A.Yu. Ivanova[4,5], G.V. Rodoman[6], K.B. Kolontarev[7,8], K.S. Skrupsky[8], E.N. Simakina[9,10], O.A. Mubarakshina[11], A.V. Taganov[12], D.Yu. Pushkar[7,8]

[1] National Research Ogarev Mordovia State University,
68, Bol'shevistskaya Str., Saransk, Republic of Mordovia, Russia, 430005
[2] Limited Liability Company "Promomed RUS"
Bld. 1, 13, Mir Ave., Moscow, Russia, 129090
[3] Ivanovo Clinical Hospital named after the Kuvaevs
Bld. 2, 52, Ermak Str., Ivanovo, Russia, 153025
[4] Regional Clinical Hospital,
Bld. A, 3, Internatsionalnaya Str., Ryazan, Russia, 390039
[5] Ryazan State Medical University named after academician I.P. Pavlova
9, Vysokovoltnaya Str., Ryazan, Russia, 390026
[6] Municipal clinical hospital No. 24, Moscow City Health Department
10, Pistsovaya Str., Moscow, Russia, 127015
[7] Moscow State Medical and Dental University named after A.I. Evdokimov
Bld. 1, 20, Delegatskaya Str., Moscow, Russia, 127473
[8] City Clinical Hospital named after S.I. Spasokukotsky, Moscow City Health Department
21, Vuchetich Str., Moscow, Russia, 127206
[9] Smolensk clinical hospital No.1
40, Frunze Str., Smolensk, Russia, 214006
[10] Smolensk State Medical University
28, Krupskoy Str., Smolensk, Russia, 214019
[11] Voronezh State Medical University named after N.N. Burdenko
10, Studencheskaya Str., Voronezh, Russia, 394036
[12] Peoples' Friendship University
6, Miklukho Maclaya Str., Moscow, Russia, 117198

E-mail: larisabalykova@yandex.ru

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Research in the development of new therapeutic agents with a wide spectrum of the antiviral activity and a low ability to develop resistance remains the main dimension in combating the global threat to public health. The need for a parenteral form of favipiravir was dictated by the necessity to increase the efficacy of therapy in COVID-19 inpatients. This dosage form has expanded the possibilities of drug therapy in the inpatients, for whom a therapeutic effect acceleration and a high safety profile of the drugs used are especially important.

The aim of the article is the evaluation of the efficacy and safety of a medicinal product containing favipiravir for the parenteral administration against the background of pathogenetic and symptomatic therapy, in comparison with standard therapy in hospitalized COVID-19 patients.

Materials and methods. An open, randomized, multicenter comparative study was conducted in 6 research centers in the Russian Federation to evaluate the efficacy and safety of favipiravir, a lyophilisate for the preparation of a concentrate for the infusion solution administrated to the patients hospitalized with COVID-19. Screening procedures and randomization were completed in 217 patients, 209 of which had completed the study in accordance with the protocol.

Results. Between the study groups, statistically significant differences have been found out, making it possible to consider the hypothesis of the drug Areplivir (favipiravir) superiority for the parenteral administration over the standard therapy, which included favipiravir (p. o.) and remdesivir. A comparative analysis has shown that a course of therapy with the paren-

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teral favipiravir drug leads to a significant improvement in the condition of patients with COVID-19, significant benefits in terms of the speed and frequency of improvement in the clinical status of patients, as well as a reduction in the hospital stay length. It has been proven that therapy with a drug containing favipiravir for the parenteral administration does not adversely affect the parameters of clinical and biochemical blood tests, urinalysis, coagulograms, vital signs and ECG, which indicates the therapy safety. The study drug is characterized by a high safety profile and tolerability.

Conclusion. The versatility and resistance to mutations of RNA-dependent RNA polymerase make it possible to consider it as the main target for combating the most common RNA viruses that cause ARVI, that determines the need further studies of favipiravir to expand the range of its indications.

Keywords: favipiravir; COVID-19; SARS-CoV-2; novel coronavirus infection; areplivir

Abbreviations: COVID-19 – novel coronavirus infection (Coronavirus disease 2019); AV – Artificial ventilation; GIT – gastrointestinal tract; ECMO – Extracorporeal membrane oxygenation; ITT – population of all included (Intent-to-treat) patients; PP – the population of patients who completed the study according to the protocol (Per protocol); NAATs – nucleic acid amplification techtiques; NYHA – New York Heart Association; e-IRK – electronic individual registration card; ARDS – Acute Respiratory Distress Syndrome; CRP – c-reactive protein; ESR – erythrocyte sedimentation rate; NAATs – Nucleic Acid Amplification Techniques; GIT – gastrointestinal tract; HR – heart rate; AspAT – aspartate transaminase; ALT – alanine aminotransferase; ULN – upper limit of normal; UDE – undesirable effects; IGs – Interim Guidelines; NSAIDs – non-steroidal anti-inflammatory drugs; GGT – gamma-glutamyl transpeptidase; CK – creatine kinase.

ЭФФЕКТИВНОСТЬ И БЕЗОПАСНОСТЬ ИНФУЗИОННОГО ВВЕДЕНИЯ ФАВИПИРАВИРА У ПАЦИЕНТОВ, ГОСПИТАЛИЗИРОВАННЫХ С COVID-19

Л.А. Балыкова[1], К.Я. Заславская[2], В.Ф. Павелкина[1], Н.А. Пятаев[1], Н.М. Селезнева[1], Н.В. Кириченко[3], А.Ю. Иванова[4,5], Г.В. Родоман[6], К.Б. Колонтарев[7,8], К.С.Скрупский[8], Е.Н. Симакина[9,10], О.А. Мубаракшина[11], А.В. Таганов[12], Д.Ю. Пушкарь[7,8]

[1] Федеральное государственное бюджетное образовательное учреждение высшего образования «Национальный исследовательский Мордовский государственный университет имени Н.П. Огарева»
430005, Россия, Республика Мордовия, г. Саранск, ул. Большевистская, д. 68

[2] Общество с ограниченной ответственностью «Промомед ДМ»
129090, Россия, г. Москва, пр-т Мира, д. 13, стр. 1

[3] Областное бюджетное учреждение здравоохранения «Ивановская клиническая больница имени Куваевых»
153025, Россия, г. Иваново, ул. Ермака, д. 52/2

[4] Государственное бюджетное учреждение Рязанской области «Областная клиническая больница»,
390039, Россия, г. Рязань, ул. Интернациональная, д. 3а

[5] Федеральное государственное бюджетное образовательное учреждение высшего образования «Рязанский государственный медицинский университет имени академика И.П. Павлова» Министерства здравоохранения Российской Федерации
390026, Россия, г. Рязань, ул. Высоковольтная, д. 9

[6] Государственное бюджетное учреждение здравоохранения «Городская клиническая больница №24 Департамента здравоохранения города Москвы»
127015, Россия, г. Москва, ул. Писцовая, д. 10

[7] Федеральное государственное бюджетное образовательное учреждение высшего образования «Московский государственный медико-стоматологический университет имени А.И. Евдокимова» Министерства здравоохранения Российской Федерации
127473, Россия, г. Москва, ул. Делегатская, д. 20, стр. 1

[8] Государственное бюджетное учреждение здравоохранения города Москвы «Городская клиническая больница имени С.И. Спасокукоцкого Департамента здравоохранения города Москвы»
127206, Россия, г. Москва, ул. Вучетича, д. 21

[9] Областное государственное бюджетное учреждение здравоохранения «Клиническая больница №1»
214006, Россия, г. Смоленск, ул. Фрунзе, д. 40

[10] Федеральное государственное бюджетное образовательное учреждение высшего образования «Смоленский государственный медицинский университет» Министерства здравоохранения Российской Федерации
214019, Россия, г. Смоленск, ул. Крупской, д. 28

[11] Федеральное государственное бюджетное образовательное учреждение высшего образования «Воронежский государственный медицинский университет имени Н.Н. Бурденко» Министерства здравоохранения Российской Федерации
394036, Россия, г. Воронеж, ул. Студенческая, д. 10

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[12] Федеральное государственное автономное образовательное учреждение высшего образования «Российский университет дружбы народов» (РУДН)
117198, г. Москва, ул. Миклухо Маклая, д. 6

E-mail: larisabalykova@yandex.ru

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Основным направлением по борьбе с глобальной угрозой здоровью населения остаются исследования в области создания новых терапевтических средств с широким спектром противовирусной активности и низкой способностью к развитию резистентности. Потребность в парентеральной форме фавипиравира была продиктована необходимостью повышения эффективности терапии у стационарных больных с COVID-19. Данная лекарственная форма расширила возможности медикаментозной терапии у стационарных пациентов, для которых особенно важно ускорение наступления терапевтического эффекта и высокий профиль безопасности применяемых препаратов.

Цель. Оценка эффективности и безопасности применения лекарственного препарата, содержащего фавипиравир для парентерального введения на фоне патогенетической и симптоматической терапии в сравнении со стандартной терапией у пациентов, госпитализированных с COVID-19.

Материалы и методы. В 6 исследовательских центрах на территории России проведено открытое рандомизированное многоцентровое сравнительное исследование по оценке эффективности и безопасности применения фавипиравира, лиофилизат для приготовления концентрата для приготовления раствора для инфузий у пациентов, госпитализированных с COVID-19. Процедуры скрининга и рандомизации прошли 217 пациентов, из них 209 завершили исследование полностью в соответствии с протоколом.

Результаты. Выявлены статистически значимые различия между исследуемыми группами, позволяющие считать доказанной гипотезу превосходства препарата Арепливир (фавипиравир) для парентерального введения над стандартной терапией, которая включала фавипиравир (перорально) и ремдесивир. Сравнительный анализ показал, что курс терапии лекарственным препаратом фавипиравир для парентерального применения приводит к существенному улучшению состояния пациентов с COVID-19, значимым преимуществам в отношении скорости и частоты улучшения клинического статуса пациентов, а также сокращению длительности пребывания в стационаре. Доказано, что терапия лекарственным препаратом, содержащим фавипиравир для парентерального введения, не оказывает негативного влияния на показатели клинического и биохимического анализа крови, общего анализа мочи, коагулограммы, на показатели жизненно важных функций и ЭКГ, что свидетельствует о безопасности проводимой терапии. Исследуемый препарат характеризуется высоким профилем безопасности и хорошей переносимостью.

Заключение. Универсальность и устойчивость к мутациям РНК-зависимой-РНК-полимеразы позволяют рассматривать ее как основную мишень для борьбы с наиболее распространенными РНК-вирусами – возбудителями ОРВИ, что определяет необходимость проведения дальнейших исследований фавипиравира для расширения спектра его показаний.

Ключевые слова: фавипиравир; COVID-19; SARS-CoV-2; новая коронавирусная инфекция; Арепливир

Список сокращений: COVID-19 – новая коронавирусная инфекция; ИВЛ – искусственная вентиляция легких; ЖКТ – желудочно-кишечный тракт; ЭКМО – экстракорпоральная мембранная оксигенация; ITT – популяция всех включенных пациентов; PP – популяция пациентов, завершившая исследование согласно протоколу; МАНК – метод амплификации нуклеиновых кислот; NYHA – Нью-Йоркская ассоциация кардиологов; э-ИРК – электронная индивидуальная регистрационная карта; ОРДС – острый респираторный дистресс-синдром; СРБ – с-реактивный белок; СОЭ – скорость оседания эритроцитов; МАНК – методы амплификации нуклеиновых кислот; ЖКТ – желудочно-кишечный тракт; ЧСС – частота сердечных сокращений; АСТ – аспартатаминотрансфераза; АЛГ аланинаминотрансфераза; ВГН – верхняя граница нормы; НЯ – нежелательное явление; ВМР – временные методические рекомендации; НПВП – нестероидные противовоспалительные препараты; ГГТ – гамма-глутамилтранспептидаза; КФК – креатинкиназа.

INTRODUCTION

Coronavirus infection (SARS-CoV-2), first recorded in December 2019 in Wuhan (China), is currently the cause of the ongoing COVID-19 pandemic. This disease is characterized by damage to the lungs tissue, the development of acute respiratory distress syndrome, kidney damage, the formation of thromboembolic complications, the development of multiple organ failure, septic shock, and deaths [1]. Most patients have a mild or moderate severity of the disease, however, in 5–10% of patients, COVID-19 acquires a severe and even life-threatening course [2, 3]. Since December 2019, SARS-CoV-2 has infected hundreds of millions of people around the world, claiming millions of lives. Thus, according to the published data of Worldometers.info[1] as of February 8, 2022, 398,671,423 cases of SARS-CoV-2 infection were registered in 225 countries of the world, 5,771,021 of which were fatal. As for Russia, on February 8, 2022, 13,147,666 cases of coronavirus infection were registered, and the number of deaths reached 336,721.

A new disease is putting an unprecedented loading on the global health system, leading to massive increases in hospitalizations. In November 2021, a new strain of Omicron was first identified in southern Africa, becoming the dominant variant of SARS-CoV-2 in most parts of the world, including Russia. It has been notified that the protection of modern vaccines in relation to the new strain is significantly reduced [4–6]. In addition, there is a huge pool of immunocompromised patients in whom vaccination is not effective enough [7, 8]. According to the Interim Guidelines of the Ministry of Health of the Russian Federation for the Prevention, Diagnosis and Treatment of the Novel Coronavirus Infection, regardless of their version, the main approach to the treatment of COVID-19 should be preemptive before the development of a full symptom complex of life-threatening conditions,

[1] Worldometer – real time world statistics. Available from: https://www.worldometers.info/coronavirus/#countries.

i. e. pneumonia, ARDS, sepsis[2]. The international medical community agrees that an early control of the viral RNA replication and the use of targeted antiviral therapy is the most important element in improving the prognosis of the disease by reducing the increasing viral load and preventing the development of complications [9–11]. At the same time, the data from the observational studies confirm that during bronchoscopy in critically ill and/or ventilated patients, a high level of SARS-CoV-2 viral load is determined; in these patients, this is the main predictor of death from COVID-19. The authors conclude that in order to improve the prognosis of patients hospitalized with COVID-19, the priority should be the therapy aimed at reducing a virus replication [12].

A number of antiviral drugs are currently under investigation and have not been approved for the treatment of COVID-19. Therefore, today it is obvious that the further development and study of specific antiviral therapy is of paramount importance for the treatment and prevention of a new coronavirus infection [13].

One of the promising drugs used for antiviral therapy of coronavirus infection is favipiravir, a selective RNA polymerase inhibitor, a synthetic antiviral drug that is active against a wide range of different RNA-containing viruses. The active form of the drug (favipiravir-RTF) acts on the RNA-dependent RNA polymerase of the virus. The first mechanism of action of the drug on the virus is the inclusion in the viral RNA chain or binding to the preserved polymerase domains, which leads to the prevention of the viral RNA replication and ultimately leads to the disappearance of the viral genome, as well as a decrease in the infection transmission. The second is the inclusion of favipiravir-RTF into the replicating viral RNA, which leads to lethal mutagenesis, which accelerates the elimination of the viral agent [14]. Thus, we can say that favipiravir has a targeted effect on the life processes of the virus in the body, and this explains the versatility of its application in the treatment of various diseases caused by RNA-containing viruses, including influenza and coronavirus infection.

The possibility of using favipiravir for the treatment of COVID-19 was proposed in February 2020. Currently, it is the most studied molecule for the targeted antiviral COVID-19 therapy with proven efficacy and a high safety profile. Analyzing the publication activity of scientific articles indexed in PubMed and ScienceDirect, mentions of the favipiravir molecule are the most frequent, which may indicate a high interest in this molecule from the medical community, especially in the context of the COVID-19 pandemic. On the multidisciplinary platform ScienceDirect (Elsevier publishing house) on request (INN + COVID-19, period 2020–2022), indexed publications: Oseltamivir – 203, Favipiravir – 686, Molnupiravir – 129, Remdesevir – 85. In the database of medical and biological publications PubMed, on request (INN+COVID-19, period 2020–2022), indexed publications: Oseltamivir – 197, Favipiravir – 672, Molnupiravir – 113, Remdesevir-13. The keywords for the search were INNs and "COVID-19". The search was carried out during the period from 2020 to 2022. As a result, the following numbers of publications were indexed on the ScienceDirect multidisciplinary platform (Elsevier publishing house)[3]: Oseltamivir – 203, Favipiravir – 686, Molnupiravir – 129, Remdesevir – 85. In the PubMed database[4], the numbers of the indexed publications were as follows: Oseltamivir – 197, Favipiravir – 672, Molnupiravir – 113, Remdesevir – 13.

At the moment, clinical studies have proven the efficacy and safety of using the tablet form of favipiravir for the SARS-CoV-2 treatment and prevention [14–19]. Moreover, according to the latest data, Omicron strains are sensitive to favipiravir [20].

One of the ways to increase the treatment efficacy is to increase the bioavailability of the drug, which affects the efficacy and safety profile. In COVID-19, lesions of the gastrointestinal tract such as an increase in the activity of gastrointestinal enzymes, are known in more than 20% of patients. The development of pseudomembranous colitis against the background of the disease, the violation of the intestinal microflora when using combinations of antibiotics, lead to a change in the pharmacokinetic parameters of oral drugs, and hence, reducing their effectiveness [21]. The international medical community has recognized the need to develop and use intravenous favipiravir to improve the therapy efficacy of the hospitalized COVID-19 patients [22, 23].

The first drug containing favipiravir and registered in the Russian Federation, the molecule with a proven activity against SARS-CoV-2, was Areplivir® (the oral form). Later, in 2020, the world's first original Areplivir® was developed and registered in the Russian Federation in the form of a dosage form for the parenteral administration (RU LP-007598).

Phase 1 clinical[5] studies have shown that favipiravir for the parenteral administration has improved pharmacokinetic parameters compared to the oral form, i.e., it provides achieving a 100% bioavailability, a faster and uniform penetration and distribution of the drug in the cells. A decrease in the "Time to reach maximum concentration" indicator, combined with an increase in the area under the pharmacokinetic curve, indicates a lon-

[2] Interim guidelines "Prevention, diagnosis and treatment of a new coronavirus infection COVID-19 (version 13.1. dated 11/17/2021, version 14 dated 12/28/2021

[3] ScienceDirect Search Results – Keywords (favipiravir covid-19). Available from: https://www.sciencedirect.com/search?qs=favipiravir%20covid-19.

[4] Favipiravir, covid 19 – Search Results. Available from: https://pubmed.ncbi.nlm.nih.gov/?term=Favipiravir%2C+covid+19.

[5] An open non-randomized clinical study to assess the safety, tolerability and pharmacokinetic parameters of various doses of AREPLIVIR, a lyophilisate for the preparation of a concentrate for solution for infusion (OOO PROMOMED RUS, Russia) in healthy volunteers. RCT No. 226 dated April 26, 2021 Protocol No. FAV-012021. Available from: https://grlsbase.ru/clinicaltrails/clintrail/11428.

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ger maintenance of a therapeutic concentration in the body while reducing the toxicological load due to the absence of favipiravir peak concentrations, which increases the safety profile of the drug. It should be notified that favipiravir for the parenteral administration is convenient for patients with gastrointestinal symptoms of COVID-19 (nausea, vomiting), as well as with difficulties in swallowing or in a stable prone position.

Parenteral therapy has advantages over the oral route of the drug delivery. It can be used in the situations where a patient is in a serious condition or unconscious, has a difficulty in swallowing or might be in conditions that prevent it (this can be important for gastrointestinal symptoms of COVID-19) and other situations where an oral administration is difficult (incl. ALV, ECMO, etc.). An intravenous route of the drug administration is used for a quick and pronounced result, since the drug immediately enters the bloodstream, the bioavailability is faster and more predictable, and there is no interaction with food or digestive enzymes [24–26].

THE AIM of the article is the evaluation of the efficacy and safety of favipiravir for the intravenous administration in COVID-19 inpatients.

MATERIALS AND METHODS

In accordance with the Recommendation of the Council of the Eurasian Economic Commission dated July 17, 2018 No.11 "On guidelines for general issues of clinical trials", the rules of Good Clinical Practice of the International Conference on Harmonization (ICH GCP), the ethical principles set forth in the Helsinki Declaration of the World Medical Association (Fortaleza, 2013) and the requirements of the Russian legislation (Federal Law No. 61-FZ dated April 12, 2010 "On the Circulation of Medicines"), a phase III clinical trial was conducted. It comprised the following: "An open randomized multicenter comparative study to evaluate the efficacy and safety of Areplivir®, a lyophilisate for the preparation of a concentrate for the preparation of an infusion solution (LLC Promomed RUS, Russia) in the patients hospitalized with COVID-19. The study was approved by the Ministry of Health of the Russian Federation RCT No. 440 (08/11/2021) FAV052021, and was also peer-reviewed in the international registry of clinical trials clinicaltrials.gov (NCT05185284)[6].

1. The research took place from 08/11/2021 to 12/15/2021 on the basis of six research centers in the Russian Federation:
2. Smolensk Clinical Hospital No. 1, Smolensk, Russia;
3. Ivanovo Clinical Hospital n. a. the Kuvaevs", Ivanovo, Russia;
4. Regional Clinical Hospital, Ryazan, Russia;
5. Municipal Clinical Hospital No. 24", Moscow City Health Department, Russia;
6. Moscow State Medical and Dental University n. a. A.I. Evdokimov Moscow, Russia;
7. National Research Mordovian State University n. a. N.P. Ogarev, Saransk, Russia.

The inclusion criteria for this study were: male and female patients aged 18 to 80 years hospitalized with moderate COVID-19; the COVID-19 diagnosis was confirmed by NAATs; obligatory clinical signs were changes at CT, typical for a viral lesion (the volume of the lesion was minimum or medium; CT was 1–2). An additional clinical sign was compliance with 1 of the following criteria: body temperature > 38°C; RR > 22/min; breath shortness during physical exertion; SpO2 < 95%; Serum CRP > 10 mg/l; consent of the patient to use reliable methods of contraception (sexual rest, use of a condom in combination with spermicide) throughout the study and for 1 month for women and 3 months for men after its completion; for men (optional): consent to avoid sexual contact with pregnant women throughout the study and for 3 months after its completion; the women who are unable to bear children, as well as men with infertility or a history of vasectomy.

The criteria for exclusion from the study were: hypersensitivity to favipiravir, remdesivir and/or other components of the study drug; the impossibility of performing a CT procedure; the vaccination history against COVID-19; the presence of a previous probable or confirmed case of COVID-19 of a moderate, severe and extremely severe course; the use of favipiravir or remdesivir within 10 days prior to screening; the need to use the drugs from the list of prohibited therapies; the presence of criteria for severe and extremely severe courses of the disease; the need for treatment in the intensive care unit; abnormal liver function (AST and / or ALT ≥ 2 ULN and / or total bilirubin ≥ 1.5 ULN) at the time of screening; the impaired renal function (GFR < 60 ml/min) at the time of screening; gout in past medical history; a positive test for HIV, syphilis, hepatitis B and / or C; chronic heart failure (III–IV FC) according to the NYHA functional classification; a history of malignant neoplasms; history of alcohol, pharmacological and/or drug dependence at the time of screening and/or in past medical history; schizophrenia, schizoaffective disorder, bipolar disorder, or other psychiatric disorder in past medical history or suspicion of their presence at the time of screening; severe, decompensated or unstable somatic diseases; any history data that, in the opinion of the investigator, may complicate the interpretation of the results of the study or create an additional risk for the patient as a result of his participation in the study; unwillingness or inability of the patient to comply with the procedures of the Protocol (in the opinion of the investigator); pregnant or lactating women, or women planning a pregnancy; participation in another clinical trial within 3 months prior to the enrollment in the study;

[6] Randomized Multicenter Study on the Efficacy and Safety of Favipiravir for Parenteral Administration Compared to Standard of Care in Hospitalized Patients With COVID-19. Promomed, LLC. 11 Jan 2022. Available from: https://clinicaltrials.gov/ct2/show/NCT05185284.

other conditions that, in the opinion of the investigator, prevent the inclusion of the patient in the study.

If any diseases or conditions appeared during the study that worsened the patient's prognosis, made it impossible for the patient to continue participating in the clinical trial, and if it was necessary to prescribe prohibited concomitant therapy/procedures, the patient was excluded from the study.

Study design

The evaluation of the efficacy and safety of the drug was carried out in comparison with the standard therapy provided by the IGs, version 11 dated 05/07/2021 or valid at the time of the study, i.e. the drugs containing favipiravir in the tablet form, the drug containing remdesivir in the form of a lyophilisate for the preparation of a concentrate for an infusion solution.

Patients were randomized using an interactive online system (Interactive web randomization system – IWRS) built into the e-RCI. The population of all included patients (Intent-to-treat) – ITT in the study was 214 (106 patients in the study drug favipiravir group + 108 patients in the standard therapy group). The population of patients who completed the study according to the protocol (Per protocol) – PP was 209 (102 patients in the study drug favipiravir group + 107 patients in the standard therapy group). This met the requirements for the minimum number of patients required for a clinical study – 200 patients (100 patients per group). The groups were comparable in anthropometric, laboratory and clinical baseline parameters (Table 1).

The most common comorbidities were arterial hypertension and other cardiovascular diseases, obesity, type 2 diabetes mellitus, gastrointestinal diseases, etc. Thus, the study included the patients with a high risk of developing life-threatening conditions and worsening prognosis. A comparative analysis of comorbidities and the general condition of patients also did not reveal intergroup differences.

The 1st group received the study drug Areplivir® (favipiravir) for the parenteral administration against the background of pathogenetic and symptomatic therapy presented in the IGs, version 11 (07.05.2021) or valid at the time of the study.

Pharmacotherapy was carried out in hospital according to the following scheme: the 1st day – 1600 mg twice a day; the 2nd–10th days – 800 mg twice a day. The administration of the drug was carried out intravenously by drop infusion for 2 hours.

The 2nd group received standard therapy in accordance with the IGs, version 11 (05/07/2021. (Table 2) or valid at the time of the study.

Discharge from the hospital was carried out in accordance with the local practice of the research center in compliance with the current sanitary and epidemiological regime.

The patients in the study drug group could not additionally receive other etiotropic drugs, as well as monoclonal antibodies with a virus-neutralizing effect (bamlanivimab in monotherapy or in combination with etsevimab, casirivimab in combination with imdevimab), or anti-covid plasma.

The study consisted of the following stages: screening – no more than 24 hours; randomization – not more than 1 day; therapy – 10 days; post-observation – not more than 19 days.

The total duration of each patient's study for was not more than 30 days. The patient's condition was monitored during 6 visits to the research center.

In case of the development of acute respiratory distress syndrome and the need to transfer the patient to the artificial lung ventilation, the use of the study drug was canceled, the patient was not excluded from the study. The patient was followed up to Visit 6, and the data obtained were taken into account to evaluate the efficacy in the ITT population.

Parameters under study

Among the studied parameters there were: a clinical status of patients on the categorial ordinal scale of clinical improvement, the degree of lung damage according to CT, virus elimination, inflammatory markers (CRP, ESR). The presented approach is consistent with the FDA Guidelines on the Development of Drugs for the Treatment and Prevention of COVID-19[7] and the Guidelines of the WHO Working Group on the Clinical Characterization and Treatment of COVID-19 Infection [27].

The primary points for evaluating efficacy were: the frequency of improvement in clinical status on a categorial ordinal scale of clinical improvement by 2 or more categories after 10 days of therapy, as well as the time (in days) to the improvement in clinical status on a categorial ordinal scale of clinical improvement (Table 3).

Additionally, the clinical status at each study visit, the proportion of patients who had achieved clinical status 0 / 1 at the study visits, the degree of lung damage (according to the CT data, according to the "empirical" scale), the rate of the virus elimination (a negative result of a laboratory test for the presence of SARS-CoV-2 RNA by NAATs), SpO2, clinical (hemoglobin, hematocrit, erythrocytes, leukocytes, platelets, ESR, leukocyte formula) and biochemical (AST, ALT, GGT, CPK, triglycerides, total protein, creatinine, urea, uric acid, total bilirubin, glucose, CRP, ferritin, lactate) blood parameters, were assessed. The degree of lung damage was assessed in accordance with the IGs of the Ministry of Health of the Russian Federation for the prevention, diagnosis and treatment of a new coronavirus infection using the scale presented in Table 4.

[7] COVID-19: Developing Drugs and Biological Products for Treatment or Prevention. Available from: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/covid-19-developing-drugs-and-biological-products-treatment-or-prevention.

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Table 1 – Initial indices

Indices	Favipiravir drug group	Standard therapy group
Average age of patients	48.56±12.25 years (from 24 to 76 years)	48.70±13.21 years (from 22 to 74 years)
Average body weight	80.57±15.84 kg (from 50 to 120 kg)	81.03±16.73 kg (from 54 to 153 kg)
Average height	170.37±7.93 cm (from 153 to 194 cm)	170.85±8.38 cm (from 149 to 190 cm)
Comorbidities (95 patients)	47.17%	46,67%

Note: among the randomized patients, there were 113 female patients (52.80%) and 101 male patients (47.20%). The groups were also comparable in terms of gender composition.

Table 2 – Recommended treatment regimens according to IGs, version 11 (07.05.2021)

	No.	Drug	Dosing regimen
Scheme 1	1	Favipiravir	For patients weighing <75 kg: 1600 mg twice daily on the 1st day and then 600 mg twice daily from the 2nd to the 10th days. For patients weighing 75 kg or more: 1800 mg twice daily on the 1st day, then 800 mg twice daily from the 2nd to the 10th days.
	2	Baricitinib	4 mg once daily for 7–14 days
		or	
		Tofacitinib	10 mg twice daily for 7–14 days
		or	
		Netakimab	120 mg in the form of two subcutaneous injections of 1 ml (60 mg) each. Administered once a week on weeks 0, 1 and 2.
	3	Anticoagulant drug for parenteral administration	
	4	NSAIDs according to indications	
Scheme 2	1	Remdesivir	Day 1: 200 mg (in a 0.9% sodium chloride solution) given as a single dose, intravenously. From the 2nd day: 100 mg intravenously once daily. The general course is no more than 10 days.
	2	Baricitinib	4 mg once daily for 7–14 days
		or	
		Netakimab	120 mg in the form of two subcutaneous injections of 1 ml (60 mg) each. Administered once a week on weeks 0, 1 and 2.
	3	Anticoagulant drug for parenteral administration	
	4	NSAIDs according to indications	
Scheme 3	1	Favipiravir	For patients weighing <75 kg: 1600 mg twice daily on the 1st day and then 600 mg twice daily from the 2nd to the 10th days. For patients weighing 75 kg or more: 1800 mg twice daily on the 1st day, then 800 mg twice daily from the 2nd to the 10th days.
	2	Olokizumab	160 mg/ml – 0.4 ml subcutaneously / 0.8 ml as a single dose, intravenously.
		or	
		Levilimab	324 mg (two pre-filled syringes of 162 mg/0.9 ml each) subcutaneously/as a single dose, intravenously.
	3	Anticoagulant drug for parenteral administration	
	4	NSAIDs according to indications	
Scheme 4	1	Remdesivir	Day 1: 200 mg (in a 0.9% sodium chloride solution) as a single dose, intravenously. From the 2nd day: 100 mg daily as a single dose, intravenously. The general course is no more than 10 days.
	2	Olokizumab	160 mg/ml – 0.4 ml subcutaneously / 0.8 ml as a single dose, intravenously.
		or	
		Levilimab	324 mg (two pre-filled syringes of 162 mg/0.9 ml each) subcutaneously/as a single dose, intravenously.
	3	Anticoagulant drug for parenteral administration	
	4	NSAIDs according to indications	
	5	In fever (t > 38°C) for longer than 3 days in a moderate course, antibiotic therapy is prescribed according to indications	

Table 4 – "Empirical" visual scale for assessing the degree of lung damage according to CT data in accordance with the IGs of the Ministry of Health of the Russian Federation for the diagnosis and treatment of COVID-19, version 11 (05/07/2021)

Description	Value
1. Absence of characteristic manifestations	KT-0
2. Minimum volume/prevalence < 25% lung volume	KT-1
3. Average volume/prevalence 25–50% of lung volume	KT-2
4. Significant volume/prevalence 50–75% of lung volume	KT-3
5. Critical volume/extent > 75% lung volume	KT-4

Table 3 – Categorial scale for determining patients' clinical condition

Patient's condition	Description	Category
Uninfected	No clinical / virological signs of infection	0
Outpatient	No activity restrictions	1
	Activity restrictions	2
Hospitalized: – mild course of the disease	Hospitalized, no oxygen therapy	3
	Oxygenation with mask or nasal cannula	4
– severe disease	Non-invasive ventilation or high-flow oxygen therapy	5
	Intubation or mechanical ventilation	6
	Ventilation + additional organ support – vasopressors, renal replacement therapy, extracorporeal membrane oxygenation (ECMO)	7
Deceased	Death	8

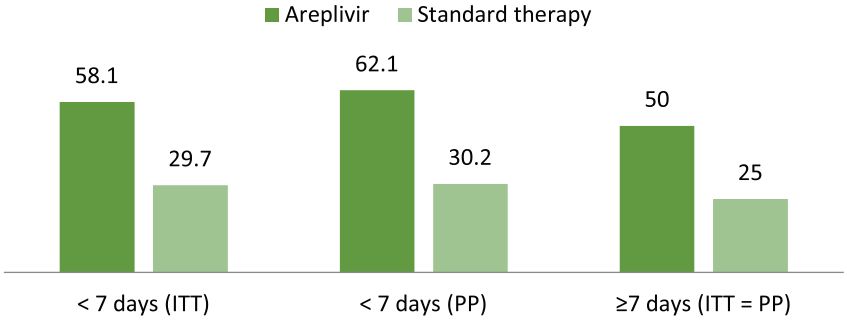

Figure 1 – Frequency (% of patients) of improvement in clinical status by 2 points or more with duration of symptoms before therapy < than 7 days and ≥ than 7 days

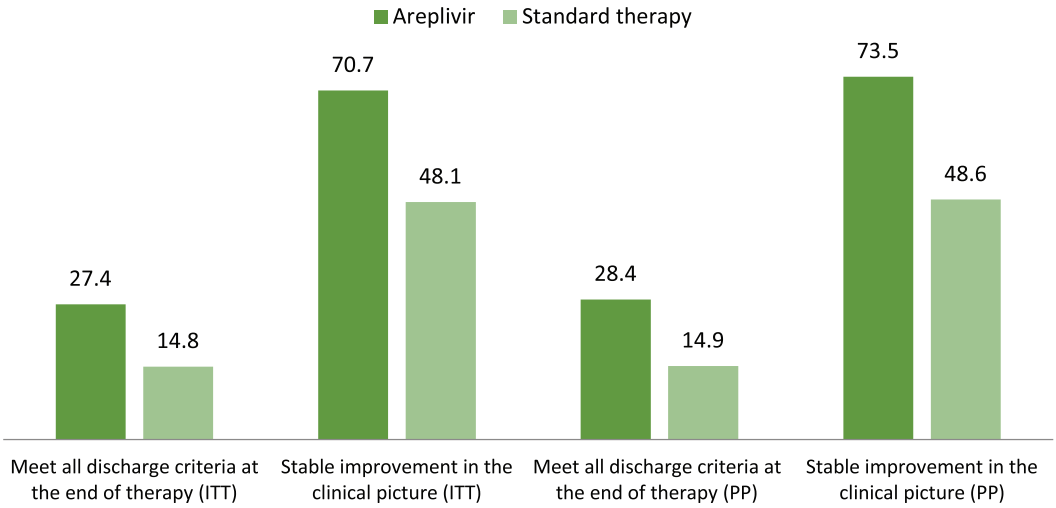

Figure 2 – Comparative analysis of patients' frequency meeting discharge criteria for current IGs at the end of therapy

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To determine the clinical condition of the patient, the characteristics of the infection course and the safety of the therapy prescribed for all patients, the information on complaints and symptoms was collected daily. The vital signs (body temperature, saturation, blood pressure, heart rate, respiratory rate) were measured at screening, then on the 5th and on the 11th days; clinical and laboratory studies (clinical and biochemical blood tests, coagulogram, urinalysis) and ECG monitoring were performed. ECG diagnostics, clinical and laboratory analyzes were performed at screening, then on the 5th and 11th days. In addition, the frequency and severity of adverse events (AEs) and serious AEs, as well as the frequency of any AEs that led to the discontinuation of the study drugs, the frequency of significant changes in vital signs and clinical and laboratory parameters, the need for non-invasive oxygen support or mechanical ventilation, as well as the incidence of deaths were assessed.

Statistical processing of results

A statistical analysis was carried out in accordance with the requirements of ICH 9, the Rules of Good Clinical Practice approved by the Eurasian Economic Commission and other applicable requirements and laws. Statistical processing of the data at the end of the study was carried out by the employees not associated with the management of patients participating in the study in order to create conditions for an independent assessment of the results obtained. For the statistical analysis, certified statistical software with validated algorithms for performing statistical analyzes and proper documentation was used Statistica version 13 (TIBCO Software Inc.). Checking for the normality of the distribution was carried out by one of the generally accepted methods (Shapiro-Wilk test). In case of a non-Gaussian distribution, non-parametric evaluation methods were used to compare efficacy and safety indicators. Significance levels and confidence intervals were calculated as two-tailed; the statistical significance of differences is two-tailed by default and refers to a significance level of 0.05 (unless otherwise indicated).

To analyze the parameter "frequency of improvement in clinical status on a categorial ordinal scale of clinical improvement by 2 or more categories at Visit 3", an intergroup comparison of shares was used with the help of a two-tailed version of Fisher's exact test (or a two-tailed version of the χ^2 («chi-square») test, in case all the expected values in the cells of the contingency table for this analysis were 5 or more). The difference in proportions between treatment groups and a 95% two-sided confidence interval for the difference in proportions were calculated using the Newcomb-Wilson method.

Student's t-test for independent samples was used to compare the efficacy between the groups in terms of "time (in days) till the improvement in clinical status on a categorial ordinal scale of clinical improvement". For a comparative assessment of the time (in days) till the improvement in the clinical status of the patient between the study groups, the Gehan-Wilcoxon test, the Cox-Mentel test, or the Log-rank test were used. The primary efficacy analyzes were performed in the ITT population (the primary analysis) and in the PP population (the additional analysis).

RESULTS

Efficacy evaluation

The efficacy evaluation of the studied drug was based on a statistical analysis of primary and secondary endpoints.

The rate of improvement in the clinical status on a categorial ordinal scale of clinical improvement by 2 or more categories in the ITT population in the Areplivir® (favipiravir) parenteral group was 54.72%, and in the standard therapy group it was 27.78% (p=0.0001). The difference in proportions between the favipiravir drug group and the standard therapy group (pa-pb) was 0.2694 (26.94%), 95% CI [0.1313; 0.3942]. In the PP population, in the favipiravir group, the proportion of patients with an improvement in the clinical status by 2 or more categories was 56.86%, in the standard therapy group it was 28.04 (p<0.0001). The difference in proportions between the favipiravir group and the standard therapy group (pa-pb) was 0.2883 (28.83%), 95% CI [0.1480;0.4139] ([14.80%;41.39%]). There were statistically significant differences in the proportions of patients between the study groups in both the ITT population (p=0.0001) and the PP population (p<0.0001). Compared with the group receiving only standard therapy for COVID-19, in the group of injectable favipiravir, almost twice more patients were recorded with an improvement in the clinical status by 2 or more points on a categorial ordinal scale.

The median time (in days) till the improvement in clinical status (in the ITT and PP populations) was 5 days in the favipiravir group and 7 days in the standard therapy group (p=0.0184 and p=0.0048, respectively).

Thus, by both primary endpoints established by the study protocol, Areplivir® showed an advantage in terms of efficacy compared with standard therapy.

Additionally, for both ITT and PP populations, a comparative analysis was performed taking into account the duration of the symptoms presence before the start of therapy (< than 7 days and ≥7 days) (Fig. 1).

The proportion of patients with an improvement in the clinical status by 2 or more points in the ITT population in the Areplivir® group was 58% (< than 7 days) and 50% (≥7 days), which was twice higher than in the standard therapy group (29.7% – < than 7 days and 25% – ≥7 days). A similar trend is observed in the PP population. Statistically significant differences in the proportions of ITT patients are: p=0.0013, PP: p=0.0004 (< than 7 days) and p=0.0154 (≥7 days). The data obtained confirm a high efficacy of the parenteral etiotropic drug

and the expediency of prescribing it even in a delayed start of treatment. Moreover, the efficacy of the therapy under consideration was analyzed in patients depending on the presence of risk factors for a severe course of a coronavirus infection (the age over 60 years, the presence of concomitant diseases, such as obesity, type 2 diabetes, etc.). As a result of a comparative analysis of the improvement cases frequency in clinical status by 2 or more categories, statistically significant differences were revealed between the study groups (p=0.0027). Thus, in the Areplivir® group, every second patient with risk factors for complications after a course of therapy achieved an improvement in clinical status by 2 points or more.

The rate of improvement in clinical status and the possibility of reducing the patients' stay length of in hospital is an important factor in assessing the appropriateness of a particular therapy, given the enormous economic burden of coronavirus infection. The study showed that in the favipiravir group, the proportion of patients with a clinical status of < than 4 which corresponds to the status of "outpatient" after a course of therapy, was 66.04% (70/106). There were statistically significant differences between the study groups (p=0.0121) for this indicator. Moreover, in the study drug group, one in four patients (25.47%) achieved a clinical status of 0 and 1 on a categorial ordinal scale, corresponding to complete recovery. In the standard therapy group, this figure was only 6.48%.

The presence of positive dynamics in the disease course in the form of a decrease in the degree of lung damage in the favipiravir group according to CT data, is evidenced by the results of an intragroup analysis. Thus, a statistically significant difference was found between Visits 1 (beginning of the therapy) and 4 (Day 14 of the observation) (ITT: p=0.0191, PP: p=0.0004). However, there was no statistically significant difference between Visits 1 and 4 in the standard therapy group (ITT: p=0.1025, PP: p=0.0733). It is also worth noting that by the end of therapy, 75.47% of the patients in the favipiravir group in the ITT population and 77.45% of patients in the PP population, had achieved improvement in the lungs condition (the degree of the lungs involvement was CT-1 and CT-0), up to complete disappearance of the disease symptoms.

An analysis of the patients' frequency with the SARS-CoV-2 virus elimination (a negative laboratory test for the presence of SARS-CoV-2 RNA by the NAATs method) showed that in the group of patients who were receiving the study drug favipiravir, the elimination of the virus occurred earlier than in the standard therapy group. On the 5th day of therapy, the elimination of the virus was observed in 73.58% of patients in the main group.

An improvement in the clinical picture is an efficacy marker of etiotropic therapy, since even in the absence of a pathogen in the oropharynx, progression of pneumonia and worsening of the general condition may occur. Against the background of the therapy, there was a pronounced positive trend in the parameters of the biochemical blood test, including such important markers of inflammation as CRP (45.5 mg/l at the screening visit, 11.3 mg/l after 5 days of therapy and 7.1 mg/l after the course of therapy); CPK (146.7 u/l at the screening visit and 78.8 u/l after the course of therapy) and ESR (20.9 mm/h at the screening visit and 9.99 mm/h after 10 days of therapy). There was normalization of body temperature and the level of blood oxygen saturation already on the 3-5th days of treatment, which indicates a decrease in the risk of developing complications of the disease and an improvement in its prognosis.

A comparative analysis of the patients frequency meeting discharge criteria on the basis of active IGs at the end of therapy showed that in the ITT population, the number of patients receiving favipiravir (27.36%) was twice the percentage of the standard therapy patients (14.81%) who had met all the discharge criteria by the end of therapy. In the favipiravir group, a stable improvement in the clinical picture was observed in 70.75%, in the standard therapy group it was 48.15%. A similar trend was observed in the PP population (Fig. 2).

A significant advantage over standard therapy in terms of achieving such "surrogate" endpoints as a faster onset of clinical improvement, shorter time to hospital discharge, and faster recovery indicates not only the clinical but also the pharmacoeconomic efficacy of Areplivir for parenteral administration and the feasibility of the chosen drug therapy in patients hospitalized with COVID-19.

Safety assessment

In this study, the analysis of all safety parameters was performed in a safety population that coincided with the ITT population of 214 patients (106 patients in the favipiravir group + 108 patients in the standard therapy group). The frequency of patients in the group of the drug favipiravir for the parenteral administration with reported cases of UDEs was 26.42%. A total of 46 UDEs was notified in 28 patients in the favipiravir group. The frequency of patients in the standard therapy group with reported cases of UDEs was 23.15%. There were no significant intergroup differences in the frequency and severity of UDEs.

Among the main UDEs, one can distinguish: an increase in ALT (39.22% in the main group, 45.45% in the comparison group), an increase in AST (15.69% in the main group, 20.45% in the comparison group), an increase in gamma-GTT (7.84% in the main group, 4.55% in the comparison group), bradycardia (3.95% and 4.95% in the main and control groups, respectively). Dizziness, hyperglycemia, and pyrexia can be distinguished out of the single UDEs, the frequency of which did not exceed 1%.

Among the reported UDEs in the favipiravir group patients, 93.48% were mild, 4.35% were moderate; in patients of the standard group therapy, 82.93% were

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mild, 17.07% – moderate. According to the physicians' study, the causal relationship with the study drug therapy was assessed as "not related" in 6.52%, "possible" in 63.04%, "doubtful" in 21.74%, "probable" – in 6.52%, "conditional" – in 2.17% of cases; a causal relationship with standard therapy was assessed as "not related" in 2.44%, "possible" in 73.17%, "doubtful" in 14.63%, "probable" in 9.76% of cases.

The analysis of the frequency of UDEs outcomes in patients showed that in the group of the study drug favipiravir, significantly more UDEs ended in "recovery without consequences" (p=0.096) and "improvement" (p=0.049). In the study drug group, most UDEs were transient, and no treatment discontinuation or dose changes due to UDEs were reported in the study drug group. There were no ventilator requirements, deaths, or serious adverse events associated with the study medication, consistent with the predictable high safety profile of parenteral favipiravir in patients with coronavirus infection.

It has been shown that favipiravir therapy does not adversely affect the parameters of clinical and biochemical blood tests, urinalysis, coagulograms, vital signs and ECG. The study physicians assessed that the study drug was well tolerated by the patients. It should be emphasized that the parenteral administration of favipiravir does not have a local irritating effect on the gastrointestinal tract, which is especially important for COVID-19 patients, taking into account both the negative impact of the virus itself and the polypharmacy characteristic of this disease treatment [28].

DISCUSSION

Compared to the group receiving only standard therapy for COVID-19, in the parenteral favipiravir group, almost 2 twice more people were recorded with an improvement in clinical status by 2 or more points on a categorial ordinal scale, which indicates a high efficacy and the feasibility of the therapy. The revealed statistically significant differences in the proportions of patients between the study groups in both the ITT population (p=0.0001) and the PP population (p<0.0001) prove the hypothesis of the Areplivir® superiority over standard therapy.

A comparative analysis of the patients' frequency with a clinical status of 0 and 1 on a categorial ordinal scale of clinical improvement in both populations (ITT, PP), both at Visit 3 (the end of therapy) and at Visit 4 (Day 14 of follow-up), showed statistically significant differences (Pearson's Chi-square, p=0.0001) in favor of the Areplivir® drug group.

The median time (in days) till the improvement in clinical status (in the ITT and PP populations) was 5 days in the favipiravir group and 7 days in the standard therapy group (p=0.0184 and p=0.0048, respectively). The revealed statistically significant differences in the time till improvement of the patients' clinical status prove the hypothesis of the superiority of the drug faviparavir over standard therapy.

Even with a delayed start of treatment, therapy with parenteral favipiravir is effective and reasonable. These data prove the efficacy of parenteral favipiravir in terms of increasing the rate and frequency of a pronounced improvement in the clinical status in the vast majority of patients hospitalized with COVID-19: statistically significant differences in the proportions of patients ITT: p=0.0013, PP: p=0 .0004 (< than 7 days) and p=0.0154 (≥7 days).

In the group of patients who received the study drug favipiravir, the elimination of the virus occurred earlier than in the standard therapy group. Already by Visit 2, virus clearance had been observed in 73.58% of patients in the ITT population and in 76.47% of patients in the PP population. The data obtained indicate a more rapid decrease in the viral load when using the parenteral form of the drug favipiravir, which helps to reduce the risks of aggravating the condition and improve the prognosis.

In the ITT population, the number of patients treated with favipiravir (27.36%) was twice the percentage of the patients on standard therapy (14.81%) who met all discharge criteria at the end of therapy. The comparative analysis revealed statistically significant differences between the study groups in terms of the frequency of patients meeting all discharge criteria at the end of therapy (ITT: p=0.0244, PP: p=0.0178), as well as the achievement of a stable improvement in the clinical picture (ITT: p=0.0008, PP: p=0.0002).

Therapy with the study drug favipiravir is accompanied by a significant improvement in the condition of the lungs according to CT, up to the complete disappearance of the disease symptoms.

Based on the statistical analysis of the data obtained, it can be argued that therapy with favipiravir for the parenteral administration significantly improves the condition of patients, accelerates recovery and reduces the time of hospital stay compared to standard therapy.

The analysis of the UDEs outcomes frequency in patients showed that in the group of the study drug favipiravir, significantly more UDEs ended in "recovery without consequences" (p=0.096) and "improvement" (p=0.049). In the study drug group, most UDEs were transient, and no treatment discontinuation or dose changes due to UDEs were reported in the study drug group. No UDEs or deaths associated with the use of favipiravir have been reported.

It has been shown that favipiravir therapy does not adversely affect laboratory parameters such as clinical and biochemical blood tests, urinalysis, coagulogram, vital signs and ECG, which indicates the safety of the therapy. During the study, the tpatients treated with the study drug showed positive changes in laboratory parameters, such as C-reactive protein, D-dimer, CPK, as well as a smaller increase in ALT and AST levels, which indicates a decrease in the intensity of inflammatory processes in

the body and the onset of a convalescence period. The study physicians assessed that the study drug was well tolerated by the patients.

CONCLUSION

The results of a comparative assessment of the parenteral favipiravir therapy efficacy in COVID-19 patients compared with standard therapy, made it possible to establish significant advantages of using the drug in terms of the speed and frequency of a pronounced improvement onset in clinical parameters. That contributed to a faster transfer of patients from hospital to the outpatient stage of observation or achieving complete clinical recovery (4 times more patients in the main group than in the comparison group). It has been shown that the use of favipiravir in the form for the parenteral administration, due to favorable pharmacokinetic parameters, makes a faster and more pronounced therapeutic effect mediated by achieving complete elimination of the virus, possible. That determines the reduction in the risk of developing a severe course and entering the ICU, and also helps to speed up the discharge of patients and improve the prognosis.

It can be argued that therapy with Areplivir® for the parenteral administration is characterized by a favorable safety profile, comparable, and in some cases, superior to that of standard therapy.

Taking into account the established reliable clinical benefits and data on the favipiravir effectiveness, regardless of a new coronavirus infection strain, the new original development of Areplivir® for the parenteral administration opens up additional opportunities to combat complicated forms of the disease and reduce the clinical and economic burden of COVID-19. The versatility and resistance to mutations of RNA-dependent RNA polymerase make it possible to consider it as the main target for combating the most common RNA viruses that cause ARVI, that determines the need further studies of favipiravir to expand the range of its indications.

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CONFLICT OF INTEREST

The clinical study was organized by LLC Promomed RUS. LLC “Promomed RUS” is a member of the Promomed group of companies. LLC “Promomed RUS” is the holder of the registration certificate for the drug “Areplivir” LP-007598 dated 11/12/2021, LP-007660 dated 12/03/2021, LP-007681 dated 12/14/2021. The manufacturer of the drug “Areplivir” is JSC “Biochemist”, which is a part of the group of companies of the state corporation “Promomed”. Zaslavskaya K.Ya. is the Director of new products of LLC “Promomed DM”.

AUTHORS’ CONTRIBUTION

Larisa A. Balykova – development and implementation of research design, research, writing and editing the text; Kira Ya. Zaslavskaya – research design development, text writing and editing; Vera F. Pavelkina – implementation of the study design, study data processing; Nikolai A. Pyataev – implementation of the study design, study data processing; Natalya M. Selezneva – implementation of the study design, study data processing; Natalya V. Kirichenko – implementation of the study design, study data processing; Anastasia Yu. Ivanova – implementation of the study design, processing of research results; Grigory V. Rodoman – implementation of the study design, study data processing; Konstantin B. Kolontarev – development and implementation of research design, text editing; Konstantin S. Skrupsky – implementation of the study design, study data processing; Elena N. Simakina – implementation of the study design, study data processing; Olga A. Mubarakshina – analysis of the research results, writing and editing the text; Aleksey V. Taganov – data collection, analysis of the research results; Dmitry Yu. Pushkar – development and implementation of research design, analysis of results, text editing.

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AUTHORS

Larisa A. Balykova – Corresponding Member of the Russian Academy of Sciences, Doctor of Sciences (Medicine), Professor, Head of the Department of Pediatrics, Director of the Medical Institute of National Research Ogarev Mordovia State University. ORCID ID: 0000-0002-2290-0013. E-mail: larisabalykova@yandex.ru

Kira Ya. Zaslavskaya –Director of New Products of LLC Promomed DM. ORCID ID: 0000-0002-7348-9412. E-mail: kiryonok@yandex.ru

Vera F. Pavelkina – Doctor of Sciences (Medicine), Professor, Head of the Department of Infectious Diseases with courses in Epidemiology, Phthisiology, Skin and Venereal Diseases, the Medical Institute of National Research Ogarev Mordovia State University. ORCID ID: 0000-0001-9582-9986. E-mail: pavelkina@rambler.ru

Nikolai A. Pyataev – Doctor of Sciences (Medicine), Associate Professor, Head of the Department of Anesthesiology and Resuscitation, the Medical Institute of National Research Ogarev Mordovia State University. ORCID ID: 0000-0002-9688-7640. E-mail: pyataevna@mail.ru

Natalya M. Selezneva — Candidate of Sciences (Medicine), Associate Professor of the Department of Hospital Therapy, the Medical Institute of National Research Ogarev Mordovia State University. ORCID ID: 0000-0002-3004-2063. E-mail: nata_rm@mail.ru

Natalya V. Kirichenko – Deputy Chief Physician of the Medical Department of Ivanovo Clinical Hospital n. a. the Kuvaevs. ORCID ID: 0000-0002-4272-9540. E-mail: igb2@ivreg.ru

Anastasia Yu. Ivanova – Assistant of the Department of Faculty Therapy, Ryazan State Medical University n. a. academician I.P. Pavlov; anesthesiologist Regional Clinical Hospital, Ryazan. ORCID ID: 0000-0002-4112-5382. E-mail: Nastya_doctor@list.ru

Grigory V. Rodoman – Doctor of Sciences (Medicine), Chief Physician of Municipal Clinical Hospital No. 24, Moscow City Health Department. ORCID ID: 0000-0001-6692-1425. E-mail: generalsurgery24@mail.ru

Konstantin B. Kolontarev – Doctor of Sciences (Medicine), Professor, Department of Urology, Moscow State Medical and Dental University n. a. A.I. Evdokimov, Head of the Oncourological Department of the City Clinical Hospital n. a. S.I. Spasokukotsky, Moscow City Health Department. ORCID ID: 0000-0003-4511-5998. E-mail: kb80@yandex.ru

Konstantin S. Skrupsky – urologist, oncourological Department of the City Clinical Hospital n. a. S.I. Spasokukotsky, Moscow City Health Department. ORCID ID: 0000-0001-6651-8142. E-mail: 89_sks@mail.ru

Elena N. Simakina – Candidate of Sciences (Medicine), Head of the Infectious Diseases Department of Smolensk Clinical Hospital No.1; Assistant of the Department of Infectious Diseases of Smolensk State Medical University. ORCID ID: 0000-0002-5709-8913. E-mail: e.simakina@mail.ru

Olga A. Mubarakshina – Candidate of Sciences (Medicine), Associate Professor of the Department of Clinical Pharmacology of Voronezh State Medical University n. a. N.N. Burdenko. ORCID ID: 0000-0001-6799-6322. E-mail: mubarakshina@mail.ru

Aleksey V. Taganov – Doctor Sciences (Medicine), Professor of the Department of Dermatovenereology with a course of cosmetology of the Faculty of Continuous Medical Education of the Medical Institute of the Peoples' Friendship University. ORCID ID: 0000-0001-5056-374X. E-mail: matis87177@yandex.ru

Dmitry Yu. Pushkar – Academician of the Russian Academy of Sciences, Doctor of Sciences (Medicine), Professor, Head of the Department of Urology, Moscow State Medical and Dental University n. a. A.I. Evdokimov; urologist, the City Clinical Hospital named after S.I. Spasokukotsky, Moscow City Health Department. ORCID ID: 0000-0002-6096-5723. E-mail: pushkardm@mail.ru

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ЛЕЧЕНИЕ НОВОЙ КОРОНАВИРУСНОЙ ИНФЕКЦИИ COVID-19 ЛЕГКОГО ИЛИ СРЕДНЕГО ТЕЧЕНИЯ У ВЗРОСЛЫХ

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***Лечение лекарственным препаратом ЭСПЕРАВИР® должно быть начато как можно раньше после постановки диагноза новой коронавирусной инфекции (COVID-19) и/или в течение 5 дней после появления первых симптомов заболевания*

[1]Открытое двухэтапное многоцентровое исследование по оценке основных фармакокинетических параметров, безопасности, а также эффективности в отношении COVID-19 лекарственного препарата JCBC00101, капсулы (ООО «ПРОМОМЕД РУС», Россия) у взрослой популяции, MOL-112021. 2022г

ИНФОРМАЦИЯ ПРЕДНАЗНАЧЕНА ДЛЯ СПЕЦИАЛИСТОВ ЗДРАВООХРАНЕНИЯ
ИМЕЮТСЯ ПРОТИВОПОКАЗАНИЯ ОЗНАКОМЬТЕСЬ С ИНСТРУКЦИЕЙ