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A FEW REPRESENTATIVES OF *ASTEROIDAE (ASTERACEAE)* SUBFAMILY AND *GERANIUM (GERANIACEAE)* GENUS IN THE BAIKAL REGION (REVIEW)

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The aim of the article was to analyze the state of knowledge of the following plants: *Heteropappus altaicus* (Willd.) Novopokr., *Solidago dahurica* L., *Leucanthemum vulgare* Lam., *Tripleurospermum inodorum* (L.), *Antennaria dioica* (L.) Gaertn., *Leontopodium conglobatum* (Turcz.) Hand.-Mazz. and *Geranium eriostemon* Fischer., *G. pratense* L., G. *wlassowianum Fisch.* ex Link. (*Geraniaceae*).

Materials and methods. To compile the review, the information from the following scientific open and available literature sources placed in scientific libraries of institutions, in electronic databases and search systems, was used: floristic summaries "Flora of Siberia"; "Flora of Central Siberia"; Electronic library of the Siberian branch of the Russian Academy of Sciences; Elibrary; PubMed; Scopus; CyberLeninka, Google Academy; The Plant List, Global *Compositae* Checklist. The search carried out, was based on the publications for the period of 2009-2020, on the information requests for names of families and subfamilies, names of plant species, biologically active compounds in English, Latin and Russian.

Results. A comparative analysis of morphological characters, common species names and the synonyms for the listed species, has been carried out. The studied objects are characterized by the presence of polyphenolic compounds and substances of a triterpene structure, in particular, flavonoids, hydroxycinnamic acids, tannides. In addition, the representatives of the *Asteroideae* subfamily (*Asteraceae*) show the accumulation of essential oils, and the representatives of the *Geranium* genus (geranium) show the accumulation of anthocyanins. The spectrum of the pharmacological activity includes anti-inflammatory, choleretic, antimicrobial, antispasmodic and other types of effects.

Conclusion. The presented review makes it possible to arrive at the conclusion about a certain knowledge level of the regional representatives of the *Asteroideae* subfamily and the *Geranium* genus. This determines the prospects of these plant objects for further pharmacognostic and pharmacological research and the creation of drugs on their basis – the sources of polyphenolic compounds.

Keywords: *Asteroideae* subfamily; *Geranium* genus; biologically active compounds; phytotherapy

Abbreviations: BAC – biologically active compound; HPLC – high performance liquid chromatography; GLC / MS – gas liquid chromatography – mass spectrometry; GC-FID – gas chromatograph with a flame ionization detector; GC-MS – gas chromatography – mass spectrometry; *A. – Antennaria* (e.g., *A. dioica*); *C. – Chrysanthemum* (e.g., *C. dentatum*); *Ch. – Chamomilla* (eg, *Ch. Inodora*); *G. – Geranium* (e.g., *G. pratense*); *H. – Heteropappus* (e.g., *H. altaicus*); *K. – Kalimeris* (e.g., *K. altaica*); *L. – Leucanthemum* (e.g., *L. vulgare*); *M. – Matricaria* (e.g., *M. perforata*); *S. – Solidago* (e.g., *S. dahurica*); *P. – Pyrethrum* (e.g., *P. elegans*); *T. – Tripleurospermum* (e.g., *T. inodorum*).

НЕКОТОРЫЕ ПРЕДСТАВИТЕЛИ ПОДСЕМЕЙСТВА *ASTEROIDAE* (*ASTERACEAE*) И РОДА *GERANIUM* (*GERANIACEAE*) ПРИБАЙКАЛЬЯ (ОБЗОР)

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ОБЗОРЫ, ЛЕКЦИИ

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Цель. В обзоре представлен анализ состояния изученности растений *Heteropappus altaicus* (Willd.) Novopokr. (гетеропаппус алтайский), *Solidago dahurica* L. (золотарник даурский), *Leucanthemum vulgare* Lam. (нивяник обыкновенный), *Tripleurospermum inodorum* (L.). (трехреберник непахучий), *Antennaria dioica* (L.) Gaertn. (кошачья лапка двудомная), *Leontopodium conglobatum* (Turcz.) Hand.-Mazz. (эдельвейс скученный) (*Asteraceae* – сложноцветные) и *Geranium eriostemon* Fischer. (герань волосистотычинковая), *G. pratense* L (г. луговая)., *G. wlassowianum* Fisch. ex Link. (г. Власова) (*Geraniaceae* – гераниевые).

Материалы и методы. Для составления обзора использовали сведения научной литературы из открытых и доступных источников, размещенных в научных библиотеках учреждений, в электронных базах данных и поисковых системах: флористические сводки «Флора Сибири»; «Флора Центральной Сибири»; Электронная библиотека Сибирского отделения Российской академии наук; Elibrary; PubMed; Scopus, Киберленинка; Google-Академия; The Plant List; Global *Compositae* Checklist. Поиск осуществлялся по публикациям за период с 2009 по 2020 гг. по информационным запросам названий семейств и подсемейств, названий видов растений, биологически активных соединений на английском, латинском и русском языках.

Результаты. Проведен сравнительный анализ морфологических признаков, общепринятых видовых названий и синонимов перечисленных видов. Для изучаемых объектов характерно присутствие полифенольных соединений и веществ тритерпеновой структуры. В частности, флавоноидов, гидроксикоричных кислот, танидов. Кроме того, у представителей подсемейства *Asteroideae* (астровые) отмечается накопление эфирного масла, а у представителей рода *Geranium* (герань) – антоцианов. Спектр фармакологической активности охватывает противовоспалительное, желчегонное, антимикробное, спазмолитическое и другие виды действия.

Заключение. Представленный обзор позволяет сделать о вывод об определенном уровне изученности региональных представителей подсемейства *Asteroideae* и рода *Geranium*. Это обуславливает перспективность данных растительных объектов для дальнейшего фармакогностического и фармакологического исследования и создания на их основе лекарственных препаратов – источников полифенольных соединений.

Ключевые слова: подсемейство *Asteroideae;* род *Geranium;* биологически активные соединения; фитотерапия

Список сокращений: БАС – биологически активные соединения; ВЭЖХ – высокоэффективная жидкостная хроматография; ГЖХ/МС – газожидкостная хроматография / масс-спектрометрия; GC-FID – газовый хроматограф с пламенно-ионизационным детектором; GC-MS – газовая хроматография – масс-спектрометрия; *A.* – *Antennaria* (н-р, *A. dioica*); C. – *Chrysanthemum* (н-р, *C. dentatum*); *Ch.* – *Chamomilla* (н-р, *Ch. Inodora*); *G.* – *Geranium* (н-р, *G. pratense*); *H.* – *Heteropappus* (н-р, *H. altaicus*); *K.* – *Kalimeris* (н-р, *K. altaica*); *L.* – *Leucanthemum* (н-р, *L. vulgare*), *M.* – *Matricaria* (*н-р, M. perforata*), *S.* – *Solidago* (н-р, *S. dahurica*), *P.*– *Pyrethrum (*н-р, *P. elegans*); *T.* – *Tripleurospermum* (н-р, *T. inodorum*).

INTRODUCTION

According to the forecasts of the World Health Organization[1], the share of herbal medicines is constantly growing and reaches 60% in the total range of medicines. This is ensured by their objective advantages and possibilities [1]. In the medical practice of the Russian Federation, about 20 thousand medicines are used, more than 40% of which are made from medicinal plant materials. Traditional medical systems of numerous national ethnic groups (Tibetan, Buryat, Russian, etc.) rely primarily on natural resources [2–4].

Currently, in the scientific literature, a lot of attention is paid to the study of metabolites of plant objects as effective antioxidants. The main representatives of such natural substances are polyphenolic compounds – flavonoids, phenol carboxylic acids, tannides, coumarins. In comparison with other natural compounds, they have the highest prevalence, a significant structural diversity and versatile pharmacological properties. Polyphenolic compounds cause antioxidant, cardiotropic, angioprotective, capillary-strengthening, hepatoprotective, choleretic, diuretic and other important effects [5–7]. It should be notified that the plant material contains a mixture of polyphenolic metabolites. This fact affects the specificity of the pharmacological action and forms the scientific basis for modeling and creating new effective herbal preparations [1, 5–10].

In this regard, it is urgent to search for new types of plant materials containing polyphenolic compounds, used to treat the most common diseases.

In this context, some species of the *Asteraceae* or *Compositae* and *Geraniaceae* families growing in the Baikal region are of the greatest interest. In particular, these are: *Heteropappus altaicus* (Willd.) Novopokr., *Solidago dahurica* L., *Leucanthemum vulgare* Lam., *Tripleurospermum inodorum* (L.), *Antennaria dioica* (L.) Gaertn., *Leontopodium conglobatum* (Turcz.) Hand.-Mazz. (*Asteraceae, Asteroidae*), and *Geranium eriostemon* Fischer., *G. pratense* L., *G. wlassowianum* Fisch. ex Link. (*Geraniaceae, Geranium*).

THE AIM of the article was to analyze and review the data from domestic and foreign scientific literature on the current state of plants knowledge in the Baikal region as promising sources of polyphenolic compounds.

MATERIALS AND METHODS

To compile the review, the information from the following scientific open and available literature sources placed in scientific libraries of institutions, in electronic databases and search systems, was used: floristic summaries “Flora of Siberia”; “Flora of Central Siberia”;

[1] WHO Traditional Medicine Strategy 2014–2023 Published by WHO, 2013.72 p.

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Electronic library of the Siberian branch of the Russian Academy of Sciences; Elibrary; PubMed; Scopus; CyberLeninka; Google Academy; The Plant List; Global *Compositae* Checklist. The search carried out, was based on the publications for the period of 2009–2020, on the information requests for names of families and subfamilies, names of plant species, biologically active compounds in English, Latin and Russian.

RESULTS AND DISCUSSION

Asteroidae Subfamily

Asteraceae, or *Compositae*, are one of the largest families of dicotyledonous plants, widely represented in all floristic systems of the Earth. *Asteraceae* include 32,913 species, united in 1911 genera [11]. This accounts for approximately 8% of all known flowering plants [12].

Representatives of *Asteraceae* are actively involved in the construction of phytocenoses, they often have a pronounced confinement to the places with certain environmental factors. Some species are endemic, relics, naturalized or feral, as well as introduced because of anthropogenic activities [13].

A multiflorous inflorescence, an anthode, is one of the priority features of this family. The anthode is a shortcut torus, with small flowers collected on them (semiflorets and false – semiflorets, tubular and funnel-shaped). The anthodes are often grouped into complex aggregate inflorescences – spicas, racemes, panicles, cymoids or capitulums. In turn, the *Asteraceae* family, depending on the type of the flowers included in the inflorescence, is subdivided into 2 large subfamilies:

– *Asteroideae* Lindl. (*Tubuliflorae*) subfamily – in the inflorescence of the false-semiflorets, the flowers are tubular, funnel-shaped;

– *Lactucoideae* L. *(Liguliflorae*) subfamily – in the inflorescence there are semiflorets and laticifer [14].

Many representatives of the *Asteraceae* family (the *Asteroideae* and *Lactucoideae* subfamilies) are official and are included in all issues of the State Pharmacopoeia of our country. Currently, about 30 species of this family plants are allowed to be used as sources of medicinal plant raw materials in Russia[2,3,4].

Pharmacopoeial representatives of *Asteraceae* are used as vitamin and diaphoretic (*Calendulae officinale flores, Bidentis tripartitae herba*), choleretic and hepatoprotective (*Tanaceti vulgarae flores, Helichrisi arenarii flores, Silibi mariani fructus,* etc.), anti-inflammatory (*Chamomillae recutita flores*), diuretics and choleretic (*Arctii radices*), anti-inflammatory and expectorant (*Tussilaginis farfarae folia*), hemostatic (*Arnicae montana flores*), immunostimulating (*Echinaceae purpureae herba*) remedies. They are also the sources of drugs that have biological antispasmodic and M-cholinolytic actions (*Senecionis platyphylloidis herba*), etc. The main groups of biologically active compounds of these plants are vitamins, flavonoids, tannins, essential oils, alkaloids, polysaccharides.

The plants of the *Asteroideae* and *Lactucoideae* subfamilies are found in all areas of the Baikal region. The *Asteroideae (Tubuliflorae)* subfamily prevails: it comprises about 85% of all genera (or 61) of the *Asteraceae* family. The constancy of their position in the ecological systems of Siberia is the basis for a long-time use of *Asteroideae* species in folk medicine of this region. About 20 representatives of this subfamily are described as medicinal, mainly from *Solidago* L., *Tripleurospermum* Sch.Bip., *Leucanthemum* Mill., *Heteropappus* Less., *Leontopodium* L., *Antennaria* Gaertn. [15–17].

The analysis of the available literature sources showed that *H. altaicus, S. dahurica, L. vulgare, T. inodorum, A. dioica, L. conglobatum* species have a specific use in folk and traditional medicine, are popular for the treatment of digestive and genitourinary diseases and are objects for study in various scientific fields.

Antennaria dioica (L.) Graeth.

In “Flora of Central Siberia” [18] and the Electronic Library of the Siberian Branch of the Russian Academy of Sciences (EL SB RAS) [19], three species belonging to the *Antennaria* (L.) Gaertn have been described. – *A. monocephala* DC., *A. dioica* (D. Don) Greene and *A. villifera* Boriss. *A. friesiana* (Trautv.) Ekman has been additionally included in the list of volume 13 “Flora of Siberia” [13]. The species *A. dioica* (L.) Graeth is more popular in folk medicine [20]. The synonymous names for this species are: *A. dioica var. australis* Gris, *A. dioica var. corymbosa* (E.E.Nelson) Jeps., *A. dioica var. dioica, A. dioica var. hyperborea* (D.Don) Greene, *A. dioica var. hyperborea* (D.Don) DC., *A. dioica var. kernensis* Jeps., *A. dioica var. marginata* (Greene) Jeps., *A. dioica var. parvifolia* (Nutt.) Torr. & A.Gray, *A. dioica var. rosea* (Greene) D.C. Eaton, *A. dioica var. rosea* Cockerell. [11, 19].

Distribution. In the Baikal region, *A. dioica* occupies all areas of the Central Siberian plateau, occurs in the Sayans and the Sayan-Baikal region, the Baikal and Stanovoe Highlands, as well as in the steppe regions of Buryatia. It prefers the following habitats: dry tundra slopes, pine forests, forest edges, dry meadows, stony-gravelly, light forests [13, 18, 19]. Besides, it grows in different countries: China, Japan, Kazakhstan, Mongolia, Europe, North America (Alaska) [21]. GLC / MS methods revealed the presence of sugars: D-glucose, D-sucrose and myo-inositol. The amino acid complex includes sixteen free and seventeen linked amino acids. In terms of the content, L-glutamic acid (up to 7.38±0.20 μg/mg) and L-aspartic acid (up to 5.38±0.12 μg/mg) prevailed [22, 23].

[2] State Pharmacopoeia of the Russian Federation. Ministry of Health of the Russian Federation. XIII ed. M., 2015. Available from: http://193.2327(13). Russian

[3] State Pharmacopoeia of the Russian Federation / Ministry of Health of the Russian Federation. XIV ed. T. I–IV. M., 2018. Available from: http://193.232.7.120 (14). Russian

[4] State Register of Medicines. Available from: http://grls.rosminzdrav.ru. Russian

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From the aerial parts of *A. dioica*, the representatives of polyphenols were isolated, in particular, phenol carboxylic acids – caffeic and chlorogenic; flavonoids – apigenin, luteolin, 7-glucoside and 4^{I}- apigenin glucoside, 7,4^{I}-luteolin diglucoside, 7-O-β-D-glucoside and 4^{I}-O-β-D-luteolin glucoside. Along with this, triterpene compounds such as ursolic acid and lupeol, steroids – β-sitosterol, sitosterol-3-O-glucopyranoside (daucosterol), which have antibacterial activity, were found [22, 24].

The composition of flavonoids, coumarins, and hydroxycinnamic acids of *A. dioica*, growing on the territory of the Vyzhnytsya district of the Chernivtsi region (Ukraine), has been studied. HPLC method revealed rosmarinic, caffeic, ferulic, coumaric, chlorogenic acids; quercetin-3-D-glucoside, luteolin, rutin, hyperoside, quercetin, apigenin, as well as umbelliferone and coumarin [25].

In the process of studying literary sources, information was found on the contents of tannins, flavonoids, vitamin K, resins and bitterness [24].

It was noted that the selenium content can be up to 0.012% of dry phytomass. Thus, *A. dioica* is considered a source of this element, an important microcomponent in the nutrition of animals and humans [26].

Traditional medicine suggests using the aerial part of *A. dioica* (D. Don) Greene – the anthode and herb. They are used orally in the form of infusions; externally in the form of powder, gruel made of raw materials, and applications [21]. The extracts from the herb of this plant are astringent. They are taken orally for dysentery, diarrhea, gastritis, stomach and duodenal ulcers. The herb infusion is used as an anti-inflammatory and emollient remedy in the form of rinses and poultices for angina, tonsillitis, stomatitis, goiter, abscesses, septic wounds, breast tumors and as a pain reliever for gout. Fresh herb is applied to the gums to relieve a toothache. Gruel from fresh leaves is applied to the fingers for panaritium, and the wounds are sprinkled with powder from dried leaves. Infusion in the form of applications is used for eye diseases, in the form of douching – for gonorrhea and leucorrhoea. In the past, healers and root doctors advised chewing flowers and taking an infusion for epilepsy and other diseases of the nervous system [21].

Kalinin E.P. et al. confirmed the hemostatic effect of biologically active compounds of *A. dioica in vitro* and *in vivo.* It was found out that the anticoagulant activity is manifested by the fraction of the protein-peptide nature [27].

Morphological and anatomical studies of the aerial parts of *A. dioica* were carried out in order to establish the diagnostic signs of the vegetative organs [28, 29].

Leucanthemum vulgare (Vaill.) Lam.

Along with *T. Inodorum, L. vulgare,* is an admixture to the official raw material of *Chamomillae recutitae flores* и *Chamomillae suaveolentis* flores[5,6,7,8]. However, they are also objects of scientific research.

L. vulgare (*L. vulgare* (Vaill.) Lam.) is the only species of *Leucanthemum* genus, represented on the territory of the Baikal region (Eastern Siberia).

The database "The Plant List" contains 37 synonyms for this name, among which 3 have the status of unconfirmed or used without an agreement with the rules of the International Code of Botanical Nomenclature (see paragraphs 1, 3, 15) [11]: *Bellis major Garsault* [Invalid], *Chamaemelum leucanthemum* (L.) E.H.L.Krause, *Chrysanthemum dentatum* Gilib. [Invalid], *C. ircutianum* Turcz., *C. lanceolatum* Vest, *C. lanceolatum* Pers., *C. leucanthemum* L., *C. leucanthemum var. boecheri* B.Boivin, *C. leucanthemum subsp. lanceolatum* (DC.) E.Mayer, *C. leucanthemum subsp. Leucanthemum, C. leucanthemum var. leucanthemum, C. leucanthemum f. leucanthemum, C. leucanthemum var. pinnatifidum* Lecoq & Lamotte, *C.leucanthemum var. subpinnatifidum* Fernald, *C. montanum* Willd. [Illegitimate], *C. montanum var. heterophyllum* (Willd.) Koch, *C. praecox* (M.Bieb.) DC., *C. pratense* Salisb., *C. sylvestre* Willd., *C. vulgare* (Lam.) Gaterau., *C. vulgare var. vulgare, Leucanthemum atratum var. heterophyllum* (Willd.) Rouy, *L. lanceolatum* DC., *L. leucanthemum* (L.) Rydb. [Illegitimate], *L. praecox* (Horvatić) Villard, *L. vulgare subsp. heterophyllum* (Willd.) Soó, *L. vulgare subsp. incisum* Arcang., *L. vulgare var. pinnatifidum* (Lecoq & Lamotte) Moldenke, *L. vulgare subsp. praecox* Horvatić, *L. vulgare var. vulgare, L. vulgare subsp. vulgare, Matricaria leucanthemum* (L.) Scop., *M. leucanthemum* (L.) Desr., *Pontia heterophylla* (Willd.) Bubani, *P. vulgaris* Bubani, *Pyrethrum leucanthemum* (L.) Franch., *Tanacetum leucanthemum* (L.) Sch. Bip.

L. vulgare has grey fruits- achenes, up to 2 mm long, with 5–10 distinct ribs, there is no coronet, as a rule, only occasionally the seeds of semiflorets show a unilateral coronet. Representatives with a unilateral coronet of achenes from the Irkutsk region are described for the *L. ircutianum* DC species and occur sporadically throughout the *L. vulgare* range in all regions of Siberia. Moreover, the presence of this trait varies even within the population, which makes it possible to consider *L. ircutianum* as variety or *L. vulgare var. ircutianum* (DC) Krylov. [13, 19].

The *L. vulgare* range covers the western and southern parts of the Central Siberian Plateau, the Sayans and the Sayan-Baikal region, the Baikal Plateau in its western part, and the northern part of the North-Baikal Plateau. Its habitat is represented by meadows, meadow slopes,

[5] GOST 2237-93. Chamomile flowers. Technical conditions. 10 pp. Russian
[6] State Pharmacopoeia of the Russian Federation / Ministry of Health of the Russian Federation. XIII ed. M., 2015. Russian
[7] State Register of Medicines. Russian
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forest glades, the border areas; it is often a ruderal species [13]. An assessment of the raw material reserves of this type has been carried out in the Irkutsk region [30].

At the beginning of the previous century, *L. vulgare* used to spread throughout Europe (excluding the Arctic zone) and in most of Asia (excluding the Arctic zone). Thanks to the anthropogenic activities, including the globalization of migration processes, this species was introduced to other climatic zones and continents (North America). In any case, it is presented as a sun-loving plant that also prefers meadows, forest-steppes, mountainous and subalpine regions. In the places of human farming or in ruderal territories, it occurs along roadsides, on the outskirts of fields, in fallow lands, in crops, in pastures and meadows. It spreads very well on fertile soils; it is characterized as a field weed [31].

L. vulgare is included in the arsenal of traditional medicine in Russia, Azerbaijan, Georgia, Italy, America, Canada, Albania, Serbia, etc. [36-38]. According to the authors Telyat'ev VV, Minaeva VG, the main active ingredients are alkaloids, inulin, ascorbic acid and carotene are found in the leaves, 11% of fatty oil in the seeds, and dyes (in the flowers) [24]. The researchers are showing interest in various groups of organic compounds of this kind. The components of the alicyclic structure were isolated from the aerial parts: hexadecacyclobut [1.2:3.4] bicyclooctene, 13-hexyloxacyclotridec-10-en-2-one (aromatic component). Among the polyyne compounds, (Z) -en-in-bicycloether was found out, and the group of aliphatic hydrocarbons and aldehydes was represented by n-nonadecane, dimethylpentadecane, n-eicosane, n-tricosane, n-pentacosane, n-octanal, n-undecanal, n-penta, (E, E) -2,4-decadienal, n-tridecanal. The chemical composition of the primary metabolites of the aerial parts of *L. vulgare* is characterized by the presence of higher fatty acids, such as octanoic, nonanoic, decanoic, n-undecanoic, cis-linoleic, rauric, myristic, palmitic and their derivatives – 2-methylbutyl-2-methylbutyrate, isoamylisovalericate, 1-octenyl acetate, n-pentylisovalerianate, γ-palmitolactone [35].

The essential oil of *L. vulgare* inflorescences is in the amount not exceeding 0.5% and includes terpenoids – mono- and sesquiterpenes: sabinene, myrcene, n-cymene, limonene, (E) -β-ocymene, 1,8-cineole, terpinene-4-ol, α-terpniol, geranyl acetate, geranilisovalerianate, α-cubeben, α-copaen, (E) -β-caryophyllene, aromadendrene, α-humulene, (E) -β-farnesene, germacrene, α-amoren, α -muurelen, δ-cadinene, γ-cadinene, isofaurinone, elemol, caryophyllene oxide, 1,2-humulene epoxide, γ-eudesmol, γ-cadinol, (Z) -lanceol, chamazulene, bisabolol A oxide, hexahydropharsylacetone, farnezolene, nerolidol, α-bisabolol [36]. A study of the essential oil of *L. vulgare* inflorescences growing on the territory of Estonia, was carried out. 115 compounds were identified by gas chromatography with flame ionization detection GC-FID and mass spectrometry GC-MS. It was found out that the composition of *L. vulgare* essential oil consisted mostly of (E)-β-farnesene (7.3%), hexadecahydrocyclobuta, dicyclooctene (5.3%), decanoic acid (4.9%), γ-eudesmol (4.5%) [36].

The composition of the essential oil from the aerial parts of *L. vulgare* growing on the territory of the Ardebal province (Iran) has been studied. In its composition, 47 compounds were identified, among which caryophyllene oxide (21.2%), aromadendrene oxide (13.7%), cis-β-farnesene (6.5%), 1-octen-3-yl-acetate (5.6%) and trans-caryophyllene (4.9%) prevailed. According to the authors, the results prove the presence of chemical varieties of this plant species [37].

Polyphenolic compounds found in the aerial parts of *L. vulgare* are represented by flavonoids, phenol carboxylic acids, and coumarins. The following flavonoids were isolated from the flowers *of L. vulgare* growing in Georgia: apigenin, cosmosin, 7-O-(3-D-glucuronide) of apigenin, vitexin, rutin, hyperin, hyperoside, quercetin, luteolin, isorhamnetin, 7-O-β-D-glucopyranoside of apigenin, chrysin, 7-O-(3-D-glucuronide) chrysin [38, 39]. Tubular flowers contain phenol carboxylic acids – chlorogenic and caffeic acids; coumarins are represented by umbelliferone and scopoletin [38, 39].

It is notified that in the folk medicine of Georgia and the Baikal region, *L. vulgare* water extracts are used for fevers, colds, coughs, pulmonary tuberculosis, eye diseases, involuntary urination, gastrointestinal colic, migraine, suffocation, pain, etc. Herbs decoction and infusions are prescribed for eye diseases, hernia, hemorrhoids. In addition, the herb and flowers are used externally – for skin rashes, lichen, ulcers, for bathing small children with intestinal spasms and convulsions. For skin diseases, a mixture consisting of crushed herbs together with flowers and rubbed with butter, is applied [15, 16, 39]. The studies devoted to the crude oil influence on the viability of *L. vulgare,* have been carried out. It was found out that this species is able not only to survive in soil exposed to crude oil, but also to reduce the concentration of this pollutant in the soil. The roots of *L. vulgare* successfully formed a symbiosis with mycorrhiza. At the same time, a positive correlation was shown between the concentration of the antioxidant compounds (including polyphenols) and the residual level of the oil concentration in the soil. The results showed that *L. vulgare* can survive under the conditions of pollution with oil products, and contribute to a decrease in their content in the substrate [40].

Tripleurospermum inodorum (L.)

According to the "Flora of Central Siberia", the *Tripleurospermum* genus of the *Asteroideae* subfamily has only one representative – *Tripleurospermum inodorum* (L.) [18]. According to the floristic summary "Flora of Siberia" [13], the genus has the name of *Matricaria* L. (*Tripleurospermum* Sch.Bip.) and includes three sections, two of which are widespread in Siberia with a population of one species – *Matricaria perforata* = *m.*

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inodora = *tripleurospermum inodorum* from the section of *Matricaria (Tripleurospermum*), and *m. tetragonosperma= tripleurospermum tetragonosperma* from the section of *Tetragonosperma*. However, the species *T. inodorum* has been included in "Flora of Siberia" conditionally, since the authors notified its possible presence in the east of Yakutia. The synonyms of the described type [13] are: *Chamomilla inodora* (L.) Gilib. [Invalid], *Ch. inodora* (L.) K.Koch, *Chamaemelum inodorum* (L.) Vis., *Ch. inodorum var. inodorum*, *Chrysanthemum inodorum* (L.) L., *Ch. maritimum var. inodorum* (L.) Bech., *Dibothrospermum agreste* Knaf., *D. pusillum* Knaf., *Matricaria inodora* L., *M. inodora var. agrestis* Weiss., *M. inodora f. agrestis* (Weiss) Fiori & Paol., *M. inodora f. biennis* Fiori & Paol., *M. inodora f. inodora., M. inodora var. inodor, M. inodora var. pusilla* Fiori, *M. maritima var. agrestis* (Knaf) Wilmott., M. *maritima subsp. inodora* (L.) Soó., *M. maritima subsp. inodora* (L.) Clapham., *M. maritima var. inodora* (L.) Soó, *M. perforata* Mérat., *M. pumila* Nyman., *Pyrethrum elegans* Pollini, *P. inodorum* (L.) Moench., *P. inodorum var. inodorum, Rhytidospermum inodorum* Sch.Bip. (permission not granted), *Tripleurospermum maritimum var. agreste* (Weiss) Briq. & Cavill. *T. maritimum subsp. inodorum* (L.) Appleq., *T. maritimum var. pusillum* (Knaf) Briq. & Cavill., *T. perforatum* (Mérat) M.Laínz, *T. perforatum* (Mérat) Wagenitz (permission not granted).

In the Irkutsk Region, *T. inodorum* is distributed in the western and southern parts of the Central Siberian Plateau and on the southern coast of Lake Baikal. The main habitats are along ditches, riverbanks, along wastelands and near roads, along the edges and glades of light and dark coniferous, as well as small-leaved forests. Since the plant is ecologically unpretentious (xero-mesophyte, mesotroph), this species colonization is often anthropogenic [13, 18].

It is carried from one place to another by seeds, while one plant can produce a huge number of achenes, according to various estimates, from 50,000–200,000 to 1.5 million and more. The seeds in the soil retain their germination capacity for a long time (up to 7 years). Due to the cold resistance, the seeds germinate early and amicably enough at low soil temperatures, and overwinter well. *T. inodorum* is a weedy species in the agricultural production. It infests not only cereals, but also tilled crops. In the Non-Chernozem zone, it is most likely to infest crops of important perennial grasses (clover and lucerne) and winter grains. A negative impact is also reflected in the fact that *T. inodorum* consumes twice as much moisture as oats and spring wheat, that negatively affects the yield. At the same time, this indicates the ecological stability of the described species. The ecological features of this representative of the *Asteroideae* subfamily serve as the basis for their rapid introduction into culture, including for the purpose of creating an additional raw material base for medicinal plants [30].

In addition to Eastern Siberia, the main areas of *T. inodorum* distribution include Western Europe, Atlantic Europe, the Balkans, Asia Minor, North America, the European part of Russia, the Caucasus, Western Siberia, and the Far East (as an invasive species) [21].

The data analysis from the available literature sources showed that *T. inodorum* contains essential oil, pyrethrin and related compounds, bitterness, mucus, gum, alkaloids, ascorbic acid; fatty oil (in seeds up to 20%) [21].

The essential oil composition of *T. inodorum* growing in Estonia has been studied in detail. The essential oil content did not exceed 0.2%. The main components are mono- and sesquiterpenoids, in particular: E-β-ocimene, artemisiaquetone, pinkarvone, geranium isobutanoate, β-caryophyllene, aromadendrene, α-humulene, E-β-farnesene, germacrene D, bicyclogermacrene, α-amorphen, spatulenol, caryophyllene oxide, bisophoxide trans-α-trans-α, Z-α-bergamotol, γ-eudesmol, γ-cadinol, chamomillol, (Z)-lanceol, bisabolol oxide A, bisabolol oxide B, bisabolone oxide A, aromadendrene oxide 2, alloaromadendrene epoxide, farnesyl acetate, hexylacahydropharenne.

The comparison of the essential oil composition of *T. inodorum* flowers and the essential oils of the *Chamomilla recutita* and *Ch*. *suaveolens* officinal species showed their significant similarity. In *T. inodorum* flowers, most were (Z, Z)-*matricaria ester* (77.9%), *(E)-β-farnesene* (3.5%), *matricaria ester isomer* (3.5%), *matricaria lactone* (3.0%) [36, 41].

Other groups of chemical compounds found in *T. inodorum,* are benzene derivatives: benzyl benzoate; aromatic compound – 1,3,4,5,6,7-hexahydro-2,5,5-trimethyl-2H-2,4a-ethanonaphthalene; a furan derivative – 2-pentylfuran. At the same time, compounds of the aliphatic series were found: n-octadecane, n-nonadecane, n-eicosane, n-tricosane, n-pentacosan, 6-methyl-5-hepten-2-one; fatty acids and their derivatives: decanoic, cis-linoleic, 2-methylbutyl-2-methylbutyrate, isoamylisovalerianate, 2-pentylisovalerianate, cis-hexenylisovalerianate, γ-palmitolactone. From the group of phenylpropanoids, including polyphenolic compounds, the presence of isoeugenol was revealed, 8-methylcoumarin, cosmosin and cynaroside in the inflorescences [42].

In addition, according to the works by a group of Estonian researchers, in the roots and aerial part of *T. inodorum*, there is information about the presence of such a specialized group of chemically active natural compounds as polyacetylenes. They are: trans-methyl-2-decene-4,6,8-triionate; matricaria ether, (Z,Z) matricaria ether, dihydromatricaria ether, (Z)-lachnophyllum methyl ether, (E)-lachnophyllum methyl ether, (Z)-ene-in-bicycloether, (E)-ene-in-bicycloether; *2cis, 8cis*-matrix triaether, *8cis-α,β*-dihydromatricaria ester, 2Z, 8Z-matrix triaether, 8Z-2,3-dihydromatricaria ester, (2E)-lachnophyllum ester, (2Z)-lachnophyllumate)-diEZ)-matrixialacton [36, 42]. On the basis of the Bryansk State Agri-

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cultural Academy, the mineral composition of weeds, including *T. inodorum*, was studied. As a result, it was found out that in the aerial parts of this species, in the process of the development and growth, sodium, magnesium, phosphorus, sulfur, silicon, manganese, copper, and molybdenum are accumulated in high concentrations [43].

A comparative phytochemical study showed that the amount of flavonoids in *T. inodorum* flowers exceeds the content of this group BACs in *Ch. recutita* flowers [44, 45].

In folk medicine, the *T. inodorum* herb is used as an analgesic, anti-inflammatory, diuretic, wound healing, antispasmodic, antihelminthic, and insecticidal agent [21].

Heteropappus altaicus (Willd.) Novopokr.

On the territory of the Baikal region, *Heteropappus* Less. genus is represented by three species – *Heteropappus hispidus* (Thunb.) Lees., *H. tataricus* (Lindl.), and *H. altaicus* (Willd.) Novopokr. [21]. "Flora of Siberia" [13], which includes descriptions of West and East Siberian plants and EL SB RAS [19], characterizes 3 species – *H. biennis* (Ledeb.) Tamamsch. *ex* grub. (syn. *H. tataricus*), *H. altaicus subsp. altaicus, H. altaicus subsp. appressifolius Koroljuk.*

According to the information base "The Plant List" [11], the name *H. hispidus* is synonymous with the species of *Kalimeris hispida* (Thunb.) Nees (*Kalimeris, Compositae* genus).

Taking into account the practical importance of the *H. tataricus* and *H. altaicus* species, a further was attention paid to these particular species.

H. tataricus Tamamsch. is the officially accepted name of the species, there are no synonyms [11]. However, there is information about the synonymy of *H. biennis* (Ledeb.) Tamamsch. ex Grub. (*H. tataricus* (Lindl.) Tamamsch.) [42]. *H. tataricus* is distributed in Western and Eastern Siberia, as well as in the Far East. It prefers valleys, steppe and dry meadows, forest edges, steppes. The species is a biennial [11, 13].

Triterpenoids such as polygalic acid, 28-O-[α-L-rhamnopyranosyl-(1→2)]-[β-D-xylopyranosyl-(1→3)]-β-D-glucopyranoside of arjunolic acid (heteropappusaponin I), as well as flavonoids – rutin, nicotiflorin, isoramnetin 3-O-rutoside – have been found in this species herb. An antifungal activity of polygalic acid isolated from the aerial organs of H. tataricus, has been experimentally established [42].

H. altaicus is a perennial herb. It inhabits the southern part of the Central Siberian plateau, in the mountains of the Eastern Sayans, in the Sayan-Baikal and Barguzinsky regions, Vitim Highlands, it is recorded in the southern part of Buryatia and Dauria. *H. altaicus* is a sun-loving xeromesophyte, preferring riverbanks, lakes, including saline ones, as well as dry and steppe meadows and steppes [13, 18 19]. *H. altaicus* (Willd.) Novopokr. (syn. *Aster altaicus* Willd. 1809, Enum. Hort. Berol. 2: 880. – *Aster altaicus* Willd. *var canescens* (Nees) Serg. – *Heteropappus canescens* (Nees) Novopokr. – *Heteropappus distortus* (Turcz. ex Ave-Lall.) Tamamsch. [18, 19].

The information base "The Plant List" [11] indicates the presence of 18 synonyms for this species: *Aster altaicus var. medius* Krylov, *A. altaicus var. uchiyamae* (Nakai) Kitam., *A. angustifolius* Lindl., *A. distortus* Turcz. ex Avé-Lall., *A. gebleri* Besser ex DC., *A. lithospermifolius* Desf., *A. medius* (Krylov) Serg., *A. millefolius* Vaniot, *A. pumilus* Fisch., *A. pyrrhopappus* Boiss., *A. spartioides* C.B.Clarke, *Brachyactis altaica* (DC.) Kitam., *Conyza altaica* DC., *Galatella altaica* Tzvelev., *G. juncea* Lindl. ex DC., *Heteropappus altaicus subsp. altaicus*, *H. altaicus var. altaicus*, *H. distortus* (Turcz. ex Avé-Lall.) Tamamsch.

The species is described in the lists of "Flora of China" [46] as *Aster altaicus var. altaicus*. The names *Aster gmelinii* Tausch, *Heteropappus altaicus* (Willdenow) Novopokrovsky, *Kalimeris altaica* (Willdenow) Nees, *K. altaica var. subincana* Avé-Lallemant are marked as synonymous. It is occurs in some China provinces, in addition, in the state of Kashmir (India), Kazakhstan, Mongolia. In the described territories, the species is found in the steppe, in meadows, salt marshes, rocky hillsides, as well as along roadsides and along riverbanks.

According to different authors' data, *H. altaicus* contains saponins, terpenoids, alkaloids, coumarins, flavonoids and tannins, essential oil. Foreign sources report that *H. altaicus* essential oil contains at least 54 components. The main ones are monoterpenes and triterpenes. Most of them are occupied by germacrine D, caryophilene, β-pinene, β-felandrene, and limonene (20%, 7%, 5%, 4 and 3%, respectively, in the total amount of identified components) [47]. The composition of mono and sesquiterpenoids of the *H. altaicus* aerial parts has been studied in detail. As a result, the presence of the following components has been established: farnesol, (-)-spatulenol, 1β 10α-caryophyllene epoxide, 4α7β-dihydroxy-10βH-guai-5-ene-1β8β-endoxide, 1β-methoxycariol-9-one; α-thuyene, α-pinene, β-pinene, camphene, sabinene, myrcene, α-fellandrene, Δ3-carene, α-terpinene, λ-terpinene, (Z) -β-ocymene, terpinolene, *n*-cymene, limonene, β-pellandrene, 1,8- cineole, cis-chrysanthenyl acetate, bornyl acetate, α-terpinyl acetate, trans-sabinyl acetate, δ-elemene, α-copaen, β-patchulene, β-bourbonene, β-elemene, β-caryofellen, β-copaen, α-humulene, germacrene D , β-selenene, cingiberen, bicyclogermacrene, trans-β-guayenne, (E, E) -α-farnesene, β-farnesene, δ-cadinene, spatulenol, caryofellene oxide, guayol [15, 16, 48].

In the aerial parts of *H. altaicus* (in flowers), the presence of diterpenoids and triterpenoids has been established. The first group includes trans-phytol, (-)-hardwickic and hautrivic acids, their derivatives – 12α-(2-methylbutyryloxy) hardwickic, lactone 12α-hydroxychautrivic-19, lactone 7α12α-dihydroxychautriv-

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ic-19, acids, 12α-(2-methylbutyryloxy) strictic acid; and also (5R, 6S, 8aS)-5-[2-3-furyl)ethyl-5,6,8a-trimethyl-4a, 5,6,7,8,8a-hexahydro-1-naphthalenecarboxylic acid] (or heteratic acid) [16].

The second group (triterpenoids) is represented by Fridelin, epifridelanol, 2β, 3β, 16α, 23-tetrahydroxyolean-12-ene-28-oic acid or polygalic acid [16, 49].

A quantitative assessment of some BACs groups of the aerial parts of *H. altaicus* growing on the territory of Western Transbaikalia, was carried out by Mazur L.V.

The composition of the herb was determined: 0.14% alkaloids, 0.76% flavonoids in terms of quercetin; 4.43% tannins and 0.88% ascorbic acid. In inflorescences, there are 0.05% alkaloids, 1.70% flavonoids in terms of quercetin, 5.92% tannins, 1.86% ascorbic acid. In the underground organs there are 0.08% flavonoids, 0.35% tannins. In addition, the study of the elemental composition revealed the following trace elements: Mn, Zn; Cu; Ni, Co, Cr [49].

In the available literature, the data on the study of *H. altaicus* in clinical medicine are insufficient; they touch on the period of 1997. The study results of the immunomodulatory and anti-inflammatory effects of triterpene saponins manifested by *Solidago virgaurea* and biennial species were presented [50]. Moreover, in folk medicine, *H. altaicus* inflorescences are prescribed for diseases of the gastrointestinal tract. The aerial part of the plant has antibacterial and protistocidal effects. In Tibetan and Mongolian medicine, the herb of this plant is used as an antipyretic, anti-inflammatory agent, for respiratory infections as an expectorant and antitussive remedy, as well as for stomach diseases (including a peptic ulcer disease [51]. The aerial organs are parts of the medicinal herbs mixtures prescribed for the treatment of measles and smallpox. In Chinese medicine, along with other plants, extracts from *H. altaicus* are used to treat sexual weakness in men, hemoptysis, and chronic bronchitis [21].

Solidago dahurica Kitag.

According to "Flora of Central Siberia" and the Siberian Branch of the Russian Academy of Sciences [18, 19], on the territory of Eastern Siberia, the Solidago L. genus has one representative – *S. dahurica* L. (syn. *Solidago dahurica* Kitag. *S. gebleri* Juz. – *S. virgaurea var. alpestris* Krylov.non. DC.). In its turn, in "Flora of Siberia" [13] it is indicated that two species grow on the territory of Eastern Siberia – *S. dahurica* and *S. spireifolia* Fisch. ex Herder. The latter is common only in the Arctic floristic region (Sakha Yakutia). According to the information base of the EL SB RAS [19], *S. gebleri*, characteristic of the Altai flora, occurs in Eastern Siberia. At the same time, *S. canadensis* L., which is an officinal species, and *S. virgaurea* L. are found on the territory of Eastern Siberia. These species belong to the wild and naturalized, and, in practice, typical species of the flora of Eastern Siberia. This is due to their widespread use as cultivated species for landscaping city streets and garden plots, as well as to the sufficient popularity of plants in the practice of traditional medicine [19, 21].

According to "The Plant List", *S. dahurica* is synonymous with *S. virgaurea subsp. dahurica* (Kitag.) Kitag., for which, in turn, the synonyms are *S. gebleri* Juz. and *S. gebleri var. gebleri* [11]. *S. dahurica* is usually a plain plant; its representatives are tall, up to 1 m, with a large panicle. The leaves are predominantly hairy along the veins. The plants from the Sayans and Stanovoe Highlands have a smaller habitus from 15 to 50 cm and have a simple racemose inflorescence, bare or almost bare leaves. Sometimes this species is distinguished as a special one – *S. gebleri* Juz. However, these differences are not constant and are not diagnostic signs [13, 18, 19].

S. dahurica is widespread in Eastern Siberia throughout the Central Siberian Plateau, in the Sayans regions along the Baikal Highlands. It does not form large thickets, but it has a massive habitus, a good supply of raw materials [52]. *S. dahurica* prefers to settle in thickets of shrubs, in forests, in clearings, along river valleys, on stony and gravelly slopes, pebble and open woodlands.

As a raw material for *S. dahurica,* the aerial parts are harvested during flowering. The found out flavonoids are astragalin, quercitrin, rutin, kaempferol, isorhamnetin, isoquercitrin. In addition, the presence of triterpene saponins, bitterness, tannins, resins, organic acids, essential oil, carotene, ascorbic and nicotinic acids, inulin, and alkaloids has been established [21].

The hydrolysis of the isolated triterpene saponins revealed eight aglycones, derivatives of virgureagenin, designated by letters A, B, C, D, I, F, G, H. The aglycone virgaureagenin A was identified as oleanolic acid, and virgaureagenin D – as a polygallic acid. The carbohydrate residue consists of glucose, rhamnose and xylose. The presence of glycosides of a polyphenolic nature, in particular, quercetin derivatives – isoquercitrin, has been established, the presence of saponins, tannins, catechins, flavones has been confirmed. The *S. dahurica* anthodium contains about 0.5% of essential oil, and about 0.7% in the leaves; the presence of saponins, catechins, tannins and flavones has been established [53].

In addition, the presence of organic acids (in particular, quinine), diterpenoids, polyacetylene compounds, phenolcarboxylic acids and their derivatives (caffeic, chlorogenic, hydroxycinnamic), coumarins (esculetin, esculin), and phytoecdysones has been revealed [53].

The chemical composition of the aerial organs ensures the use of *S. dahurica* as an expectorant, diuretic and hypoazotemic agent for the treatment of chronic nephritis and renal failure. In different countries, the *Solidago* species are used in a similar way. In particular, the herbs *S. dahurica, S. virgaurea* and *S. canadensis* have been long prescribed for the diseases of kidneys and bladder, for dissolving kidney and bladder stones, with a high protein content in urine, as a diuretic for dropsy, and also as effective remedies for rheumatism, gout, bronchial asthma, pulmonary tuberculosis, cholelithia-

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sis and other diseases. A successful use of these types externally are for angina rinsing, in gum sponginess, for strengthening sensitive teeth, for purulent wounds applications and bone fractures [24].

From the aerial parts of *S. dahurica*, mixtures are made – ointments, anatriptics on the basis of cream, pork fat or butter. Such remedies are recommended for skin tuberculosis, dermatitis, burns, rheumatism, and leucorrhoea. The peoples of Komi and Siberia use *S. dahurica* extracts internally for ulcerative cystitis, hepatitis, prostate adenoma, impotence, frequent emissions and chronic prostatitis (mixed with other plants), as well as externally for rinsing and washing with acute laryngitis, eczema [54].

In the folk medicine of the Caucasus, alcoholic tincture from the underground parts of the plant is used as a wound healing agent [21, 54]. The herb *S. virgaurea* is included in the British Herbal Pharmacopoeia as a diaphoretic and antiseptic [55]. In homeopathy [56], the essence of fresh inflorescences is used for chronic inflammatory diseases of kidneys, accompanied by skin rashes, swelling of the glands, edema, catarrh and rheumatic pains.

***Leontopodium conglobatum* (Tursz.) Hand.-Mazz.**

Leontopodium R. Br. ex Cass. genus in the East Siberian flora is represented by 4 species:

Leontopodium camesrte (Ledeb) Hand., *Leontopodium conglobatum* (Tursz.) Hand.-Mazz, *Leontopodium leontopodioides* (Wild.) Beauv., *Leontopodium ochroleucum* Beauv. [23, 24]. The floristic summary "Flora of Siberia" [13] describes the following species: *Leontopodium camesrte* (Ledeb) Hand., *Leontopodium conglobatum* (Tursz.) Hand.-Mazz и *Leontopodium ochroleucum* Beauv. as *Leontopodium conglobatum subspecies*.

The Global *Compositae* Checklist database, which is an integrated database of nomenclature and taxonomic information of the *Asteraceae* family, included in the Catalog of Life: 2015 Annual Checklist [57] and "The Plant List" information base [11] indicate that the names *Leontopodium ochroleucum var. conglobatum* (Turcz.) Grubov and *Leontopodium sibiricum var. conglobatum* Turcz. are synonymous with the name *Leontopodium conglobatum* (Turcz.) Hand.-Mazz. The comparative analysis of indicators is presented in the table.

Table – Comparative analysis of names, morphological characters, species distribution and intraspecific taxa *Leontopodium conglobatum* of Eastern Siberia

Sources (database)	Indicators			
	Species name	Distribution	Morphological characters	Habitat
Flora of Central Siberia, vol. 2	*Leontopodium conglobatum* (Turcz.) Hand. -Mazz.	Central Siberian plateau in the East of the Irkutsk region (in the area of the Lena River basin), in the Sayan-Baikal region on the southern coast of Lake Baikal, the Irkut river, in the eastern and western parts of the Stanovoy Highlands, including the South of the North-Baikal Highlands, in the steppe regions of the Republic of Buryatia in the Selenga River basin, in Selenga and Dzhida districts	– Stems are usually single, less often they are several, and then in small tussocks, the leaves on the stem are numerous (up to 15). Floral bracts are ovate-lanceolate; lanceolate or oblong in the upper half with curled edges, sharply narrowed; twice-3 times longer than the inflorescence, the inflorescence is often branched in the form of a shield of several "stars", less often solitary.	Steppe dry and valley meadows, forest edges, dry forests, stony-gravelly steppes.
Flora of Siberia, vol. 13	*Leontopodium ochroleucum subsp. conglobatum* (Turcz.) V. Khan. stat. et comb. nov.	In the Irkutsk region – the Angara-Sayan floristic region; In the Republic of Buryatia – the North Buryat and South Buryat floristic regions		
Electronic Library, Siberian Branch of the Russian Academy of Sciences Synonym	*Leontopodium ochroleucum var. conglobatum* (Turcz.) Grub.	Western Siberia: Altai. Central Siberia: Krasnoyarsk Territory, Republic of Khakassia, Republic of Tyva. Eastern Siberia: Irkutsk, Chita regions, Buryatia, Republic of Sakha (Yakutia)		
	Leontopodium sibiricum. var. conglobatum Turcz. 1847 in Bull. Soc. Nat. Moscou, 20,3.			
	Leontopodium ochroleucum Beauverd conglobatum (Turcz.) V. Khan.			
The Plant List Global *Compositae* checklist – Intra-species taxon, synonym	*Leontopodium ochroleucum var. conglobatum* (Turcz.) Grubov.	30 – Siberia, Irkutsk (IRK), Irkutsk (IRK-OO); 31 – Russian Far East, Amur (AMU), Amur (AMU-OO); … 31 – Russian Far East, Khabarovsk (KHA), Khabarovsk (KHA-OO); 30 – Siberia, Yakutskiya (YAK), Yakutskiya (YAK-OO); 3 – Asia-Temperate, 30 – Siberia, Chita (CTA), Chita (CTA-OO);		
	Leontopodium sibiricum var. conglobatum Turcz.			

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The data presented in the table makes it possible to conclude that an equivalent species is described under the accepted and synonymous names of *Leontopodium conglobatum*. At the same time, *Leontopodium conglobatum* has clearly pronounced morphological features, a fairly wide range on the territory of Eastern Siberia, and accessible habitats.

In the available literary sources, there is no information about clinical studies of this species. However, *Leontopodium conglobatum* (Turcz.) Hand.-Mazz. is included in the arsenal of medicinal plants of Tibetan medicine. For therapeutic purposes, the entire aerial part of the plant is used as a wound healing, expectorant, analgesic and sedative remedy; it is prescribed for cholelithiasis, as well as externally for cauterization during acupuncture [22]. The level of biological activity was studied according to the influence degree of the 1:20 infusion from *Leontopodium conglobatum* on the foaming reaction in a suspension of *Saccharomyces cerevisiae* [58]. No data on chemical composition has been found.

Geranium Genus

The *Geranium* L. (*Geraniaceae*) genus includes more than 300 species represented by herbs and shrubs. The representatives of the *Geranium* genus are native to the world and are found in various climatic zones. They can grow in mountains and rocks. More than 20 species of Geranium are cultivated and are the material for the cultivation of highly decorative varieties. The representatives of the *Geranium* genus form radical leaf rosettes on long petioles. At the same time, the leaf blade is finger-dissected into lobes of various shapes – from rather wide to thin, almost filiform. In the representatives of the *Geranium* genus, the flowers are actinomorphic, saucer-shaped, usually large and beautiful, and consist of five almost round petals. The petals can grow together to form a flat or calyx shape. In this case, the petals are either bent towards the rim, or, conversely, bent towards the petiole. The color of geranium flowers can be white, purple, blue, violet, lilac of various shades. The fruit is a box of a specific shape with the remaining calyx petals. The shape of the fruit resembles the beak of a crane, hence the name of the genus and the family [60].

The representatives of the *Geranium* genus of the north-west European Russia flora, were studied by Razarenova K.N. on the basis of the "St. Petersburg State Chemical and Pharmaceutical Academy". The objects of the research were: *Geranium pratense* L., *G. sylvaticum* L., *G. palustre* L., *G. sanguineum* L., *G. sibiricum* L., *G. robertianum* L., *G. pusillum* L. and a cultivated *G. lividum* species [59].

The floristic lists and identification guides describe a different number of the representatives of the Geranium genus inhabiting the Baikal region. In particular, in "Flora of Siberia" [61], 20 species are described. In "Flora of Central Siberia" [18], in the Program of the Siberian Branch of the Russian Academy of Sciences [19], there is information about 10 species; a photo guide "Plants of the western coast of Lake Baikal" and a revision summary "Abstract of flora of the Irkutsk region (vascular plants)" [62] contains a description of 4 species.

There is information about the use of geraniums various types in folk medicine as a means of treating insomnia, epilepsy, fever, rheumatism, diarrhea, as a hemostatic in gynecological diseases. In Tibetan medicine, these types are prescribed for the treatment of eye diseases, including cataracts [21, 60].

Among the Baikal representatives of the *Geranium* genus, the most common species have been identified: *Geranium eriostemon* Fischer., *G. pratense* L., *G. wlassowianum* Fischer.

Geranium eriostemon Fischer.

According to the information base [11], the name *G. eriostemon* is in the Illegitimate status, i.e. the use of this name is not consistent with the rules of the International Code of Botanical Nomenclature. At the same time, the name *G. eriostemon* is synonymous with the *G. platyanthum Duthie* species. In the floristic summaries "Flora of Siberia" [61], "Flora of Central Siberia" [18], and the Siberian Branch of of the Russian Academy of Sciences [19], the *G. eriostemon* species is described as independent.

The habitats are light forests, thickets of bushes, forest edges. The main areas of distribution are the western and southern regions of the Irkutsk region, the Central Siberian plateau, the southwestern outskirts of the Irkutsk region (Eastern Sayan), as well as the southern coast of Lake Baikal [18, 19, 61, 62].

Phytotechnological studies were carried out to obtain an extract containing the maximum amount of flavonoids of the *G. eriostemon* herb. Based on a comparison of the results obtained on a mathematically predictable model and experimental data, it was found out that the optimal conditions are the ratio "raw material : extractant" = 1 : 40, herewith the extractant was a 60% ethanol and the duration of the extraction was 35 min [63]. The content of tannides was determined by HPLC in some species of geraniums growing in China; the sum of these compounds in the *G. eriostemon* herb was 0.88% [64].

The component composition of *G. eriostemon* was studied by Du S. et al. established the presence of inositol derivatives – scillite β-sitosterol. The presence of polyphenols – phenolcarboxylic acids and flavonoids – was notified. In particular, the first group is represented by proto-catechic acid and gallic acid derivatives: 1,6-di-O-galloyl-α-L-glucose, 1,2,3,6-tetra-O-galloyl-β-D-glucose, corilagin. The second group is flavonoids, which belong to flavonols – quercetin, myricetin, 7-O-α-L-arabinofuranoside and 3-O-α-L-arabinofuranoside kaempferol. Shikimic acid was found in the herb [64].

A phytochemical study of the methanol extract from the *G. eriostemon* aerial part showed the presence of oleanolic acid, three lignans – (-)-kobusin, (-) – eude-

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smin, (+) – (+) – magnolin, lilac acid, and four flavonoids – quercetin, juglandin, juglalin and hyperin. Moreover, (+)-(+) – magnolin, lilac acid and quercetin showed a moderate cytotoxic activity against 4 human cancer cell lines *in vitro* [64].

The dynamics of tannins accumulation in the aerial and underground parts of *G. eriostemon,* growing on the territory of Buryatia, has been studied. The underground parts of this species are maximally rich in tannides, their content reached 4.14% [65].

A fairly high antimicrobial activity level of the sum of tannides isolated from underground *G. eriostemon* parts was experimentally established. The determination of bactericidal activity was carried out visually in Petri dishes according to the size of the growth inhibition zone of the most common representative of gram-positive bacteria, *Staphylococcus aureus*[9] [66].

G. pratense L.

G. pratense has a common name in the World List of Plant Families (World Checklist of Selected Plant Families – WCSP) [12] and is represented by 24 synonyms of different status: *G. acknerianum* Schur., *G. alpinum* Kit. ex Kanitz., *G. batrachioides* Bubani., *G. caeruleum* Gilib. [Invalid], *G. coelestinum* Schur., *G. kemulariae* Kharadze, *G. mariae* Sennen, *G. napellifolium* Schur., *G.neapolitanum* Nyman., *G. pratense var. affine* (Ledeb.) C.C. Huang & L.R. Xu, *G. pratense var. albidum* Regel, *G. pratense f. albiflorum* Q.Zhu & J.Wang, *G. pratense f. leucanthemum* B.Boivin., *G. pratense var. litwinowii* Woronow, *G. pratense var. pallidum* Regel, *G. pratense var. parviflorum* Regel, *G. pratense f. pratense, G. pratense var. pratense, G. pratense var. pubescens* Regel, *G. pratense var. schmidii* Y.J.Nasir, *G. pratense subsp. sergievskajae* Peschkova, *G. pratense subsp. stewartianum* Y.J. Nasir, *G. pratense var. tenuisectum* Regel, *G. valde-pilosum* Schur *ex* Nyman.

In the Irkutsk region, the species prefers the following habitats – meadows, forest edges, clarified forests. It occurs in the Sayans, on the northern coast of Lake Baikal, on the territory of the Baikal-Lensky Nature Reserve [18, 19, 62].

In the *G. pratense* aerial part, phenolcarboxylic acids and their derivatives – geraniin and isoheraniin – were found, in the underground part there were caffeic, gallic, dehydrogallic, ellagic, chebulagic acids; methylgallate, 6-galloylglucose [64, 67, 68].

Among the compounds of the flavonoid structure, the presence of rutin, quercetin, and apigenin has been established [67]. Among other polyphenolic compounds in the aerial organs of *G. pratense*, the following were identified: myricetin 3-O-(2”-O-haloyl)-β-D-glucopyranoside, 5 quercetin derivatives: 3-O-β-D-glucopyranoside, 3-O-β-D-galactopyranoside, 3-O-(2”-O-haloyl)-β-D-glucopyranoside, 3-O-(2”-O-haloyl)-β-D-galactopyranoside, 3-O-α-D-arabinopyranoside, as well as 3-O-β-D-glucopyranoside kaempferol [67–68], as well as (-)-6-chloroepigallocatechin, methyl gallate and tryptophan. In the underground organs, there were (+)-catechin and (-)-Epicatechin The isolated compounds were found to be effective against disorders of the endothelium-dependent relaxation in an isolated segment of the rat aorta [70].

The aerial parts of *G. pratense,* which grows in the western part of Russia, were examined for the amino acid composition. In particular, in the aerial parts of *G. pratense* harvested in the Republic of Bashkortostan, 20 amino acids were identified and their contentss were determined [67, 68, 71].

The color of geranium flowers is provided by anthocyanin – malvidin diglycoside; leukoanthocyanidin is present in the seeds [68].

The Baikal species *G. pratense* is one of the first plant objects studied at the Department of Pharmacology of the Irkutsk State Medical Institute in the 60[s] of the last century. The studies were related to the effect study of the extract from this plant on the central nervous system and its toxicity [72]. At the same time, in the experiments on laboratory animals, the wound-healing effect of the polysaccharides sum of this plant has been studied. The effect of this group of compounds on the regeneration process of de-epilated animal skin, pretreated with a 20% potassium hydroxide solution, was studied. A noticeable decrease in the area of the wound surface, the acceleration of the regeneration processes and proliferation were found [73]. *G. pratense,* a species native to Mongolia, has a high antioxidant activity [74, 75]. In isolated plasma, methanol extracts inhibited the action of the α-amylase enzymatic activity by more than 40% [74]. The prospect of using the sum of this type polyphenolic compounds against opportunistic microorganisms – strains *B. cereus, E. coli, P. aeruginosa, S. aureus* – was established [76].

Agrobiological studies of *G. pratense* were carried out. Was studied the antimicrobial activity of the dried powder from the roots of this plant after the ground application with a potato crop. This procedure reduced a further morbidity of the inoculum. It was found out that the fraction with geraniine exhibits the antimicrobial activity at its content of up to 15% of the dry weight of the root (HPLC method). The antimicrobial activity of this fraction corresponded to 1.25% of the streptomycin effect (a paper disk method). The results of the study indicate that the use of *G. pratense* as an organic supplement or an accompanying crop for controlling the microbial contamination of potatoes is promising [77].

Geranium wlassowianum Fischer

According to the information database, *G. wlassowianum* Fischer. has no synonyms [11]. In the Irkutsk region, *G. wlassowianum* is found in the east and south of the region, more often in the Tulunsky region. It is also

[9] GOST 10444.2-94. Food products. Methods for detecting and quantifying Staphylococcus aureus. Russian

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typical for the southern coast of Lake Baikal, is included in the list of vegetation of the Baikal-Lensky reserve, while it prefers habitats similar to the above-listed geraniums species [18, 19, 62].

Throughout the whole vegetation season, a consistently high content of tannides was noted in the underground and aerial parts of the *G. wlassowianum* species growing on the territory of Buryatia [69]. The bactericidal activity level of tannins of *G. wlassowianum* vegetative organs against Staphylococcus aureus, was determined [66].

CONCLUSION

The data presented in the review show that species of the *Asteroidae* subfamily and the *Geranium* genus are sources of biologically active compounds of various groups – polyphenols, terpenoids, essential oil components, saturated and unsaturated fatty acids, trace elements, vitamins, etc. The presence of phenolcarboxylic acids, tannides is notified in all species. In folk medicine, the described plant objects are prescribed for the treatment of various diseases. The effect on the central nervous system has been experimentally confirmed. The following types of pharmacological actions – hemostatic, diaphoretic, antiseptic, antioxidant, cytostatic, anti-influenza, antiherpetic, antiprotozoal, antimicrobial and anti-inflammatory – have been confirmed, too.

The ranges of the presented species cover almost all the continents of the globe. This indicates broad adaptive capabilities of the plant objects described. Many of the listed species are the bases for the introduction of varieties with special decorative, economic and biological characteristics into cultivation; the ones with purposefully improved pharmaceutical values have also been included there. In this regard, in relation to the Baikal species *Heteropappus altaicus, Solidago dahurica, Leucanthemum vulgare, Tripleurospermum inodorum, Antennaria dioica, Leontopodium conglobatum* and *Geranium eriostemon, G. pratense, G. wlassowianum,* it is advisable to conduct a deep complex phytochemical and pharmacological research in order to create medicines for the treatment of the most common diseases.

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The author declares no conflict of interest.

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Elena G. Privalova – planning, collecting literature data, writing and editing the review.

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ОРИГИНАЛЬНАЯ СТАТЬЯ

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DEVELOPMENT OF PERORAL HYPOLIPIDEMIC FORMULATION BASED ON SULFATED ARABINOGALACTAN IN THE FORM OF POTASSIUM SALT

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An original heparinoid, sulfated arabinogalactan in the form of potassium salt, possessing anticoagulant and hypolipidemic activities, has been developed at the A.E. Favorsky Irkutsk Institute of Chemistry Siberian Branch of the Russian Academy of Sciences.

The aim was to develop solid peroral dose forms (capsules and film-coated tablets) for the prevention and treatment of atherosclerotic lesion of blood vessels on the basis of potassium salt of sulfated arabinogalactan which would be suitable for further clinical trials of these forms.

Materials and methods. The following materials were used in the work: sulfated arabinogalactan in the form of potassium salt, obtained at the A.E. Favorsky Irkutsk Institute of Chemistry Siberian Branch of the Russian Academy of Sciences; Ludipress®; AEROSIL® 200 Pharma; calcium stearate; Aquacoat ECD. The powder mixtures were briquetted followed by tableting and application of the finished film coating Aquacoat ECD, and encapsulation in hard gelatin capsules.

Results. Composition and technological characteristics of capsules and film-coated tablets were determined using physico-chemical and technological properties of sulfated arabinogalactan in the form of potassium salt. Technological parameters and quality indicators were determined for the solid pharmaceutical dose forms in accordance with the requirements of the State Pharmacopoeia of the Russian Federation of the XIVth edition.

Conclusion. The optimum compositions and technology for the preparation of capsules and film-coated tablets based on potassium salt of sulfated arabinogalactan for the prevention and treatment of atherosclerotic lesion of blood vessels, were developed. The data obtained were used for the regulatory documentation design.

Keywords: sulfated arabinogalactan; film-coated tablets; capsules; production technology

Abbreviations: RF – the Russian Federation; SB RAS – Siberian Branch of the Russian Academy of Sciences; FDF – finished dosage form; GPhM – General Pharmacopoeial Monograph; FFC – finished film coating.

РАЗРАБОТКА ПЕРОРАЛЬНОЙ ЛЕКАРСТВЕННОЙ ФОРМЫ ГИПОЛИПИДЕМИЧЕСКОГО ДЕЙСТВИЯ НА ОСНОВЕ СУЛЬФАТИРОВАННОГО АРАБИНОГАЛАКТАНА В ВИДЕ КАЛИЕВОЙ СОЛИ

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RESEARCH ARTICLE

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В Иркутском институте химии им. А.Е. Фаворского Сибирского отделения Российской академии наук разработан оригинальный гепариноид – сульфатированный арабиногалактан в виде калиевой соли, обладающий антикоагулянтной и гиполипидемической активностями.

Цель. Создание на основе сульфатированного арабиногалактана в виде калиевой соли твердых дозированных лекарственных форм для перорального применения в виде таблеток, покрытых пленочной оболочкой, и капсул для профилактики и лечения атеросклеротического повреждения кровеносных сосудов, которые в дальнейшем будут пригодны для клинического исследования.

Материалы и методы. Для получения твердых дозированных лекарственных форм использовались: сульфатированный арабиногалактан в виде калиевой соли, полученный в Иркутском институте химии им. А.Е. Фаворского СО РАН; Ludipress®; AEROSIL® 200 Pharma; кальция стеарат; Aquacoat ECD. Применялось брикетирование порошковых масс с последующим таблетированием и нанесением готового пленочного покрытия Aquacoat ECD и капсулирование в твердые желатиновые капсулы.

Результаты. На основе изученных физико-химических и технологических свойств сульфатированного арабиногалактана в виде калиевой соли обоснован состав и технология производства таблеток, покрытых пленочной оболочкой, и капсул. Для разработанных твердых дозированных лекарственных форм определены технологические параметры и показатели качества в соответствии с требованиями Государственной фармакопеи РФ XIV издания.

Заключение. Разработаны оптимальные составы и технологии получения таблеток, покрытых оболочкой, и капсул на основе сульфатированного арабиногалактана в виде калиевой соли для профилактики и лечения атеросклеротического повреждения кровеносных сосудов. Полученные данные положены в основу разработки нормативной документации.

Ключевые слова: сульфатированный арабиногалактан; таблетки, покрытые пленочной оболочкой; капсулы; технология производства

Список сокращений: РФ – Российская Федерация; СО РАН – Сибирское отделение Российской академии наук; ГЛФ – готовые лекарственные формы; ОФС – общая фармакопейная статья; ГПК – готовое пленочное покрытие.

INTRODUCTION

Atherosclerotic lesion of blood vessels is one of the most widespread and serious disorders of the homeostatic system. The use of drugs with a polyvalent mechanism of pharmacological action for the prevention and treatment of this pathology is justified, and the application of heparinoids for this purpose is very promising [1–8].

Foreign hypolipidemics based on natural and semi-synthetic heparinoids are limited [9–13] due to their physico-chemical properties (they are extremely easily destroyed in the digestive tract that impairs their absorption and transport to blood)[1].

In the Russian Federation (RF), heparinoids are represented by sulodexide-derived drugs, which exert angioprotective, profibrinolytic and antithrombotic effects[2]. Sulodexide is an organopreparation, extracted from the mucous membrane of the small intestine of a pig. It is a natural mixture of glycosaminoglycans (80% of heparin-like fraction with a molecular weight of 8 kDa and 20% of dermatansulfate) [14–15]. The effective application of Sulodexide for the secondary prevention of coronary atherosclerosis and its complications is proven by clinical multicenter placebo-controlled trials [16–18]. Sulodexide-based medicines cause serious side effects in view of the peculiarities of the technology for obtaining organopreparations. For instance, these drugs contain trace proteins and histamine-like substances that can lead to allergic reactions[3].

Thus, the search for heparinoids devoid of similar disadvantages is an urgent problem in medical and pharmaceutical sciences.

A directed chemical modification of arabinogalactan, the main polysaccharide of Siberian larch, made it possible to develop the original pharmacologically active compound – sulfated arabinogalactan in the form of potassium salt (Agsular®), in A.E. Favorsky Irkutsk Institute of Chemistry Siberian Branch of the Russian Academy of Sciences[4]. This is a semi-synthetic heparinoid exhibiting anticoagulant and hypolipidemic activities, having a high relative bioavailability after the peroral administration (54.4%) [19]. The improved technology for the synthesis of potassium salt of sulfated arabinogalactan made it possible to obtain a pharmaceutical grade substance [20, 21]. The safety and efficiency of the latter has been proven by the pre-clinical trials conducted together with the Research Institute of Toxicology (St. Petersburg) in 2009–2011 [22, 23].

Thus, sulfated arabinogalactan in the form of potassium salt is a promising building block for the development of peroral medicines. Today, the most popular are such peroral finished dose forms (FDFs) as tablets and capsules, which have a wide range of possibilities and advantages [24–25].

[1] Ryzhenkov VE, Makarov VG, Remezova OV, Makarova MN. Metodicheskie rekomendacii po izucheniyu gipolipidemicheskogo i antisklerotycheskogo dejstviya lekarstvennyh sredstv. [Methodical recommendations for the study of hypolipidemic and antisclerotic action of drugs]. In: Vol. 1, Rukovodstvo po provedeniyu doklinicheskih issledovanij lekarstvennyh sredstv. [Guidelines for conducting preclinical studies of drugs]. Moscow: Grif and K; 2012. p. 445–452. Russian

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[4] A.E. Favorsky Irkutsk Institute of Chemistry Siberian Branch of the Russian Academy of Sciences, assignee. Agsular®. Russian Federation trademark No. 398618. 2010 Jan 19. Russian

ОРИГИНАЛЬНАЯ СТАТЬЯ
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THE AIM was to develop solid peroral dose forms (capsules and film-coated tablets) for the prevention and treatment of atherosclerotic lesion of blood vessels, on the basis of potassium salt of sulfated arabinogalactan which would be suitable for further clinical trials of these forms.

MATERIALS AND METHODS

Materials

The following materials were used in the work: sulfated arabinogalactan in the form of potassium salt (Agsular®), obtained according to the developed methods [20, 21]; Ludipress® (BASF, Germany); colloidal silicon dioxide – AEROSIL® 200 Pharma (Evonik Degussa, Germany); calcium stearate, analytically pure, reagent grade (Technical conditions 2432-061-56856807-04); Aquacoat ECD (FMC, USA); purified water (Pharmacopoeia Monograph.2.2.0020.18)[5].

Study of physico-chemical properties

The shape and size of the particles were determined by scanning electron microscopy using a SEM 525-M scanning electron microscope (Philips, the Netherlands) and by dynamic light scattering using a Zetasizer Nano ZS analyzer (Malvern Instrument, UK). The measurements were carried out at the angles of 13° and 173° in plastic cuvettes (1×1 cm). The average hydrodynamic diameter was calculated by the analysis of fluctuations in the intensity of light scattering of spherical particles. The results were processed using the Dispersion technology Zetasizer family software v7.01.

The mass-average molecular mass was determined by high performance size exclusion chromatography on an Agilent 1260 Infinity chromatograph (Agilent Technologies, Germany), a PL aquagel-OH 408 nm column, 300×7.5 mm, a refractometer detector, a sample concentration of 1 mg/ml, a sample volume of 20 µL. The elution was carried out with a 0.1 M aqueous solution of lithium nitrate ($LiNO_3$) at 30°C with the eluent flow rate of 1 ml/min. The column was calibrated using D-galactose and dextrans with molecular weights of 5, 12, and 25 kDa (Sigma, United States). The calculations were performed using the Agilent ChemStation software.

The elemental analysis was performed on a Flash2000 automatic elemental analyzer (Thermo Scientific, Italy).

The solubility was determined according to the method described in General Pharmacopoeial Monograph (GPhM) 1.2.1.0005.15 “Solubility”[6].

The average density (ρ_{av}, kg/m^3) was determined by the ratio of the sample mass (m≥0.3 kg) to the entire volume occupied by it (V) in natural conditions, including the cavities and pores in it (V_{pore}), and calculated by the formula:

$$\rho_{av} = \frac{m}{V} = \frac{m}{V_{s.ph.} + V_{pore}}, \quad (1)$$

where: ρ_{av} is an average density, kg/m^3; m is the mass of the sample, kg; V is the sample volume, m^3; $V_{s.ph.}$ is a volume of a solid phase, m^3; V_{pore} is a volume of cavities and pores, m^3.

Specific surface area ($S_{sp.s.}$, m^2/kg) was determined as the sum of the surface of all particles, the total mass of which was 1 kg, and calculated by the formula:

$$S_{sp.s.} = \frac{k}{d_{s.ph.} \times \rho_{av}}, \quad (2)$$

where: $S_{sp.s.}$ is a specific surface area, m^2/kg; k is a particle shape factor; $d_{s.ph.}$ is a particle diameter of the solid phase, m; ρ_{av} is an average density, kg/m^3.

The hygroscopicity (H, %) was determined under the extreme conditions (in a chamber with a relative humidity of 100% for 24 hours) [26]. The kinetics of the moisture absorption was calculated by the formula:

$$H = \frac{(m - m_0) \times 100\%}{m}, \quad (3),$$

where: H is a hygroscopicity, %; m_0 is the mass of the powder before being kept in a chamber with a 100% air humidity, g; m is the mass of the powder after the exposure in the chamber with a 100% air humidity, g.

Study of technological properties

A fractional (granulometric) composition was determined according to the method described in GPhM.1.1.0015.15 “Sieve analysis”[7] on an automatic WEB scatterer (MLW-Labortechnik, Germany).

The flowability (g/s), the angle of repose (°) and the bulk density (g/ml) were determined according to the methods described in GPhM.1.4.2.0016.15 “The degree of flowability of powders”[8] on a device for determining the flowability TK-1 (Pharmatech, Ukraine) and on a device for vibration compaction of powders 545-P-AK-3 (Pharmatech, Ukraine).

The compressibility (breaking strength, N) was determined according to the following procedure: a weighed portion (0.3 g) of the powder was compressed in a matrix with a diameter of 9 mm on a PGR 400 manual hydraulic press (Infraspek company, Russia) at the pressure of 120 MPa. After pushing the tablet out of the matrix, the compressive strength was determined using a tablet and granule strength tester TT-03 (China) in kg of load, which was recalculated in N [26].

The Hausner index was calculated by the formula:

[5] State Pharmacopoeia of the Russian Federation XIV ed., Vol. I–IV. Available from: http://femb.ru/pharmacopea.php
[6] Ibid.
[7] Ibid.
[8] Ibid.

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$$Hausner_{index} = \frac{\rho_{bd1}}{\rho_{bd0}}, \quad (4),$$

where: ρ_{bd0} is a bulk density before the compaction, kg/m³; ρ_{bd1} is a bulk density after the compaction, kg/m³ [27].

The Carr index (%) was calculated by the formula [28]:

$$Carr_{index} = \frac{100\% \times (\rho_{bd1} - \rho_{bd0})}{\rho_{bd1}}, \quad (5),$$

where: ρ_{bd0} is a bulk density before the compaction, kg/m³; ρ_{bd1} is a bulk density after the compaction, kg/m³.

Technology for tablets production

Briquetting was carried out from a powder mixture of active ingredients and auxiliary substances obtained in a PSM "plough" mixer (Pharmag, Germany) at 50-250-450 rpm for 30 min, on a hydraulic hand press PGR 400 (matrix diameter – 25 mm) (Infraspek company, Russia) under the pressure of 200 bar. The briquettes were grinded in a dry granulator DG (Pharmag, Germany).

The obtained granules were fractionized on an automatic diffuser (WEB MLW-Labortechnik, Germany); for tableting, granules with a size of at least 1 mm were used.

Tableting was performed using the punches to the tablet press CPR-6 (Dott. Bonapace & C s.r.l., Italy), allowing biconvex tablets with a diameter of 12 mm to be obtained.

The application of 30% aqueous dispersion of the finished film coating Aquacoat ECD was performed in a CP-9 coater (Pharmag, Germany) with a fixed operating angle of the device (45°). The coated tablets were dried using infrared light (150 W).

Technology for capsules production

The encapsulation was carried out using the finished semi-closed hard-gelatin capsules Empty Hard Gelatin Capsules (Shaanxi Genex Bio-Tech Co., Ltd., China) with the sizes No. 00-1. The capsules were filled with the help of manual machines MC-1.2 (Multipharma, Italy) of the appropriate size.

FDF quality assessment

The quality of the obtained FDFs was evaluated according to the standard pharmacopoeial methods[9]:

1. GPhM.1.4.2.0009.15 "Uniformity of the mass of dosage forms", the determination of the uniformity of the mass of the capsules contents, was carried out using laboratory electronic scales LV 120-A (Sartogosm, Russia);

2. GPhM.1.4.2.0008.15 "Dosage Uniformity", the determination of the distribution uniformity of the active substance among individual dosage units of FDFs (capsules and film-coated tablets) was carried out according to method 2 using laboratory electronic scales LV 120-A (Sartogosm, Russia);

3. GPhM.1.4.2.0013.15 "Disintegration of tablets and capsules", the determination of disintegration, was carried out in purified water using the laboratory identifier of the disintegration process 545 AK-00-00 (Pharmatech, Ukraine).

4. GPhM.1.4.2.0014.15 "Dissolution for solid dosage form", the determination of the amount of active substance released from the pharmaceutical dose forms for a certain period of time, was carried out in purified water using a device for determining the solubility 545 AK7-00-00 (Pharmatech, Ukraine) by spectrophotometric method on a UV/VIS spectrometer Lambda 35 (Perkin Elmer, USA).

5. GPhM.1.4.1.0015.15 "Tablets", the determination of the excipients amount aerosil and calcium stearate) was carried out by the gravimetric method using laboratory electronic scales LV 120-A (Sartogosm, Russia).

Statistical processing of results

All obtained data were statistically processed (P = 95%) using the Student's test in accordance with the requirements of GPhM.1.1.0013.15[10].

RESULTS AND DISCUSSION

Physico-chemical and technological properties

The most economically beneficial and technological method for tableting of powders is their direct compression. However, despite the advantages of this method, it is applicable for a limited number of pharmaceutical substances and depends on their physico-chemical and technological properties [29].

Sulfated arabinogalactan in the form of potassium salt (Fig. 1) is a hydrophilic functionalized biopolymer, where sulfate groups are located at the C2 and C4 atoms of the main galactan chain and at the C6 atom of the terminal galactose residues of the main and side chains of the polysaccharide. According to the data of elemental analysis and characteristics of the molecular weight distribution, the degree of substitution of the macromolecule is 0.4, i.e. about one sulfate group is per one structural unit of the biopolymer [21].

[9] Ibid.

[10] Ibid.

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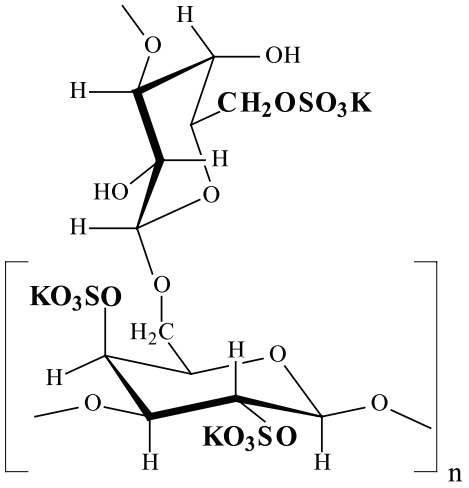


Figure 1 – Fragment of sulfated arabinogalactan in the form of potassium salt

Visually, it is a white or white-creamy amorphous powder with a weak characteristic odor. According to the data of scanning electron microscopy (Fig. 2A), its particles have a spherical shape and form large agglomerates with the size in the range of 200-600 nm (Fig. 2B).

To evaluate suitability of a sulfated arabinogalactan potassium salt for tableting by direct compression methods, its physico-chemical (Table 1) and technological properties (Table 2) were studied.

The results of the carried out experiments showed that sulfated arabinogalactan in the form of potassium salt is finely dispersed amorphous powder with high hygroscopicity (20.07%). It is almost uniform in the particle size distribution, which does not meet the requirements to powders for direct pressing, since it has low technological properties.

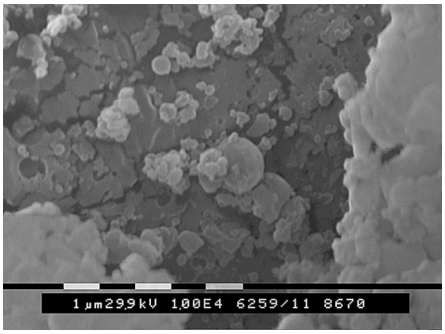
Powders with a particle size of 0.5–1 mm are known to be the best for a direct compression [24]. This parameter for sulfated arabinogalactan in the form of potassium salt is only 8.6%, and the amount of the fine fraction is more than 79%. That significantly affects the material flowability, leading to its almost complete absence (1 g/s). In this case, not the entire sample spilled out by gravity from the device funnel, there was a partial freezing, dusting and static-charge accumulation of the powder. The Carr (24.91%) and the Hausner (1.33) indices confirm a very poor compressibility (24.5 N) of sulfated arabinogalactan in the form of potassium salt, accompanied by the complexity of filling the matrix and ejecting the finished tablet from it. All these can lead to a violation of the active substance dosage accuracy in the FFC.

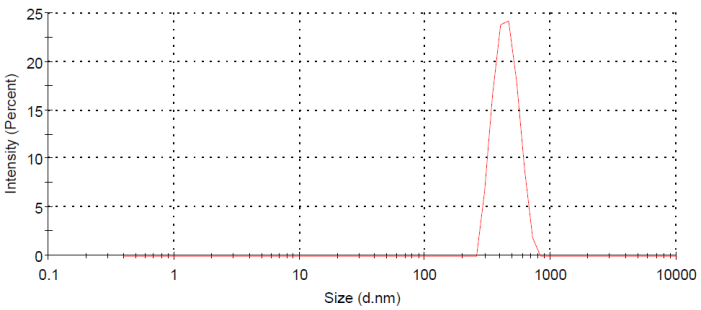
In this regard, the next stage was to bring technological properties of sulfated arabinogalactan in the form of potassium salt into compliance with the properties of a technologically conditioned mass, suitable for obtaining tableted FFC by the direct compression method.

Technology for tableted FDF preparation

Previously, a tableted FDF based on sulfated arabinogalactan in the form of potassium salt with a dosage of the active component of 500 mg, had been designed. Anhydrous lactose, low-molecular polyvinylpyrrolidone and aerosil were used as adjuvants [34]. The shelf life of the tablets, obtained by direct compression with a monocomponent composition of adjuvants, was no more than two and a half years because of a high hygroscopicity of such sulfated arabinogalactan and the absence of protective coatings from the atmospheric moisture in the tablets.

Therefore, in order to increase stability and extend the shelf life of tablets derived from sulfated arabinogalactan, the composition and technology (the selection of modern combined adjuvants to improve flowability and compressibility; the selection of film moisture-protective coatings for tablets; the approbation of wide used granulation method for the directed enlargement of the particles), were experimentally optimized.





A **B**

Figure 2 – Shape and size of sulfated arabinogalactan particles in the form of potassium salt

Note: A – scanning electron microscopy (200–600 nm); B – dynamic light scattering method (585.9 nm)

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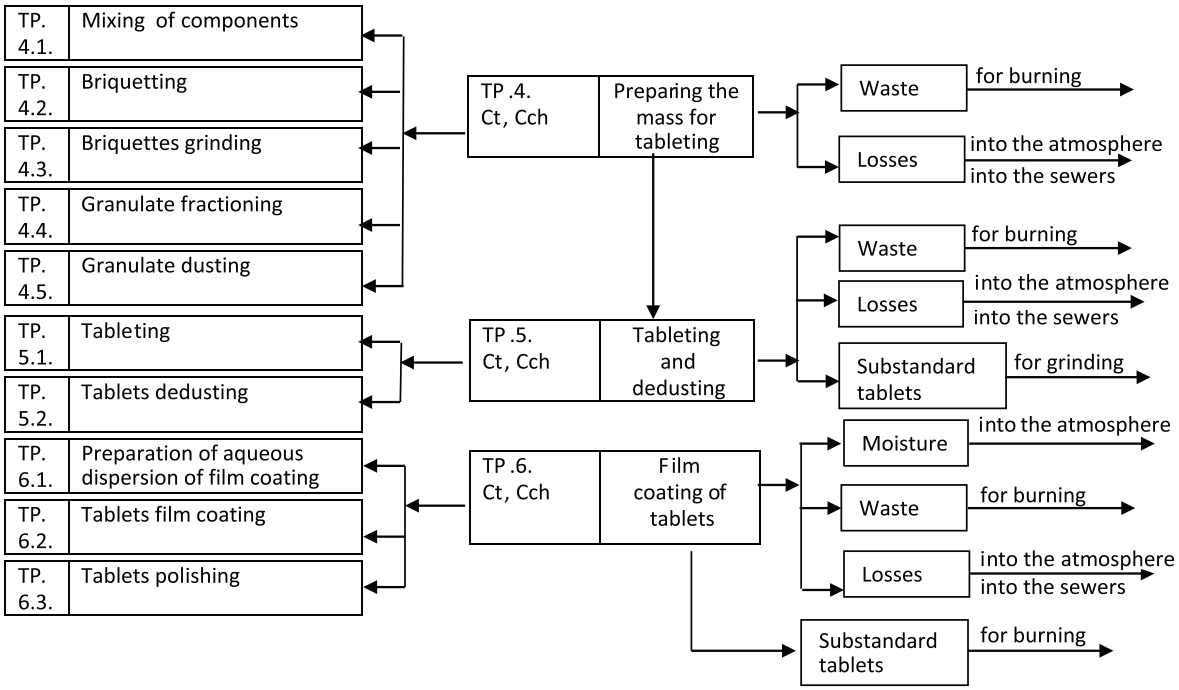

Figure 3 – Fragment of technological scheme for manufacture of "Agsular® film-coated tablets 500 mg"
Note: Ct, Cch are technological and chemical control, respectivel

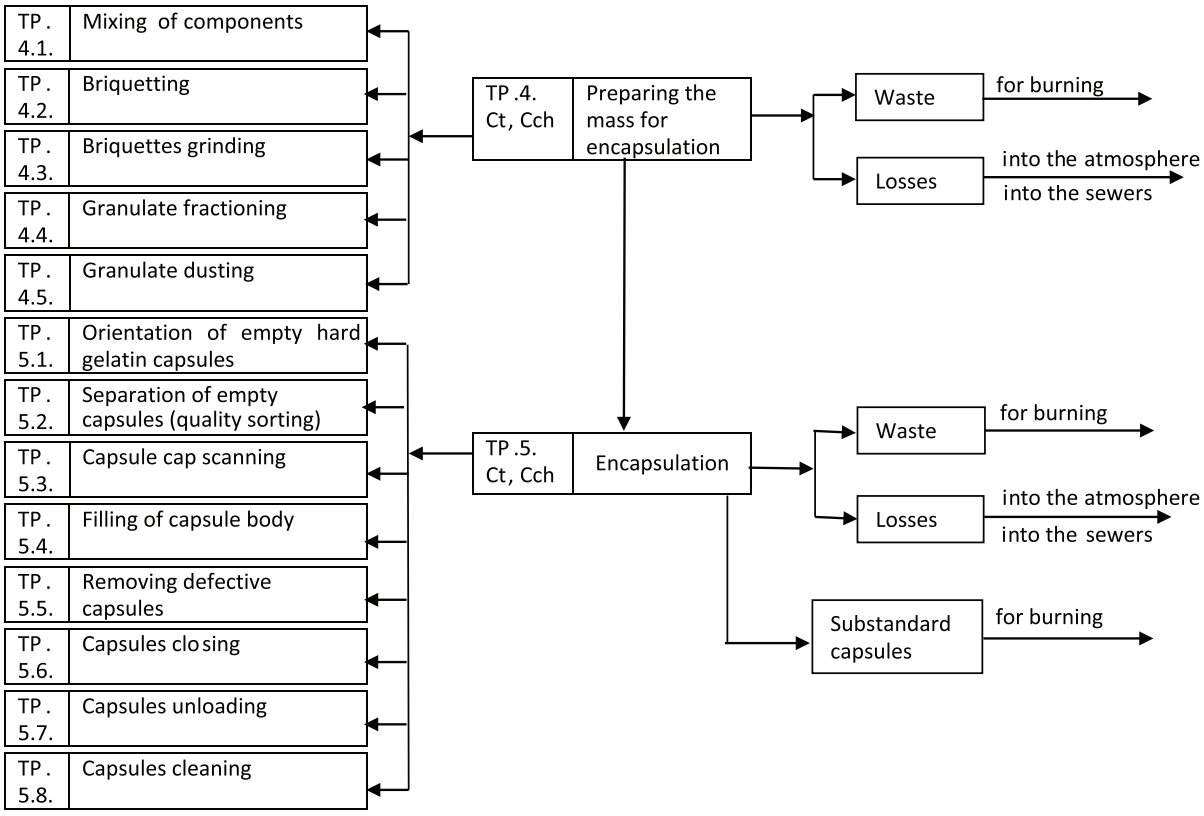

Figure 4 – Fragment of the technological scheme for the manufacture of "Agsular® capsules 500 mg"
Note: Ct, Cch are technological and chemical controls, respectively

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Table 1 – Physico-chemical properties of sulfated arabinogalactan in the form of potassium salt

Average molecular weight, kDa	Solubility, kg/m^3	Average density, kg/m^3	Specific surface area, m^2/kg	Hygroscopicity, %	Found (%) Calculated (%)			
					C	H	S	K
27.5	300; H_2O	843.17	13.88	20.07	26.63 25.39	2.26 3.23	11.01 11.48	15.55 13.99

Table 2 – Technological parameters of sulfated arabinogalactan in the form of potassium salt

Technological characteristics	Experimental data	Reference values
Granulometric composition	Fractions: < 0.25 mm 19.8%; 0.25–0.5 mm 59.5%; 0.5–1.0 mm 8.6%; 1.0–2.0 mm 10.5%; 2.0–3.0 mm 1.4%; > 3.0 mm 0.2%	Granulometric composition: more than 60% should be fractions with a particle size of 0.5–2 mm; less than 20% – fractions with a particle size of up to 0.2 mm [30].
Flowability, g/s	1.00±0.05	8.6–12 g/s – excellent; 6.6–8.5 g/s – good; 3–6.5 g/s – satisfactory; 2–3 g/s – allowable; 1–2 g/s – bad; 0.3–1 g/s – very bad [31].
The angle of the natural slope	43–45°	25–30° – very good flowability; 31–35° – good flowability; 36–45° – satisfactory degree of flowability; 46–55° – unsatisfactory degree of flowability (additional stirring or vibration is required; 56–65° – poor flowability; > 66° – very poor flowability [11]
Compressibility (breaking strength), N	24.50±1.14	> 70 N – good 40–70 N – medium < 40 N – bad [26].
Bulk density before compaction, g/ml	0.633±0.032	> 2.0 g/ml – very heavy; from 1.1 to 2.0 g/ml – heavy; from 0.6 to 1.1 g/ml – medium; < 0.6 g/ml – light bulk materials [31].
Bulk density after compaction, g/ml	0.843±0.042	
Hausner index	1.33	1.00–1.11 – very good compressibility; 1.12–1.18 – good compressibility; 1.19–1.25 – average compressibility; 1.26–1.34 – satisfactory compressibility; 1.35–1.45 – poor compressibility; 1.46–1.59 – very poor compressibility; > 1.60 – a very poor compressibility [32].
Carr index, %	24.91	10 – very good compressibility; 11–15 – good compressibility; 16–20 – average compressibility; 21–25 – satisfactory compressibility; 26–31 – poor compressibility; 32–37 – very poor compressibility; > 38 – a very poor compressibility [33].

[11] Ibid.

Table 3 – Composition and technological characteristics of tablet mixtures based on sulfated arabinogalactan in the form of potassium salt

Components, technological parameters	Tablet mixtures, wt.%			
	No. 1	No. 2	No. 3	No. 4
Sulfated arabinogalactan in the form of potassium salt	47.15	61.88	73.33	82.92
Ludipress	47.15	30.94	18.34	13.27
Aerosil	4.70	6.18	7.33	3.31
Calcium stearate	1.00	1.00	1.00	0.50
Flowability, g/s	5.5±0.2	2.6±0.1	2.0±0.1	1.5±0.1
Technological parameters of mixtures after briquetting				
Flowability, g/s	10.7±0.5	6.4±0.3	4.6±0.2	3.1±0.1
Compressibility (breaking strength), N	44.5±2.2	15.0±0.7	11.2±0.5	9.7±0.4
Bulk density before compaction, g/ml	1.349±0.067	1.270±0.063	1.215±0.061	0.497±0.025
Bulk density after compaction, g/ml	1.448±0.073	1.434±0.072	1.427±0.071	0.646±0.032
Hausner index	1.07	1.13	1.17	1.30
Carr index, %	6.84	11.44	14.86	23.07

Table 4 – Quality indicators of tableted FDF based on sulfated arabinogalactan in the form of potassium salt

Key indicators	Methods [12]	Standards	Result
Description	Visual	Tablets, coated with white or almost white films, round, smooth, biconvex. On a cross section, the core of the tablet is from white to white with a slightly creamy shade.	Compliant
Authenticity	1. Polysaccharide fragments.	When a drop of 95% ethyl alcohol is added, the solution becomes cloudy.	Performed
	2. Reaction of metachromasia.	When a 0.005% solution of toluidine blue is added, the color of the dye solution changes from blue to lilac.	Performed
	3. Potassium ions (reaction B) (GPhM.1.2.2.0001.15)	When diluted acetic acid and sodium cobalt nitrite solution are added, a yellow crystalline precipitate is formed.	Performed
Uniformity of dosage	Weight (GPhM.1.4.2.0008.15)	The content of the substance of sulfated arabinogalactan in the form of potassium salt in coated tablets is determined according to method 2. Sample size is 10. First acceptance rate AV ≤15%.	Performed
Disintegration	Visual (GPhM.1.4.2.0013.15)	At least 16 out of 18 samples should completely disintegrate in 30 minutes; dissolution medium is purified water.	25.8 min Compliant
Dissolution	Spectrophotometric (GPhM.1.4.2.0014.15)	Release (not less than 75%) of the substance of sulfated arabinogalactan in the form of potassium salt is within 45 minutes; dissolution medium is purified water.	94.2% Compliant
Determination of auxiliaries	Gravimetric (GPhM.1.4.1.0015.15)	The content of aerosil and calcium stearate in film-coated tablets should be from 0.054 g to 0.066 g (0.06 g±10%) and not more than 11% of the average tablet weight (1.082 g) – 0.119 g.	0.059 g Compliant
Quantification	Spectrophotometric	Content of the substance of sulfated arabinogalactan in the form of potassium salt in one film-coated tablet should be from 0.475 g to 0.525 g (0.5 g±5%).	0.496 g Compliant

[12] Ibid.

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Table 5 – Size of capsules for tablet mixtures based on sulfated arabinogalactan in the form of potassium salt, depending on their technological properties

Parameters	Tablet mixtures		
	No. 1	No. 2	No. 3
Average capsule mass contents, g	1.061	0.808	0.682
Acceptable deviation, %		7.5	
Flowability, g/s	10.7±0.5	6.4±0.3	4.6±0.2
Bulk density before compaction, g/ml	1.349±0.067	1.270±0.063	1.215±0.061
Range of the volume occupied by the granulate, ml	0.77–0.81	0.62–0.66	0.55–0.58
Size of hard gelatin capsules	00	0	0
Bulk density after compaction, g/ml	1.448±0.073	1.434±0.072	1.427±0.071
Volume range occupied by granulate, ml	0.71–0.75	0.55–0.58	0.47–0.49
Size of hard gelatin capsules	00	0	1

Table 6 – Quality indicators of encapsulated FDF based on sulfated arabinogalactan in the form of potassium salt

Key indicators	Methods[13]	Standards	Results
Description	Visual	Hard gelatin capsules No. 1 or 0 or 00 with a white body and a pink lid. The content of the capsules is a compact mass from white to white-creamy.	Compliant
Authenticity	1. Polysaccharide fragments.	When a drop of 95% ethyl alcohol is added, the solution becomes cloudy.	Performed
	2. Reaction of metachromasia.	When a 0.005% solution of toluidine blue is added, the color of the dye solution changes from blue to lilac.	Performed
	3. Potassium ions (reaction B) (GPhM.1.2.2.0001.15)	When diluted acetic acid and sodium cobalt nitrite solution are added, a yellow crystalline precipitate is formed.	Performed
Mass uniformity	Weight (GPhM.1.4.2.0009.15)	The average weight of the contents of capsules No. 00 is from 0.981 to 1.141 g (1.061±7.5%). The average weight of the contents of capsules No. 0 is from 0.747 to 0.869 g (0.808±7.5%). The average weight of the contents of capsules No. 1 is from 0.631 to 0.733 g (0.682±7.5%). No more than two individual masses may deviate from the average mass by an amount exceeding the acceptable deviation. In this case, no individual mass should deviate from the average mass by an amount that is twice as high as the acceptable deviation.	Capsules No. 00 – 1.084 g; Capsules No. 0 – 0.798 g; Capsules No. 1 – 0.696 g. Compliant
Dosage uniformity	Weight (GPhM.1.4.2.0008.15)	The content of sulfated arabinogalactan in the form of potassium salt in capsules is determined according to method 2. The sample size is 10. The first acceptance rate AV ≤15%.	Performed
Disintegration	Visual (GPhM.1.4.2.0013.15)	At least 16 out of 18 samples should completely disintegrate in 30 minutes; the dissolution medium is purified water	Capsules No. 00 – 27.4 min; Capsules No. 0 – 26.2 min; Capsules No. 1 – 26.6 min; Compliant
Dissolution	Spectrophotometric (GPhM.1.4.2.0014.15)	The release (not less than 75%) of sulfated arabinogalactan in the form of potassium salt is within 45 minutes; the dissolution medium is purified water.	Capsules No. 00 – 91.8%; Capsules No. 0 – 90.8%; Capsules No. 1 – 91.3%. Compliant
Determination of auxiliaries	Gravimetric (GPhM.1.4.1.0015.15)	The content of aerosil and calcium stearate in capsules No. 00 should be from 0.054 g to 0.066 g (0.06 g±10%) and not more than 11% of the average weight of the capsule contents (1.061 g) – 0.117 g. The content of aerosil and calcium stearate in capsules No. 0 should be from 0.052 g to 0,.064 g (0.058 g±10%) and not more than 11% of the average weight of the capsule contents (0.808 g) – 0.089 g. The content of aerosil and calcium stearate in capsules No. 1 should be from 0.051 g to 0.063 g (0.057 g±10%) and not more than 11% of the average weight of the capsule contents (0.682 g) – 0.075 g.	Capsules No. 00 – 0.058 g; Capsules No. 0 – 0.057 g; Capsules No. 1 – 0.062 g. Compliant
Quantitative determination	Spectrophotometric	The content of sulfated arabinogalactan in the form of potassium salt in one capsule should be from 0.475 g to 0.525 g (0.5 g±5%).	Capsules No. 00 – 0.501 g; Capsules No. 0 – 0.511 g; Capsules No. 1 – 0.477 g. Compliant

[13] Ibid.

From a large assortment of modern combined adjuvants, Ludipress®, consisting of a complex of α-lactose monohydrate (93.4%), polyvinylpyrrolidone (Kollidon 30) (3.2%) and 3D polyvinylpyrrolidone (Kollidon CL) (3.4%)[14], was chosen. Ludipress has the same qualitative composition of ingredients that had been used earlier to obtain tablets based on sulfated arabinogalactan in the form of potassium salt, but it is characterized by the optimal quantitative combination. That makes it possible to obtain sufficiently stable tablets under a low pressure of compression and to improve the technological properties of the active substance [35].

Since sulfated arabinogalactan in the form of potassium salt is a hygroscopic and thermolabile compound, the briquetting technique (a kind of dry granulation method) was employed to improve the flowability of the tableting mixture and prevention of its separation [36–37].

For the experiments, the model compositions of tablet mixtures were obtained, and their main technological properties were studied (Table 3).

As Table 3 shows, only tablet mixture No. 1 satisfies the technological requirements of the tableting process, because it has an excellent flowability, an average breaking strength and a very good compressibility [31, 38]. The results obtained confirm the right choice of its optimal composition and technology. Tablet mixtures No. 2 and No. 3 can be used to prepare FDFs for the peroral administration as capsules.

To protect sulfated arabinogalactan in the form of potassium salt from the effects of the atmospheric moisture, an effective film coating for tablets was selected. Hydrophobic polymers or hydrophobic polymers in combination with hydrophilic additives or hydrophilic polymers in combination with hydrophobic additives are most often used as moisture-proof coatings [39]. Such structure-forming moisture-protecting compounds as ethyl cellulose (insoluble in water, but permeable to aqueous solutions) [40] and cetyl alcohol, possessing emulsifying and stabilizing properties, were chosen as film-forming agents [41].

In modern pharmaceutical engineering, finished commercial film-forming compositions (FFCs) are widely used in the form of solutions consisting of a film-forming agent, plasticizer, colorant and solvent taken in the optimum ratio, as well as semi-products in the form of granules (powders). From the latter, a film-forming solution (dispersion) is prepared immediately prior to application using an appropriate solvent [42–45]. From all the nomenclature of FFCs containing ethyl cellulose and cetyl alcohol, Aquacoat ECD film coating was selected for the experimental studies on the protection of tablets from the atmospheric moisture; the tablets were based on sulfated arabinogalactan in the form of potassium salt. This FFC is used as a 30% aqueous dispersion obtained immediately prior to use. The advantage of the specified HPC is the possibility of its application not only for creating a barrier coating (the application in the amount of 1–2% of the tablet weight) but also for maintaining the pH-independent mode of the release (the application in the amount of 8–15% of the tablet weight[15]. In addition, Aquacoat ECD is compatible with most alcohol- and propyleneglycol-soluble dyes, as well as various types of plasticizers. It promotes the formation of high-resistant, stable, easily dissolvable (in the stomach) coating, giving the tablet a pleasant appearance and effectively protecting the active substance from the atmospheric moisture [46].

The technology for the preparation of a solid pharmaceutical for a peroral administration in the form of film-coated tablets based on sulfated arabinogalactan in the form of potassium salt according to the formulation of the tablet mixture No. 1, was elaborated using Pharmag equipment and a tablet press with one CPR-6 compression station (Fig. 3).

As a result, biconvex tablets with a diameter of 12 mm of high strength (44.5±2.2 N) were obtained.

Quality evaluation of the tableted FDF

The quality of the tablets obtained was evaluated according to the following parameters: description, authenticity, dosage uniformity, disintegration, dissolution, determination of auxiliaries (aerosil and calcium stearate) and quantitative determination (Table 4).

According to the studies, the developed solid dosage formulation of FDF for the oral administration in the form of film-coated tablets based on sulfated arabinogalactan in the form of potassium salt, withstands all tests and fully complies with the requirements of the GPhM.1.4.1.0015.15 “Tablets”[16].

The data obtained form the basis for the development of regulatory documents and laboratory regulation for the production of “Agsular® film-coated tablets 500 mg”.

Technology for preparation of encapsulated FDF

In order to prepare FDF based on sulfated arabinogalactan in the form of potassium salt in the form of capsules, the granulate, according to the recipes of tablet mixtures No. 1–3, was placed in hard gelatin capsules of size No. 00-1, respectively (Table 5).

The elaboration of technology for the preparation of a solid pharmaceutical for the peroral administration in the form of capsules based on sulfated arabinogalactan in the form of potassium salt, was carried out using

[14] Ludipress® – Enabling efficient direct tabletting. Available from: http://www.pharmaceutical.basf.com/en/Drug-Formulation/Ludipress.html

[15] Aquacoat ECD – Aqueous Dispersion Coating System. Available from: http://www.zhion.com/pharmaceutics/Aquacoat_ECD.html

[16] State Pharmacopoeia of the Russian Federation XIV ed., Vol. I–IV. Available from: http://femb.ru/pharmacopea.php

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Pharmag equipment and sets of manual machines for filling hard gelatin capsules (MC-1.2) of the appropriate size. That was due to the forced feeding of the tablet mixture into the capsule and additional pre-pressing of the material, according to the proposed technological scheme (Fig. 4).

Quality evaluation of FDF

The finished capsules were controlled according to the developed specification (Table 6). The following quality indicators were investigated: description, authenticity, mass uniformity, dosage uniformity, disintegration, dissolution, determination of auxiliaries (aerosil and calcium stearate), quantitative determination.

According to the experiments, the developed peroral solid dosage formulation of FDF in the form of capsules based on sulfated arabinogalactan in the form of potassium salt withstands all tests and fully complies with the requirements of the GPhM.1.4.1.0005.15 "Capsules"[17].

The data obtained form the basis for the development of regulatory documents and laboratory regulation for the production of "Agsular® capsules 500 mg".

CONCLUSION

In conclusion, the optimum compositions and technology for preparation of solid peroral dosage forms have been experimentally developed on the basis of potassium salt of sulfated arabinogalactan (capsules and coated tablets) for the prevention and treatment of blood vessels atherosclerosis. The data obtained make possible to plan a placebo-controlled clinical trial of the 1st phase.

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CONFLICTS OF INTEREST

Authors declare no conflicts of interest.

AUTHORS' CONTRIBUTION

Yana A. Kostyro – experimental work execution, research results processing, text writing; Konstantin V. Alekseev – concept and design of the study development, management of experimental work, text editing.

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RESEARCH ARTICLE

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STUDY OF CLINICAL AND PATHOGENETIC EFFECTS OF ANTI-VIRAL DRUG BASED ON FAVIPIRAVIR IN COMORBID PATIENTS WITH COVID-19 AT THE OUTPATIENT STAGE OF TREATMENT

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In many ways, arterial hypertension and obesity determine the likelihood of a severe course and lethal outcomes in COVID-19. This fact justifies the expediency of an early use of drugs with a direct antiviral action, the analysis of their efficacy not only in the acute, but also in the postcovid period.

The aim of the research was to analyze the outpatient cards and case histories of the COVID-19 patients to study the effect of the early (up to the 5th day after the onset of the first symptoms of the disease) use of the drug based on favipiravir, on the frequency of patients' hospitalizations with arterial hypertension and obesity, as well as to determine the cytokine status characteristics of this patient category in the postcovid period.

Materials and methods. "An open prospective comparative study of the "Areplivir®" (favipiravir) efficacy in the debut of COVID-19 in comorbid patients" was carried out in the Republic of Mordovia (the analysis of the hospitalizations frequency and blood levels of M-CSF, EPO in 218 patients, in terms of the use of the antiviral preparation).

Results. According to the results of the analysis, it was found out that, despite the presence of comorbid conditions that increase the risk of developing a severe course of COVID-19, i.e. obesity and essential arterial hypertension, in the group of patients taking favipiravir, the need for hospitalization was twice as low ($p < 0.05$), in relation to the comparison group. The analysis of the cytokine status revealed that in the postcovid period, in the group that took the drug based on favipiravir at the outpatient stage, the average level of M-CSF was significantly lower ($p > 0.05$), and EPO was higher ($p > 0.05$) than in the patients from the group "without antiviral drugs at the outpatient stage". Indirectly, according to the previously obtained data, that acts as a potential marker for reducing the risk of long-term cardiovascular complications of COVID-19.

Conclusion. This study showed that an early prescription of favipiravir contributes to a decrease in the rate of COVID-19 patients' hospitalization even against the background of concomitant hypertension and obesity, due to a decrease in the likelihood of moderate and severe courses of the disease, and also leads to an earlier objective and subjective recovery. The results demonstrated a high potential benefit of an early favipiravir use in the novel coronavirus infection and in the prevention of postcovid complications.

Keywords: favipiravir; COVID-19; novel coronavirus infection; Areplivir

Abbreviations: AH – arterial hypertension; DM – diabetes mellitus; ACA – Acute Cerebrovascular Accident; TCA – Transient Cerebrovascular Accident; MI – myocardial infarction; ALT – alanine aminotransferase; AspAT – aspartate aminotransferase; BMI – body mass index; CT – computerized tomography; PCR – polymerase chain reaction; ECG – electrocardiogram; VED – Vital and Essential Drugs; EAH – essential arterial hypertension; MCSF – macrophage colony stimulating factor; RNA – ribonucleic acid; enzyme-linked immunosorbent assay; ELISA – enzyme immunoassay; CI – confidence interval; GCS(s) – glucocorticosteroids; WHO – World Health Organization; TNF-α – tumor necrosis factor alpha; 1β-IIβ – interleukin.

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ИЗУЧЕНИЕ КЛИНИКО-ПАТОГЕНЕТИЧЕСКИХ ЭФФЕКТОВ ПРОТИВОВИРУСНОГО ПРЕПАРАТА НА ОСНОВЕ ФАВИПИРАВИРА У КОМОРБИДНЫХ ПАЦИЕНТОВ С COVID-19 НА АМБУЛАТОРНОМ ЭТАПЕ ЛЕЧЕНИЯ

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Артериальная гипертензия и ожирение, во многом, определяют вероятность тяжелого течения и летальных исходов при COVID-19. Этот факт обосновывает целесообразность раннего применения лекарственных средств прямого противовирусного действия с анализом эффективности не только в остром, но и постковидном периоде.

Цель. Провести анализ амбулаторных карт и историй болезни пациентов с COVID-19 для изучения влияния раннего (до 5-го дня от момента появления первых симптомов болезни) применения препарата на основе фавипиравира на частоту госпитализаций у пациентов с артериальной гипертензией и ожирением, а также определить особенности цитокинового статуса пациентов данной категории в постковидном периоде.

Материалы и методы. Проведено «Открытое проспективное сравнительное исследование эффективности применения препарата «Арепливир®» (фавипиравир) в дебюте COVID-19 у коморбидных пациентов» в Республике Мордовия (анализ частоты госпитализации и содержания в крови M-CSF, EPO у 218-ти больных в зависимости от применения противовирусного препарата).

Результаты. По результатам проведенного анализа установлено, что, не смотря на наличие коморбидных состояний, повышающих риск развития тяжелого течения COVID-19, а именно ожирения и эссенциальной артериальной гипертензии, в группе пациентов, принимавших фавипиравир, необходимость в госпитализации была в 2 раза ниже ($p<0,05$), по отношению к группе сравнения. Анализ цитокинового статуса выявил, что в группе, принимавшей препарат на основе фавипиравира на амбулаторном этапе, средний уровень M-CSF в постковидном периоде был достоверно ниже ($p>0,05$), а EPO – выше ($p>0,05$), чем у пациентов из группы «без противовирусных препаратов на амбулаторном этапе», что косвенно, по полученным ранее данным, выступает потенциальным маркером снижения риска отдаленных сердечно-сосудистых осложнений COVID-19.

Заключение. Раннее назначение фавипиравира способствует снижению частоты госпитализации пациентов с COVID-19 даже на фоне сопутствующих АГ и ожирения из-за уменьшения вероятности среднетяжелого и тяжелого течения заболевания, а также приводит к более раннему объективному и субъективному выздоровлению. Показана высокая потенциальная польза раннего применения фавипиравира при новой коронавирусной инфекции и в отношении профилактики постковидных осложнений.

Ключевые слова: фавипиравир; COVID-19; новая коронавирусная инфекция; Арепливир

Список сокращений: АГ – артериальная гипертензия; СД – сахарный диабет; ОНМК – острое нарушение мозгового кровообращение; ТНМК – транзиторное нарушение мозгового кровообращения; ИМ – инфаркт миокарда; АлТ – аланинаминотрансфераза; АсТ – аспартатаминотрансфераза; ИМТ – индекс массы тела; КТ – компьютерная томография; ПЦР – полимеразная цепная реакция; ЭКГ – электрокардиограмма; ЖНВЛП – жизненно необходимые важнейшие лекарственные препараты; ЭАГ – эссенциальная артериальная гипертензия; M-CSF – макрофагальный колониестимулирующий фактор; РНК – рибонуклеиновая кислота; ИФА – иммуноферментный анализ; ДИ – доверительный интервал; ГКС – глюкокортикостероиды; ВОЗ – Всемирная организация здравоохранения; TNF-α – фактор некроза опухоли альфа; 1β–Iiβ – интерлейкин.

INTRODUCTION

The pandemic of the novel coronavirus infection continues to pose new challenges to the health care system. The issues of diagnosis and prevention of COVID-19 have been mainly determined, but the treatment strategies continue to be developed [1]. The latest six versions of the domestic temporary guidelines emphasize the need for early specific etiotropic therapy (including favipiravir) at the outpatient stage of the disease (Temporary guidelines version 7–13[1]).

Arterial hypertension and obesity (the most frequent comorbidities in coronavirus patients) mainly determine the likelihood of a severe course and lethal outcomes in COVID-19 [2]. This fact justifies the expediency

[1] Temporary guidelines “Prevention, diagnosis and treatment of new coronavirus infection (COVID-19)” of the Ministry of Health of Russia (version 13.1 of 11/09/2021). Russian

of an early use of drugs with a direct antiviral action, followed by the analysis of their efficacy not only in the acute, but also in the postcovid periods [3, 4].

Previously, the efficacy of favipiravir preparations had been established in terms of the rate of the virus elimination, positive dynamics of the lung tissue state according to the computerized tomography (CT) data during SARS-CoV-2 infection [5]. However, the published data demonstrating a decrease in the incidence of a severe course and lethal outcomes of the coronavirus infection while taking favipiravir drugs, are insufficient [6], especially in high-risk comorbid patients (with arterial hypertension, obesity, and diabetes mellitus). When carrying out a comparative analysis of the favipiravir and arbidol efficacy for the treatment of COVID-19, Chen S. et al. found out the following. In the patients with concomitant hypertension and/or diabetes mellitus in a moderate course of disease, the time period before the normalization of the body temperature and the disappearance of cough was significantly shorter in the group using favipiravir, compared with group using arbidol ($p<0.001$) [7].

The published results demonstrate the maximum efficacy of antiviral therapy [8, 9] when carried out in the first 5 days of the disease, which actualizes the use of the drug and the analysis of its efficacy starting from the outpatient stage of treatment, especially in the patients at risk. That is why, by order of the Government of the Russian Federation dated October 14, 2021 No.2626-r[2], favipiravir was included in the List of Vital and Essential Drugs (VED), which made it possible to compensate for the costs of purchasing drugs from the compulsory medical insurance fund and start the treatment as soon as possible.

In the Republic of Mordovia, since September 2021, a complication of the situation with the novel coronavirus infection has been notified. So, in October 2021, according to recent reports of the autonomous public health care institution "Medical Information and Analytical Center for the Republic of Mordovia"*, 13,414 cases[3] of the novel coronavirus infection COVID-19 have been registered (1722.0 per 100 thousand population), which is 7098 cases more than in September 2021 (6316 cases – 810.8 per 100 thousand population). In other words, the increase in the incidence was 112.4%, and the features of the course of the coronavirus infection caused by the delta strain, are the rapid progression of the disease and a tendency to a severe course. In this situation, the Executive Board of the republic took a number of organizational measures in the form of an increase in the additional bed fund of the infectious profile by 185% (from 1503 to 2780 beds). Nevertheless, even in this situation, inpatient beds worked at full capacity, which actualized the need for a more active treatment of COVID-19 at the outpatient stage to potentially reduce the number of patients requiring hospitalization. As a part of the charitable program, on 01.10.2021 and 12.10.2021, the Ministry of Health of the Republic of Mordovia received 16,038 packages of the drug "Favipiravir" (Areplivir®) (film-coated tablets, 200 mg No. 40) for the outpatient treatment of the novel coronavirus infection, which made it possible to determine the aim of the study.

THE AIM of the research was to analyze the outpatient cards and case histories of the COVID-19 patients to study the effect of the early (up to the 5th day after the onset of the first symptoms of the disease) use of the drug based on favipiravir, on the frequency of patients' hospitalizations with arterial hypertension and obesity, as well as to determine the cytokine status characteristics of this patient category in the postcovid period.

MATERIALS AND METHODS

Approval by the local ethics committee at National Research Ogarev Mordovia State University (Protocol No. 5 dated May 17, 2020) "An open prospective comparative study of the efficacy of the drug "Areplivir®" (favipiravir) in the debut of COVID-19 in comorbid patients" was conducted in the Republic of Mordovia. The study included 1200 patients who received outpatient treatment at the outpatient clinics of Saransk. For 340 hospitalized patients of them, the data from the case histories from March to May 2021, as well as during October 2021 (the time of the maximum use of favipiravir at the outpatient stage), were analyzed. The COVID-19 diagnosis was aligned with the current interim guidelines for the prevention, diagnosis and treatment of the novel coronavirus infection[4].

The study included patients of both sexes with laboratory and / or clinically confirmed new mild and moderate kinds of coronavirus infection. They were aged 48-80 years, suffered from the combination of obesity and essential arterial hypertension (EAH), stage II, established before getting infected with SARS-CoV-2 and controlled by antihypertensive drugs. Herewith, a duration of COVID-19 before treatment was to be no longer than 5 days. 2 groups of patients were formed. The comparison group consisted of the patients who received basic anti-inflammatory, anticoagulant, symptomatic (according to the indications – antibacterial) therapy for the coronavirus infection, according to temporary guidelines[5], and the ones who did not receive, for various reasons, antiviral drugs. The second group consisted of the patients selected according to the case-control type, who, alongside with anti-inflammatory, anticoagulant and symptomatic therapy, received the antiviral drug Areplivir® already at the outpatient stage.

In accordance with the instructions for the use of a medicinal product, the drug based on favipiravir, was to be administered orally 30 minutes before meals, accord-

[2] Order of the Government of the Russian Federation dated 12.10.2020 No.2626-r. Available from: http: //publication.pravo.gov.ru/Document/View/0001202010140014. (Date of access 24 Nov 2021). Russian

[3] GAUZ of the Republic of Mordovia "Medical Information and Analytical Center". Available from: http://miacrm.ru.

[4] Temporary guidelines "Prevention, diagnosis and treatment of new coronavirus infection (COVID-19)" of the Ministry of Health of Russia (version 13.1 of 11/09/2021). Russian

[5] Ibid.

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ing to the following scheme: the patients weighing less than 75 kg were to be administrated with 1600 mg (8 tablets) twice on the 1st day of therapy, then, from the 2nd to the 10th day – 600 mg (3 tablets) twice/day; the patients weighing more than 75 kg were to be administrated with 1800 mg (9 tablets) twice on the 1st day of therapy, then (from the 2nd to the 10th day of therapy) – 800 mg (4 tablets) twice/day)[6].

The exclusion criteria were: the history of associated clinical conditions – acute cerebrovascular accidents (ACVs), myocardial infarction (MI), angina pectoris, coronary revascularization, renal failure, type 1 diabetes mellitus, autoimmune, allergic diseases, symptomatic hypertension, the use of glucocorticosteroids, hydroxyl-chloroquine, the use of other antiviral drugs (except "Areplivir®") and/or immunomodulators at the outpatient stage, vaccination for the prevention of COVID-19 in history, a patient's refusal from a long-term participation in the study. At the postcovid stage, for 4 months (once every 2 months), a survey of 218 patients of the indicated groups was carried out, with the registration of the features of the postcovid period according to the developed questionnaire and the verification of the changes based on the analysis of the outpatient records. The indicator of "the number of days before the subjective recovery" was assessed according to the period, which was named by the patient during the telephone survey, subjectively notifying the restoration to working condition. From the 1st day of health encounter, after signing a written informed consent, a blood sample was taken with the release of serum, which was frozen for storage and subsequent determination of the level of cytokines. There were 48 patients (out of 91) whose treatment had been completed without hospitalization and who were taking favipiravir on the outpatient basis, and 42 patients (out of 53) who were not taking any antiviral drugs. During the period of therapy, the analysis of the outpatient records focused on the levels of ALT, AST, and blood creatinine with the calculation of the glomerular filtration rate, as well as the control of dyspeptic complaints of all the patients, was carried out. The characteristics of the patients included in the study, are presented in Table 1.

Obtaining biological material (blood) for research was carried out taking into account the provisions of the Helsinki Declaration of the World Medical Association (WMA) (2013)[7] and the Protocol of the Council of Europe Convention on Human Rights and Biomedicine (1999), taking into account the additional protocol to the Convention on Human Rights and Biomedicine in the field of biomedical research (2005)[8]. In this category of patients, additional blood sampling was carried out 10, 60, 180 days after two negative results of the polymerase chain reaction (PCR) for the presence of SARS CoV-2 RNA in the morning on an empty stomach (12 hours without food). The blood was centrifuged, followed by separation of serum and storage in labeled tubes at –30°C for no longer than 45 days. The levels of erythropoietin (EPO) and the macrophage colony-stimulating factor (MCSF) were determined by ELISA in the laboratory of the Department of Immunology, Microbiology, Virology of National Research Ogarev Mordovia State University on an enzyme immunoassay analyzer "Personal Lab TM" (Adaltis, Italy). The choice of the above-listed cytokines is based on the data of the authors' earlier studies, which included 32 cytokines. The research is devoted to the study of the cytokine-dependent mechanisms of the essential arterial hypertension progression (10 years of 480 patients' dynamic follow-up), with the inclusion of the analysis in the period after the coronavirus infection (1.5 years of the dynamics). This made it possible to identify the prognostic significance of an increase in MCSF (above 389 pg/ml) [4] and a decrease in EPO as risk factors for cardiovascular complications in patients with EAH in the postcovid period, and the markers of the efficacy of the outpatient use of favipiravir in relation to isolated COVID-19 related complications.

The average age of patients was 59 (75% CI [48–80]) years.

Statistical processing of results

Statistical processing of the data obtained was carried out using Stat Soft Statistica 13.5. The results are shown with the indication of the median (Me) and percentiles (Q 0.25–Q 0.75). The distribution of indicators differed from the normal Gaussian-Laplace distribution, therefore, when comparing dependent samples, the Wilcoxon test was used; for unconnected samples, the Mann-Whitney U-test. Absolute and relative risks were calculated with determination of 95% confidence interval (CI), sensitivity and specificity, χ^2 with Yates correction.

RESULTS

According to the results of the analysis, it was found out that, despite the presence of comorbid conditions that increase the risk of developing a severe course of COVID-19 – obesity and essential hypertension, in the group of patients taking favipiravir, the need for hospitalization was twice as low ($p<0.05$), in relation to the comparison group (in the group "favipiravir" – 29 people were hospitalized (24.2%), in the group "without antiviral drugs" – 45 people (45.9%), including the decrease ($p<0.01$) in the number of people with severe COVID-19 (hospitalization was carried out according to the criteria described in the recommendations[9]. As follows from the

[6] State Register of Medicines of the Russian Federation. Areplivir®. Available from: https://grls.rosminzdrav.ru/LP-007609-171121. Russian

[7] Association WM. World Medical Association Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects. JAMA. 2013;310(20):2191-4.

[8] Kholodova EI, Turshuk LD. Bioethics and Human Rights: International Legal Regulation and Ways of its Implementation. Actual Problems of Russian Law. 2017;(3):193–198. Russian. DOI: 10.17803/1994-1471.2017.76.3.193-198.

[9] Temporary guidelines "Prevention, diagnosis and treatment of new coronavirus infection (COVID-19)" of the Ministry of Health of Russia (version 13.1 of 11/09/2021). Russian

data presented in Table 2, during hospitalization against the background of the disease progression in patients who had not received antiviral drugs, the risk of a severe course of the disease was 3.36 times higher (95% CI [1.57–7.23]%. The sensitivity was 0.86; the specificity was 0.6, χ^2 with Yates' correction equal to 10 (p=0.002); Pearson's coefficient was 0.31 (the relationship was more moderate than in the patients receiving the drug based on favipiravir).

The early antiviral therapy based on favipiravir, reduces the length of a hospital stay: the number of bed-days without antiviral therapy at the outpatient stage is 21.7 days (18–31), with antiviral therapy (favipiravir) – 14.3 (9.7–17.1, p<0.01). It also increases the frequency of significant improvements in the lung tissue state according to the CT data on the 10th day of treatment. Positive dynamics in CT at discharge (by 2 or more points on the WHO scale) without antiviral therapy at the outpatient stage comprises 23 people (51.1%), with antiviral therapy (favipiravir) – 21 people (72.4%), p<0.01.

During the recovery period (2 and 4 months after the illness), the analysis of the telephone survey data determined a 2-fold decrease in the number of days before the subjective recovery in COVID-19 convalescents with hypertension and obesity, when starting antiviral therapy, no later than the 5th day of illness compared to the group without early antiviral therapy (p<0.001). In the group of patients who did not take antiviral drugs, in a greater number of cases (34 people (75.5%) versus 10 people (34.5%), in the group with the early use of favipiravir at the stage of inpatient treatment, antibacterial drugs were prescribed due to the secondary bacterial infection.

The analysis of the cytokine status revealed that in the group who took the drug based on favipiravir at the outpatient stage, the average level of MCSF in the postcovid period was significantly lower (p>0.05), and EPO was higher (p>0.05) than in the patients from the group "without antiviral drugs at the outpatient stage" (Table 3). At the same time, at the onset of the disease, the patients of both groups were comparable in terms of the content of these cytokines.

The patients with COVID-19, concomitant EAH and obesity, who had not required hospitalization and had not taken outpatient antiviral drugs, had lower average serum EPO levels on days 10, 30 and 180 after clinical and laboratory recovery than the patients who had been treated with a drug based on favipiravir (p<0.001), at the outpatient stage. The opposite trend was revealed when analyzing the content of MCSF in blood serum: higher average levels of this cytokine were recorded in the patients "without antiviral therapy" with the progression of negative dynamics in the postcovid period.

Thus, even in patients with hypertension and obesity, at an early start of taking Areplivir®, there was no imbalance in the production of the cytokines EPO and M-CSF, which indirectly, according to previously obtained data, may be a marker for reducing the risk of cardiovascular COVID-19 complications.

The results of the questionnaire (survey) of the coronavirus infection convalescents in hypertension and obesity patients who had received treatment on an outpatient basis without hospitalization 180 days after the recovery showed that at an early start of the antiviral therapy with a drug from the favipiravir group, the crisis course of hypertension was observed less frequently than in the control group. Moreover, in the considered group, there was no need to change the regimen and dose of antihypertensive drugs, less often symptoms of asthenia were observed: increased fatigue, weakness, dizziness, emotional lability, pain in joints and muscles (Table 4).

It is important to notify that in the group of COVID-19 and EAH convalescents who had not taken antiviral drugs at the outpatient stage of treatment (98 people) within 180 days of the observation after the clinical and laboratory recovery, 5 cases of cardiovascular complications (5.1%) were recorded: 3 cases were transient cerebrovascular accidents, 1 case was an acute cerebrovascular accident, 1 case was myocardial infarction. Of these, 4 patients were characterized by a significant (twice or more times) individual increase in the M-CSF content in the blood during the observation period against the background of a 35% decrease in EPO. In the group treated with favipiravir at the outpatient stage, there were no cases of cardiovascular complications during the convalescence period after COVID-19 (the patients with an individual increase in M-CSF blood by 100% or more were not identified.

Thus, on the one hand, an early initiation of favipiravir therapy reduced the risk of hospitalization and aggravation of the condition, and, on the other hand, it was beneficial in a faster recovery from the coronavirus infection and reduced risk of the associated cardiovascular complications.

According to the analysis of the outpatient records during the treatment of the acute phase of the coronavirus infection, an increase in the level of ALT and/or AST was detected in 4 patients out of 91 (4.39%) in the group taking the drug based on favipiravir and in 1 out of 53 (1, 88%) in the group "without antiviral drugs". Subjective complaints of nausea, heaviness in the right hypochondrium in the favipiravir group were notified in 8 people (8.79%), in the group "without antiviral drugs" – in 4 patients (7.55%). No increase in the level of creatinine (a glomerular filtration rate), and of urea above the reference values, was recorded. The data obtained, confirm the predictable favorable safety profile of the investigational drug based on favipiravir (Areplivir®) and are consistent with the data of the international studies [5, 7, 10, 11].

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Table 1 – Characteristics of patients with COVID-19, included in the study

Patient history and condition parameters	Without anti-viral medication at the outpatient stage (n=98)	With anti-viral medication (favipiravir) at the outpatient stage (n=120)
Duration of illness before starting therapy (days)	3.12 [1.28–4.73]	3.17 [1.33–4.47]
Percentage of lung damage on the 5th day of illness (%)	6.82 [0-10.7]	8.12 [0–13.2]
Presence of comorbid diseases	–	–
AH	100%	100%
Type 2 diabetes mellitus	31.6% (31 persons)	51.6% (62 persons
Obesity	100%	100%
BMI	36.2 [34.2–39.6]	37.6 [35.4–40.5]
SpO_2, %	98.4 [97.6–99.8]	97.9 [96.3–99]
C-reactive protein, mg/l	6.3 [6.76–8.9]	5.7 [4.17–8.33]
D-dimer, ng/ml	242 [180–430]	310 [287–402]
Glucose, μ/L	5.41 [4.8–11.2]	6.22 [4.66–10.9]
Hematoglobulin, g/l	125 [112–137]	122 [117–141]

Table 2 – Use of favipiravir group drugs at the outpatient stage and features of the COVID-19 course in patients with EAH and obesity (Me [$Q_{25\%}$–$Q_{75\%}$]

Groups considered	Group number	Number of disabled days	Time until temperature returns to normal (days)	Period to subjective recovery (days)*	Severity of the course, pers (%)
Patients without hospitalization during the period	–	–	Without GCS	–	–
Without antiviral therapy at the outpatient stage, n = 53 people	1	15.3 [12.4–18.8]	5.47 [4.32–7.45]	61 [32–95]	Mild course – 42 pers. (79.2%) Moderate course – 11 pers. (20.8%)
Use of Favipiravir at the outpatient stage, n = 91 people	2	12,7 [10.1–14.3]	3.78 [2.16–4.22]	31 [16–49]	Mild course – 84 pers. (92.3%) Moderate course – 7 pers. (7.7%)
Statistical significance	–	p^{1-2}<0.05	p^{1-2}<0,05	p^{1-2}<0,001	p^{1-2}<0.05
The hospitalized after the 5th day of outpatient therapy	–	–	With GCS	–	–
Without antiviral therapy at the outpatient stage, n=45 people	3	34,7 [21.5–42.1]	3,97 [2.45–4.23]	74 [41–120]	Moderate course – 21 (46.7%) Severe course – 22 (48.8%) CT-2 (4.5%)
Use of favipiravir at the outpatient stage, n=29 people	4	25.9 [15.2–28.4]	4.12 [2.66–4.68]	35 [29–58]	Moderate course – 20 (68.9%) Severe course – 8 (27.6%) CT-1 (3.5%)
Statistical significance	–	p^{3-4}<0.01 p^{1-3}<0.001 p^{2-4}<0.01	p^{3-4}>0.05 p^{1-3}<0.05 p^{2-4}>0.05	p^{3-4}<0.01	p^{3-4}<0.01

Note: * – according to the survey data after the laboratory recovery, the Mann-Whitney test was used for related aggregates; when specifying the degree of confidence.

Table 3 – Comparative characteristics of EPO and MCSF levels (pg/ml) in the blood serum of patients with hypertension and obesity 10, 30 and 180 days after COVID-19 (Me [$Q_{25\%}$–$Q_{75\%}$])

Without antiviral therapy, n=42 people	EPO	M-CSF
Initiation of therapy	98.3 [89,9–107]	287 [254–327]
10 days after recovery	102 [95.5–108]	587 [538–702]
30 days after recovery	105 [97.1–126]	724 [623–810]
180 days after recovery	127 [98.8–140]	742 [669–856]
Statistical significance	$p>0.05^{1–2, 2–3,3–4}$ $p<0.05^{1–4}$	$p<0,001^{1–2, 1–3,1–4, 2–3, 2–4}$ $p>0,05^{3–4}$
Favipiravir, n = 48 persons	EPO	M–CSF
Initiation of therapy	95.4 [91,7–121]	307 [269–336]
10 days after recovery	148 [110–169]	297 [248–410]
30 days after recovery	162 [155–176]	307 [204–416]
180 days after recovery	177 [159–202]	299 [242–457]
Statistical significance	$p>0,05^{1–5}$ $p<0,05^{6–7,7–8}$ $p<0.001^{5–6, 5–7, 5–8, 2–6, 3–7, 4–8, 6–8}$	$p>0.05^{1–5, 5–6, 5–7, 5–8, 6–7, 7–8, 6–8}$ $p<0.001^{2–6, 3–7, 4–8}$

Note: the confidence level in accordance with the specified group based on the Wilcoxon criterion for related aggregates and the Mann-Whitney U-test for unrelated aggregates).

Table 4 – Analysis of subjective and objective characteristics of patients with hypertension and obesity 180 days after suffering COVID-19 (without hospitalization)

Estimated characteristics	Without antiviral therapy, n=53 pers.	Favipiravir, n=91 pers.
Crisis course of AH	26 pers. (49%)	8 pers. (8.79%)
Changing antihypertensive therapy regimen	21 pers. (39.6%)	12 pers. (13.2%)
Fatigue	42 pers. (79.2%)	32 pers. (35.1%)
Dizziness	7 pers. (13.2%)	2 pers. (2.2 %)
Muscle and joint pain	24 pers. (45.3%)	14 pers. (15.4%)

Taking into account the ongoing charity event (16,672 packages of the drug were distributed among 8,336 sick patients), the analysis of the pharmaco-economic effect of increasing the frequency of the early prescription of Areplivir® in the Republic of Mordovia in the period from October 1, 2021 to November 1, 2021, revealed a decrease in the hospitalization rate by 1757 people for 1 month when compared with September 2021. So, according to the Ministry of Health of the Republic of Mordovia, despite the fact that the number of infected people during the period under review had increased by more than three times, the total number of patients in the need for the inpatient treatment (24/7 beds of an infectious profile), decreased from 49.3% to 36.2%. Taking into account the average cost of treating one patient in hospital under the compulsory medical insurance system in the Republic of Mordovia, this made it possible to reduce budgetary costs by more than 260 million rubles.

The tariff agreement in the system of compulsory health insurance of the Republic of Mordovia was validated on 02/14/2021 (as amended on 09/30/2021)][10].

Moreover, according to the presented study, the use of the drug based on favipiravir, made it possible to reduce the duration of hospital stay by more than 1.5 times compared to the group of the patients who had not received antiviral therapy at the outpatient stage, which provided additional budget savings. It is also worth noting a decrease in the burden on the social security system of citizens due to a decrease in the number of days of disability in the patients receiving favipiravir at the onset of the disease compared with patients without antiviral therapy.

DISCUSSION

To date, the results of more than 1,000 studies devoted to the research of the anticovid activity of drugs based on favipiravir, are available. At the same time, the data on the effect of the drug on such check points as mortality, duration of respiratory support, as well as the duration of virus elimination based on PCR results vary widely, which is confirmed by the results of published systematic reviews [12, 13].

By reference to the immunopathogenesis of COVID-19, a number of researchers emphasize the need to study the efficacy of drugs from the favipiravir group

[10] Tariff agreement in the compulsory health insurance system of the Republic of Mordovia dated 02.14.2021 (as amended on 09.30.2021). Available from: https: //docs.cntd.ru/document/571071803. Russian

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for the early outpatient prescription [14,15], followed by the study of its effect on the frequency of hospitalizations. According to the data obtained, it was the early (up to 5 days from the onset of the first symptoms) use of favipiravir that twice reduced the frequency of hospitalizations in the group of comorbid COVID-19 patients against the background of hypertension and obesity as the conditions that increased the risk of a severe course and unfavorable outcomes of the coronavirus infection. This fact has undoubted clinical and social and medico-economic significance, given the difference in the costs of treating an outpatient and an inpatient. It is worth noting that, according to the instructions for medical use, the prescription of favipiravir is possible for both confirmed SARS-CoV-2, and for a probable case of the disease diagnosed on the basis of characteristic clinical symptoms, which is associated with the direct antiviral effect of the drug against a wide range of RNA-containing viruses.

The immunopathogenetic and clinical effects of a drug based on favipiravir proved in this study, are most likely mediated by the suppression of viral replications and a decrease in the peak viral load [16] due to the selective blockade of the key replication enzyme SARS-CoV-2, RNA-dependent RNA polymerase [17–20]), which increases the likelihood of an effective immune response without a hyperinflammatory phase. However, even in case of hospitalization with the progression of the disease in the group of patients with an early start of COVID-19 treatment using the drug favipiravir compared with the group without antiviral therapy, a decrease in the likelihood of a severe course of COVID-19 was determined. That emphasizes the beneficial effect of an early start of therapy on the characteristics of the inpatient stage. In addition, within the framework of this study, based on routine biochemical tests, the safety of using the drug based on favipiravir, has been confirmed once again.

A serious consequence of SARS-CoV-2 infection is an imbalance in the system of pro- and anti-inflammatory immunoregulatory peptides (cytokines), which can persist after the recovery and determine the progression of existing chronic diseases and pathological conditions, such as hypertension and atherosclerosis, as well as be a component of a postcoid syndrome.

The previously published data on the role of the M-CSF family members [22] in the progression of hypertension in the postvoid period, can explain the effect of antiviral drugs based on favipiravir not only on reducing the risk of severe COVID-19, but also in the aspect of preventing the progression of chronic diseases in the postcovid period, in particular the development of fatal and non-fatal cardiovascular complications. The relationship between the use of a drug based on favipiravir and the absence of a decrease in EPO in the period after COVID-19, opens up prospects for a new direction of scientific research: the effect of a therapy regimen on the characteristics of the postcovid period in patients with hypertension and obesity. It is important to note that the data on a decrease in serum EPO levels were obtained in the group of patients who did not need hospitalization and were mainly characterized by a higher percentage of cases of a mild COVID-19 course despite the concomitant pathology. EPO is a factor that regulates not only erythropoiesis, but also a number of immune mechanisms due to the wide presentation of its receptors on the cells of the body. Tissue hypoxia, typical for patients with COVID-19, should activate the synthesis of this cytokine [23], but, as it has been established in the course of this study, in the patients receiving antiviral therapy at the onset of the disease, the dynamics notified was opposite.

A pathophysiological regulation of EPO and proinflammatory TNF-α, IL-1β cytokines obeys the principle of a negative feedback [24]. Consequently, the revealed decrease in EPO in the patients who did not receive early antiviral therapy is associated with a decrease in the anti-inflammatory effect of this cytokine, which, possibly, determines a wider spectrum and frequency of manifestations of a postcovid syndrome identified in this category of patients. It is necessary to identify the problem of false clinical well-being of such patients, since, according to the data presented, they remain at risk of developing cardiovascular complications.

The data reflecting the dynamics of the M-CSF content in the blood of convalescents of the coronavirus infection are of scientific interest. An increase in its content within 180 days of the post-infectious period may be associated with the progression of endothelial dysfunction or, possibly, an increase in the pro-inflammatory activity of adipocytes, which can maintain a viral replication for a long time and determine an additional release of pro-inflammatory cytokines into circulation [25]. As established in this study, an early prescription of an antiviral drug based on favipiravir in the treatment regimen for comorbid patients with COVID-19, reduced the incidence of ACA, TCA, MI, compared with the patients who did not receive antiviral drugs. Of course, the data obtained require a further study on an expanded sample of patients.

The relevance of the further feature analysis of the drugs based on favipiravir, is indisputable. According to the doctors' data, the prescription of a drug is not accompanied by a significant increase in the frequency of undesirable effects (increased ALT, AST, dyspeptic phenomena). That can be associated with the use of a drug from the favipiravir group: a comparison was made with the group without any use of antiviral therapy in the patients with COVID-19 against the background of hypertension and obesity. Previously, the review data on the potential risk of mutagenic effects of the drugs based on favipiravir against SARS-CoV-2 have been published [26]. It is important to note that the authors confirm the effectiveness of the drug in blocking SARS-CoV-2 viral replication, but there is a problem, which, first of all, is

relevant if the manufacturer's recommended doses and duration of the drug use are not followed. At the same time, fundamental studies of the action mechanism of the drug show [27–31] those abnormalities in nucleotide sequences that determine violations of RNA virus replications, cause processes of lethal mutagenesis, which lead to the destruction of virus particles, rather than its variability. The resulting fragments of RNA do not even represent subgenomic fragments and cannot biologically represent mutations. The issue of the relevance of its modification in order to maintain its high efficacy in subsequent years requires additional research.

CONCLUSION

This study showed that an early prescription of favipiravir contributes to a decrease in the rate of COVID-19 patients' hospitalization even against the background of concomitant hypertension and obesity, due to a decrease in the likelihood of moderate and severe courses of the disease, and also leads to an earlier objective and subjective recovery. The results demonstrated a high potential benefit of an early favipiravir use in the novel coronavirus infection and in the prevention of postcovid complications. The results obtained open up prospects for further studies analyzing individual cytokine-mediated variants of a postcoid syndrome in patients with a high risk of cardiovascular complications against the background of the use of drugs with molecular-targeted antiviral effects. The demonstrated effect of the Areplivir® drug on reducing the socio-economic burden of the coronavirus infection, which has been shown off, emphasizes the advisability of an early prescription of antiviral therapy in high-risk comorbid COVID-19 patients.

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CONFLICT OF INTEREST

The clinical study was organized by the National Research Mordovia State University. Promomed DM LLC is a member of the group of companies JSC Promomed. Promomed RUS LLC is a part of the group of companies JSC Promomed. Promomed RUS LLC is the holder of the registration certificate for the drug "Areplivir" LP-006288 dated 23 June 2020. The manufacturer of the drug "Areplivir" is JSC Biokhimik, which is part of the group of companies JSC Promomed. Promomed RUS LLC is the initiator of a charitable program to provide the population of the Republic of Mordovia with favipiravir. Administration of the Head of the Republic of Mordovia is the coordinator of the charitable program. Promomed RUS LLC is the rightholder of a patent for an antiviral composition - Patent for invention No. 2751108 (application No.2020119747, priority 15 June 2021; date of state registration 07 Aug 2021). Zaslavskaya K.Ya. – director for new products of Promomed DM LLC.

AUTHORS' CONTRIBUTION

Larisa A. Balykova – development and implementation of research design, text writing and editing; Olga A. Radaeva – setting the study aim, study goal setting, results analysis, text writing; Kira Ya. Zaslavskaya – text writing and editing; Yulia A. Kostina – statistical processing of the experimental results; Maria S. Iskandyarova – statistical processing of the experiment results, setting the aim of the study, Elena V. Negodnova – statistical processing of the experiment results, setting the aim of the study, results analysis; Vitaly V. Eremeev – statistical processing of the experiment results, setting the aim of the study, results analysis; Lenar F. Sabirov – control of material sampling; Elena V. Semeleva – development and implementation of research design.

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ОРИГИНАЛЬНАЯ СТАТЬЯ

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CLINICAL AND ECONOMIC JUSTIFICATION OF SOFTWARE SCREENING PERFORMANCE OF COLORECTAL CANCER AT THE REGION LEVEL

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The aim of the study is to assess the clinical and economic effectiveness of the practical implementation results of programmed screening for colorectal cancer (CRC) in the Primorsky Territory using clinical and economic research methods.

Materials and methods. In the study, the following kinds of data were used: the statistical data from the regional clinic's cancer registry on the structure of the morbidity and average life expectancy of CRC patients in the Primorsky Territory; the data on the cost of screening studies and the stages of anticancer therapy in accordance with the "Territorial Tariff Agreement on Payment for Medical Care (Medical Services) in the System of Compulsory Health Insurance in the Territory of Primorsky Krai", 2021. Two methods of clinical and economic analysis with the corresponding calculation formulas have been applied. The cost of medical interventions were estimated in accordance with the screening standards and clinical guidelines for the treatment of malignant neoplasms of the colon and rectum, approved by the Scientific and Practical Council of the Ministry of Health of the Russian Federation, 2020.

Results. The evidence-based substantiation of screening clinical effects has been obtained: the structure redistribution of colorectal cancer incidence towards the prevalence of early forms by 16.81%; the average increase in the life expectancy of patients with the studied disease is 12.8 months. A natural consequence of these events is the predicted decrease in the mortality rate from CRC in the territory of the subject in the subsequent years. The economic justification of CRC screening software which guarantees a significant saving in health care resources amounting to 23% compared to an alternative strategy, has been demonstrated. It can influence the management decisions on the further strategy of the mass introduction of this medical technology.

Conclusion. Currently, CRC screening is the most effective way to reduce morbidity and mortality from this disease. The predominance of the early diagnosis of the disease is extrapolated to significant savings in public health care. A promising direction for further research in the field of CRC screening is the study of its long-term effects, in particular, a detailed clinical and economic analysis of the diagnostics effectiveness and the elimination of premalignant neoplasms.

Keywords: colorectal cancer; screening; clinical and economic analysis; morbidity; cost of treatment; resource saving; special pharmacotherapy; cost; pharmacotherapeutic interventions; effectiveness

Abbreviations: CRC – colorectal cancer; IARC – International Agency for Research on Cancer; ASCO – American Society of Clinical Oncology; WHO – World Health Organization; FIT – fecal immunochemical test; gFOBT – Guaiac fecal occult blood test; FCS – fibrocolonoscopy; CDI – cost difference indicator; CMA – cost/minimization analysis MN – malignant neoplasm; DC – direct cost; CER – cost-effectiveness ratio; Ef – effectiveness; DRG – Diagnosis-Related Group; CHIS – Compulsory Health Insurance System; WPT – willingness-to-pay threshold; LYS – life year saved; QALYS – quality-adjusted life-year saved; GDP – gross domestic product; CSAMIs – Central Storage Archive of Medical Images.

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КЛИНИКО-ЭКОНОМИЧЕСКОЕ ОБОСНОВАНИЕ ЦЕЛЕСООБРАЗНОСТИ ПРОГРАММНОГО СКРИНИНГА КОЛОРЕКТАЛЬНОГО РАКА НА УРОВНЕ СУБЪЕКТА

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Цель. Оценить клиническую и экономическую эффективность результатов практической реализации программного скрининга колоректального рака (КРР) на территории Приморского края с использованием методов клинико-экономического исследования.

Материалы и методы. В исследовании использованы статистические сведения канцеррегистра краевого онкологического диспансера о структуре заболеваемости и средней продолжительности жизни больных КРР в Приморском крае; данные о стоимости скрининговых исследований и этапов противоопухолевой терапии в соответствии с «Территориальным тарифным соглашением по оплате медицинской помощи (медицинских услуг) в системе обязательного медицинского страхования на территории Приморского края», 2021. Применены два метода клинико-экономического анализа с соответствующими расчетными формулами. Затраты на медицинские вмешательства оценивались в соответствии со стандартами скрининга и клиническими рекомендациями по лечению злокачественных новообразований ободочной и прямой кишки, одобренными Научно-практическим Советом Минздрава РФ, 2020.

Результаты. Получено доказательное обоснование клинических эффектов скрининга: перераспределение структуры заболеваемости КРР в сторону преобладания ранних форм на 16,81%; среднее увеличение ожидаемой продолжительности жизни пациентов с изучаемым заболеванием на 12,8 мес. Закономерным следствием данных событий является прогнозируемое снижение уровня летальности от КРР на территории субъекта в последующие годы. Продемонстрирована экономическая целесообразность программного скрининга КРР, что гарантирует существенную экономию ресурсов здравоохранения, составляющую 23% по сравнению с альтернативной стратегией, и может повлиять на принятие управленческих решений по дальнейшей стратегии массового внедрения данной медицинской технологии.

Заключение. В настоящее время скрининг КРР является самым эффективным направлением в снижении показателей заболеваемости и смертности от данного заболевания. Преобладание диагностики ранних стадий заболевания экстраполируется в значимую экономию средств системы государственного здравоохранения. Перспективным направлением дальнейших исследований в области скрининга КРР является изучение его долговременных эффектов, в частности, детальный клинико-экономический анализ эффективности диагностики и элиминации предраковых новообразований.

Ключевые слова: колоректальный рак; скрининг; клинико-экономический анализ; заболеваемость; стоимость лечения; экономия ресурсов; специальная фармакотерапия; затраты; фармакотерапевтические вмешательства; эффективность

Список сокращений: КРР – колоректальный рак; IARC – Международное агентство по изучению рака; ASCO – Американское общество клинической онкологии; ВОЗ – Всемирная организация здравоохранения; FIT – фекальный иммунохимический тест; gFOBT – Гваяковая фекальная проба на скрытую кровь; ФКС – фиброколоноскопия; CMA – показатель разницы затрат; ЗНО – злокачественное новообразование; DC – прямые затраты; CER – показатель «затраты-эффективность»; Ef – эффективность; КСГ – клинико-статистическая группа; ОМС – обязательное медицинское страхование; ПГП – порог готовности платить; LYG – сохраненный год жизни; QALYS – скорректированный на качество сохраненный год жизни; ВВП – валовой внутренний продукт; ЦАМИ – Централизованный архив медицинских изображений.

INTRODUCTION

Colorectal cancer (CRC) is one of the most commonly diagnosed cancers worldwide. According to the information provided by the International Agency for Research on Cancer (IARC[1]), colorectal cancer is the third most common cancer among men after lung and prostate kinds of cancer (10.6% or 1,065,960 cases in 2020). Among women with cancer, colorectal cancer ranks second in prevalence after breast cancer (9.4% or 865,630 cases in 2020) [1]. According to the Global Cancer Observatory (GCO[2]), the global burden of CRC

[1] The World Health Organization (WHO). International Agency for Research on Cancer, IARC. [Электронный ресурс]. – Режим доступа: https://www.iarc.fr/.

[2] Global Cancer Observatory, (GCO). Available from: https://gco.iarc.fr/.

ОРИГИНАЛЬНАЯ СТАТЬЯ
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is expected to increase by 60% (more than 2.2 million new cases and 1.1 million deaths) by 2030 [2]. Morbidity and mortality from colorectal cancer show wide geographical differences around the world: the highest rates are recorded in Australia and New Zealand, the lowest - in West Africa [3]. In Russia, in 2019[3], colorectal cancer (CRC) took the fourth place in the structure of the incidence of malignant neoplasms. Most of the cases are people aged 50 and over, but according to ASCO[4] forecasts, 12% of colorectal cancer cases will be diagnosed in people under 50 years of age [4]. At the same time, an early diagnostics provides a 50–60% 5-year survival rate, while at stage IV it is less than 10% [5, 6].

The multistage theory of colon carcinogenesis explains the development of carcinoma through the stage of adenoma [7–9]. Depending on the evolution path of a malignant intestinal tumor [10], its manifestation can be realized from 4–5 to 20 years after its onset [11, 12]. This confirms a long asymptomatic course of this disease with the absence of active complaints in the patient [5]. Thus, a timely diagnostics and removal of colon and rectum adenomas are a priority to reduce not only mortality, but also the incidence of colorectal cancer [3, 13, 14].

There are two most effective screening strategies for colorectal cancer [15, 16]. First, colonoscopy is to be performed every 10 years, that provides the highest long-term clinical results, and it is the least expensive. Second, it is the annual fecal immunochemical test (FIT) [17]. This strategy is considered the best as well as the most cost-effective screening option, with a willingness-to-pay threshold more than €15,000 for each acquired life year [18]. Currently, a generalized 2-step screening standard for CRC is the examination of persons aged 50 to 75 years. The first stage is a laboratory determination of occult blood in feces (hemocult test, gFOBT, or, preferably, an immunochemical method – FIT). The second stage is fibrocolonoscopy (FCS) [13, 19, 20].

In the context of age restrictions for CRC screening, it should be notified that, in contrast to the decline in the incidence of colorectal cancer among the elderly, this indicator has almost doubled among young people since the early 1990s [21]. These are people younger than 50 years old who do not meet the screening recommendations [4, 22]. On the other hand, it has been proven that endoscopic resection of colon polyps is safe even for elderly patients aged 80 and older [23]. In this regard, nowadays, the optimal age for CRC screening is being revised [21]. Taking into account the growing economic burden of CRC, the cost of program screening and the economic effects of its implementation, a number of experts proposed the age of 32 years as the minimum threshold for screening studies [24].

THE AIM of the study is to assess the clinical and economic effectiveness of the practical implementation results of programmed screening for colorectal cancer (CRC) in the Primorsky Territory using clinical and economic research methods.

MATERIALS AND METHODS

This study was carried out in the area of diagnostics and treatment of colorectal cancer in the Primorsky Territory as a retrospective analysis of the data from 2016 to 2018. The choice of the period is due to the following facts: in 2016, screening for colorectal cancer was not carried out in the region; in 2017, the gradual introduction of screening began but did not have a regulatory basis; in 2018, in accordance with the Order of local health authority[5], the implementation of the CRC screening program began.

The practical foundation of the research, implemented in specific spatial-temporal conditions, made it possible to exclude the need to build a conceptual model of the initial stage implementation of the medical technology under consideration. By CRC, a set of malignant neoplasms of the colon and rectum corresponding to the C18-21 ICD-10 codes[6] is meant. The study is based on two methods of clinical and economic analysis: a cost/minimization analysis (CMA) and a cost/effectivness analysis (CEA)[7] [25]. In the first case, the cost difference indicator (CDI) was calculated using the formula:

$$CMA = DC1 – DC2,$$

where: DC1 is the cost when applying the 1st method; DC2 is the cost when applying the 2nd method.

In the second case, the cost/effectiveness ratio was determined:

$$CER = DC/Ef,$$

where: DC is the cost; Ef is the effectiveness (in this study, the unit of effectiveness is a month of life after making the diagnosis of colorectal cancer).

The resulting CER values corresponding to the study periods were compared. The optimal (smallest) cost-effectiveness indicator has been identified.

[3] Kaprin AD, Starinskiy VV, Shakhzadova AO. The state of cancer care for the population of Russia in 2019. – M.: MNIOI them. P.A. Herzen is a branch of National Medical Research Center of Radiology. 2020: 239 p. Russian

[4] American Society of Clinical Oncology, (ASCO). Available from: https://www.asco.org/.

[5] Order of November 13, 2017 N 977-o "On the introduction of centralized screening of malignant neoplasms in the Primorsky Territory" (as amended on December 20, 2019). Available from: http://docs.cntd.ru/document/446618102/. Russian

[6] International classification of diseases 10th revision (ICD-10). Available from: https://mkb-10.com/. Russian

[7] GOST R 57525-2017. Clinical and economic research. General requirements Available from:http://protect.gost.ru/v.aspx?control=8&baseC=6&page=2&month=7&year=2017&search=&RegNum=1&DocOnPageCount=15&id=210129&pageK=270D5A13-9BFF-4EE4-B026-A43786F3620F/. Russian

In order to conduct this doublet of complementary clinical and economic studies, the average diagnostics cost and treatment of one CRC patient, depending on the stage of the disease, was determined, as well as the average predicted life expectancy of these categories of patients after the diagnosis had been made. The data obtained were converted into averages for each patient array identified in 2016, 2017, and 2018 and used to perform the corresponding calculations. As a result of the application of the above-listed research methods, the analysis of "the impact on the budget" of the implementation of the considered treatment and diagnostics strategy was carried out and presented in the study. Besides clinical and economic methods, the following additional research methods were used: statistical (summary and grouping of statistical observation materials) and sociological (collection and analysis of quantitative documentary information).

The statistical information used is provided by the databases of the Cancer Registry and the Software Department of the Primorsky Regional Oncological Dispensary. To obtain and process the data necessary for the research work, Microsoft Office Excel 2007 software was used, as well as medical information systems DOKA+, Oncor and the Centralized Storage Archive of Medical Images (CSAMIs). The analyzed treatment strategy corresponds to the current clinical guidelines approved by the Scientific and Practical Council of the Ministry of Health of the Russian Federation[8]. The calculations estimated the direct costs of medical interventions: screening studies and an anticancer treatment program. The expenses were accounted for in accordance with the current Territorial Tariff Agreement on Payment for Medical Care (Medical Services) in the Compulsory Health Insurance System in the Primorsky Territory[9]. Inflationary expectations had not been considered.

Thus, the analysis eliminated the impact of changing price conditions on medical interventions during the period covered by the study. There is an assumption in the work: the patients with an unknown stage of the disease were not taken into account (their share among the identified patients in 2016 was 5.4%, in 2017 – 5%, in 2018 – 2.8%). The clinical and economic effects of detecting precancerous diseases were not the subject of this study and were not used to carry out the corresponding calculations.

RESULTS

In Primorye, CRC screening was set up in 2017. At that time, it did not have a regulatory framework and was opportunistic in nature. The implementation of centralized screening for colorectal cancer began in 2018 after the approval by the local health authority of the corresponding Order dated November 13, 2017 N 977-o "On the introduction of centralized screening of malignant neoplasms in the Primorsky Territory" (as amended on December 20, 2019)[10].

Despite the absence of the screening program in 2017, 641 laboratory fecal occult blood tests were carried out as the first stage of CRC screening and subsequent FCC in the amount of 104 manipulations. The price expression of one hemotest is 846 rubles/$12[11], colonoscopy – 1,283 rubles/$18. The cost of performing blood tests was 542,286 rubles/$7,474, of endoscopic examinations – 133,432 rubles/$1,839. As a result, in 2017, the cost of a 2-stage CRC screening amounted to 675,718 rubles/$9,313.

In 2018, as a result of the introduction of centralized CRC screening, 13,245 laboratory occult blood tests were performed. The second, endoscopic stage of screening, was carried out by performing FCS in the amount of 1,045 procedures. The cost of performing hemotests was 11,205,270 rubles/$154,438, of carrying out endoscopic examinations – 1,340,735 rubles/$18,479. In 2018, the total cost of CRC screening amounted to 12,546,005 rubles/$172,916. Thus, the resource consumption of screening studies with the introduction of the Order on their planned implementation, increased by 11,870,287 rubles/$163,603.

In 2016, in the absence of CRC screening, 687 cases of CRC were diagnosed for the first time. In 2017, when screening appeared, there were 711 such cases, and in 2018, with the introduction of centralized screening, there were 769 cases. Therefore, taking into account the presented costs, the cost of screening corresponding to one case of a newly diagnosed CRC in 2017 was 950 rubles/$13, in 2018 – 16,315 rubles/$225, or 15,364 rubles/$212 more than before the screening program implementation (Fig. 3). The change in the structure of the CRC cases identified in 2016-2018, is presented in Table 1.

As it has been demonstrated, with the introduction of screening, the diagnostics of colorectal cancer improved. That was reflected in an increase in newly diagnosed cases of the disease, as well as in an increase in the number of patients diagnosed at stages I–II (Fig. 1).

[8] Clinical guidelines. Malignant neoplasms of the colon and rectosigmoid section 2020. Association of Oncologists of Russia. Russian Society of Clinical Oncology. Available from: https://oncology-association.ru/files/clinical-guidelines-2020/zno_obodochnoj_kishki.pdf/. Russian

[9] Territorial tariff agreement on payment for medical care (medical services) in the compulsory medical insurance system in the Primorsky Territory for 2021. Available from: http://omspk.ru/upload/iblock/701/ТТС%20на%202021%20год%20(на%20сайт).doc/. Russian

[10] Order of November 13, 2017 N 977-o "On the introduction of centralized screening of malignant neoplasms in the Primorsky Territory" (as amended on December 20, 2019).

[11] 72.6022 RUB (rubles) for 1 USD (US dollar) – exchange rate of the Central Bank of the Russian Federation as of November 19, 2021.

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Table 1 – Structure of CRC incidences in 2016-2018

CRC localization	Disease stage	2016		2017		2018	
		Number of cases	%	Number of cases	%	Number of cases	%
Colon malignant neoplasms	I	46	11.7	70	17.24	98	21.8
	II	85	21.7	129	31.78	132	29.4
	III	94	24	88	21.67	102	22.7
	IV	167	42.6	119	29.31	117	26.1
	Total	392	100	406	100	449	100
Rectal malignant neoplasms	I	24	8.1	40	13.11	45	14.06
	II	61	20.7	95	31.15	96	30
	III	84	28.5	80	26.23	89	27.81
	IV	126	42.7	90	29.51	90	28.13
	Total	295	100	305	100	320	100
Total number of CRC cases	I	70	10.19	110	15.47	143	18.6
	II	146	21.25	224	31.5	228	29.65
	III	178	25.91	168	23.63	191	24.84
	IV	293	42.65	209	29.4	207	26.91
	Total	687	100	711	100	769	100

Table 2 – Predicted life expectancy of CRC patients depending on the stage of the disease

CRC stage	Average life expectancy of patients, months	Weighing coefficient of CRC contingent enrollment and corresponding average life expectancy, months					
		2016		2017		2018	
I	99	0.102	10.1	0.156	15.4	0.186	18.4
II	83	0.213	17.7	0.315	26.1	0.297	24.7
III	35	0.259	9.1	0.236	8.3	0.248	8.7
IV	13	0.427	5.6	0.294	3.8	0.269	3.5

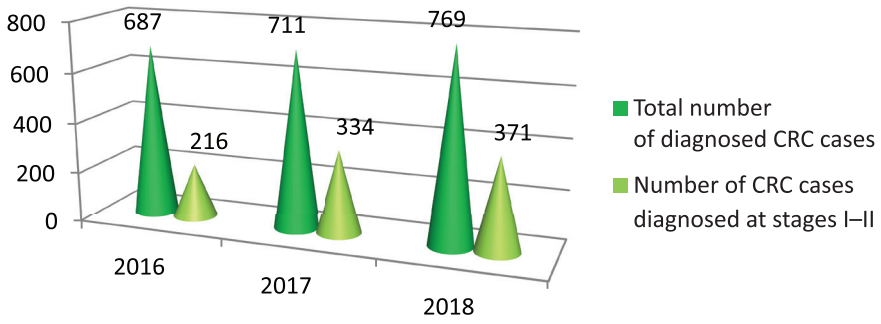

Figure 1 – Increase in diagnosed CRC cases and number of patients diagnosed at stages I–II of the disease in 2016–2018

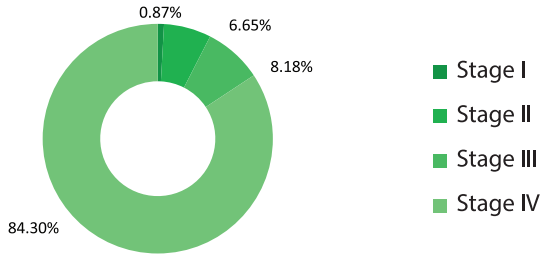

Figure 2 – Costs of CRC treatment depending on the disease stage in 2016

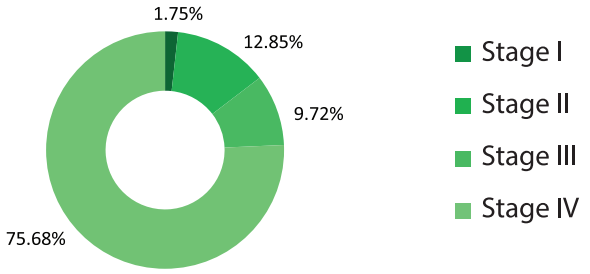

Figure 3 – Costs of CRC treatment depending on the disease stage in 2017

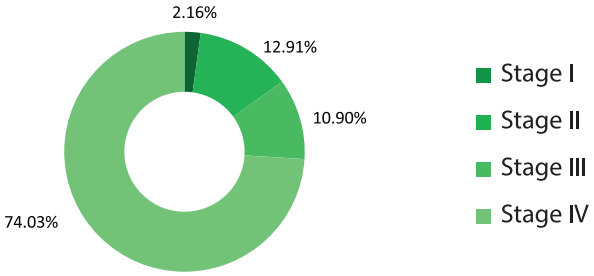

Figure 4 – Costs of CRC treatment depending on the disease stage in 2018

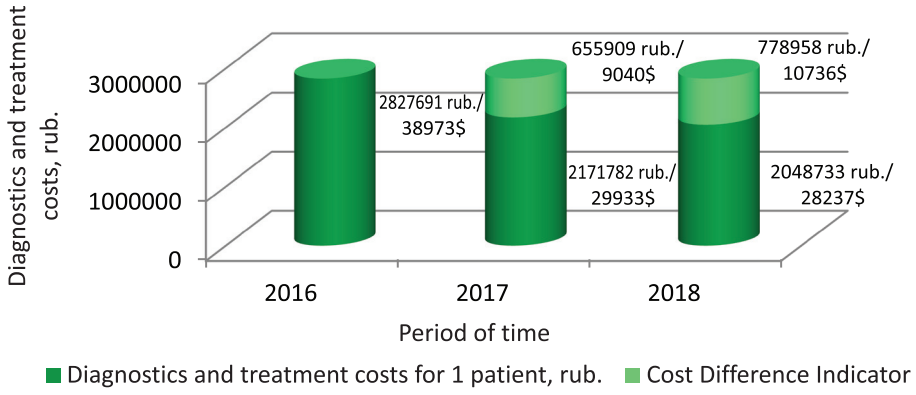

Figure 5 – Diagnostics and treatment costs for 1 CRC patient in 2016–2018

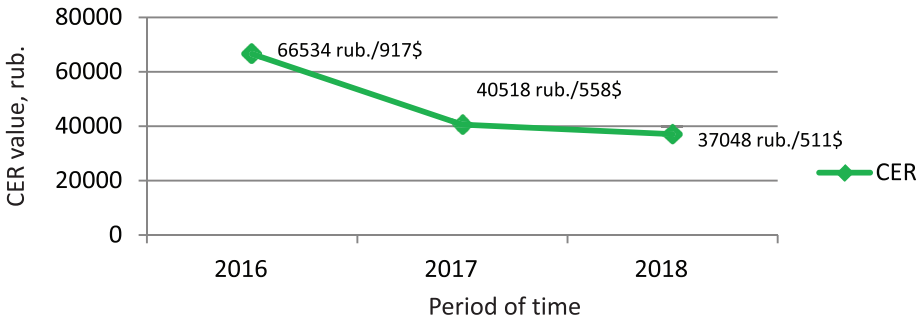

Figure 6 – Changes in the "cost-effectiveness" ratio (CER) in 2016–2018

According to the clinical guidelines approved by the Scientific and Practical Council of the Ministry of Health of the Russian Federation[12] and the National Guidelines for the Drug Treatment of Malignant Tumors[13], depending on the stage of the disease, a few worked out options should be used in the treatment of colorectal cancer. They are as follows: at stage I – surgical treatment; at

[12] Clinical guidelines. Malignant neoplasms of the colon and rectosigmoid section 2020. Association of Oncologists of Russia. Russian Society of Clinical Oncology. Available from: https://oncology-association.ru/files/clinical-guidelines-2020/zno_obodochnoj_kishki.pdf/. Russian

[13] Fedenko AA, Tryakin AA, Zhukova LG, Zeinalova PA, Moiseenko FV, Stroyakovsky DL, Smolin AV, et al. National guidelines for the drug treatment of malignant tumors. M., 2020: 408 p. Russian

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stages II and III – surgical treatment followed by adjuvant drug therapy XELOX or FOLFOX for up to 6 months; at stage V, as well as in the progression of the disease – sequentially prescribed lines of special pharmacotherapy (combined and independent modes of cytostatic and targeted agents). When the process is localized in the rectum, the treatment program, as a rule, includes radiotherapy or its combination with a pharmacotherapeutic component.

Making use of Diagnostic Related Groups (DRGs)[14] Decoding for medical care and the Territorial Tariff Agreement on Payment for Medical Care (Medical Services) in the CHI system in the Primorsky Territory for 2021, the authors calculated the cost of treating a CRC case depending on the stage of the disease. So the cost of treating a patient at stage I of the tumor process was 187,718 rubles/$2,587; at stages II and III – 819,830 rubles/$11,299. Chemoradiation therapy as a part of the rectal cancer treatment program adds 154,488 rubles/$ 2,129 to its cost. Stage IV, like the progression of the disease, requires the use of the most expensive treatment option – palliative, including molecular targeted pharmacotherapy. Taking into account the average duration of its use (9–11 months), the cost of treating one patient ranges from 2,015,680 rubles/$27,781 to 9,163,500 rubles/$ 126,297, which is on average equal to the amount of 5,589,590 rubles/$77,039. Knowing the above-mentioned numerical structure of CRC detected in 2016-2018, the authors calculated the budget for treating the contingent of patients at stages I, II, III, and IV.

The costs of colorectal cancer treatment in 2016 (Fig. 2) were as follows: at stage I – 16,847,972 rubles/$ 232,209 (0.87%), at stage II – 129,118,948 rubles/$1,779,594 (6.65%), at stage III – 158,906,732 rubles/$2,190,146 (8.18%), at stage IV – 1,637,749,870 rubles/$ 22,572,436 (84.3%). The total costs of treating CRC patients in 2016 amounted to 1,942,623,522 rubles/$26,774,385. The average cost of treating one patient is 2,827,691 rubles/$38,973.

The costs of colorectal cancer treatment in 2017 (Fig. 3) were as follows: at stage I – 26,828,500 rubles/$369,766 (1.75%), at stage II – 19,8318,280 rubles/$2,733,340 (12.85%), at stage III – 150,090,480 rubles /$2,068,636 (9.72%), at stage IV – 1,168,224,310 rubles/$16,101,157 (75.68%). In 2017, the total costs of treating CRC patients amounted to 1,543,461,570 rubles/$21,272,899. The average cost of treating one patient is 2,170,832 rubles/$29,920.

In 2017, the costs of colorectal cancer treatment (Fig. 3) were as follows: at stage I – 26,828,500 rubles/$369,766 (1.75%), at stage II – 198,318,280 rubles/$2,733,340 (12.85%), at stage III – 150,090,480 rubles/$2,068,636 (9.72%), at stage IV – 1,168,224,310 rubles/$16,101,157 (75.68%). In 2017, the total costs of treating CRC patients amounted to 1,543,461,570 rubles/$21,272,899. The average cost of treating one patient is 2,170,832 rubles/$29,920.

In 2018, the costs of colorectal cancer treatment (Fig. 4) were as follows: stage I – 33,795,634 rubles/$465,791 (2.16%), stage II – 201,752,088 rubles/$2,780,666 (12.91%), stage III – 170,336,962 rubles/$2,347,685 (10.9%), stage IV – 1,157,045,130 rubles/$15,947,079 (74.03%). The total costs of treating patients with CRC in 2018 amounted to 1,562,929,814 rubles/$21,541,222.

The average cost of treating one patient is 2,032,418 rubles/$ 28,012.

Thus, compared with 2016, after the introduction of screening advent in 2017, the average treatment cost of 1 patient decreased by 656,859 rubles/9,053$, and after the introduction of software screening in 2018 – by 795,273 rubles/10,961$. Taking into account the fact that, at the same time, the CRC diagnostics cost increased by only 950 rubles/$13 in 2017 and by 15,364 rubles/$212 in 2018, the authors show the amount of obvious savings in healthcare resources. They have justified their conclusions by applying one of the main methods of clinical and economic research – the analysis of "cost minimization". The amount of cost for the diagnostics and treatment of 1 CRC patient in the absence of screening (2016) averaged 2,827,691 rubles/$38973, and after the appearance of screening (2017) – 2,171,782 rubles/$29,933, in the first year of the program implementation (2018) – 2,048,733 rubles/$ 28,236.85. According to the formula for calculating the cost difference indicator, when comparing the expenses in 2016 and 2017, the authors show its value as 655,909 rubles/$9,040, and when comparing the expenses in 2016 and 2018, its value is proved to be 778,958 rubles/$10,736.

The presented calculations convincingly demonstrate the cost savings for each case of screened CRC (Fig. 5).

To obtain more convincing evidence of the economic justification of program screening, a cost-effectiveness analysis as the most objective of the methods of clinical and economic research was applied. For this purpose, the Cancer Registrar information on the average life expectancy of CRC patients after the registration, depending on the stage of the disease, was used. Taking into account the number and structure of CRC cases, the authors calculated the average predicted life expectancy of CRC patients identified in 2016, 2017 and 2018. The corresponding calculated data are presented in Table 2.

Thus, the average predicted life expectancy of CRC patients detected in 2016, is 42.5 months (3.5 years), in 2017 – 53.6 months (4.5 years), in 2018 – 55, 3 months (4.6 years). Applying the formula for calculating the cost-effectiveness ratio, the average of diagnostics and treatment costs for 1 patient is put in the numerator, the average predicted life expectancy of CRC patients,

[14] Decoding of clinical and statistical groups (CSG) for medical care // Joint letter of the Ministry of Health of the Russian Federation No. 11-7 / I / 2-20621, Federal MHI Fund No. 00-10-26-2-04 / 11-51 dated 12/30/2020 of the year, "On methodological recommendations on methods of paying for medical care at the expense of compulsory medical insurance funds for 2021". Available from: http://omspk.ru/upload/iblock/a02/Расшифровка%20групп%20КС%202021.xlsx/. Russian

months, is put in the denominator. The CER calculated by the authors in 2016, was 66,534 rubles/917 $, in 2017 – 40,518 rubles/$558, in 2018 it was 37048 rubles/511 $. The optimal value is considered to be the lowest value of the ratio, which corresponds to the program screening strategy in this study (Fig. 6).

The results obtained make it possible to analyze "the impact of medical technology on the budget". The cost of providing CRC patients with medical care in 2016, amounted to 1,942,623,522 rubles/$26,774,385 (specialized treatment cost), in 2018 – to 1,575,475,819 rubles/$21,634,376 (screening and specialized treatment costs). The reduction in the resource consumption of the whole diagnostics and treatment process of the entire CRC array, identified in the Primorsky Territory in 2018, amounted to 367,147,703 rubles/$5140009. Thus, the introduction of CRC screening software provides savings in the financial support of medical care for the disease under study by 23%, compared with an alternative diagnostics and treatment strategy.

DISCUSSION

According to WHO principles, cancer screening is aimed at the early detection of the disease or its precursors [19]. Centralized CRC screening is currently actively used in most European countries, Canada, separate regions of the Americas, Asia and Oceania. The Netherlands showed the highest level of participation in the program (68.2%), and some parts of Canada - the lowest one (16%) [3].

A decrease in mortality from colorectal cancer observed in the latest 10 years, which is, in fact, a significant clinical achievement, is explained, first of all, by screening (53%) and, only second and third, by improved treatment (12%) and a controlled decrease in the influence of risk factors (35%) [4, 26]. It has been estimated that increasing the prevalence of CRC screening up to 80% in the next 2 years, will prevent 277,000 CRC cases which is more than 75% of the potential incidence rate, and 203,000 deaths by 2030 [4, 27]. The implementation of CRC screening programs is a rare example of effectiveness in oncological practice, yielding only to screening for cervical cancer in its clinical results [5].

At the same time, all over the world, more and more attention is paid to the cost and value of cancer treatment; among them, CRC is a nosological unit with a leading resource consumption. It is with this disease that the highest direct costs of the healthcare system of the Russian Federation are associated (52 milliard rubles per year), which are mainly attributable to anticancer pharmacotherapy [28–30]. Within the framework of the RF project "Combating Cancer"[15], in the period of 2019–2024, financing of the drug supply with anticancer preparations will be from 70 to 140 milliard rubles a year. However, the choice of a strategy for medical interventions is associated with an idea of a limited nature of the health care economic resources. This dictates the need for obtaining evidence not only of the clinical, but also of the economic justification of alternative medical technologies.

Professional oncological communities such as ESMO, ASCO are trying to analyze the cost-effectiveness of various treatment and diagnostic options for colorectal cancer. The complexity of this task lies in different costs of medical technologies in different countries. This fact does not make it possible to extrapolate the data on cost effectiveness from one country to another [31]. So, in Australia, thanks to the screening programs, the cost of one year of life extension for CRC patients is $16,632, in the USA – up to $22,000, in Europe – up to $5,000 [17, 19,23]. However, nowadays, the undoubted economic justification of colorectal cancer screening is beyond dispute among specialists in the United States and most European countries [16].

On the basis of two main methods of clinical and economic analyses, identical results, indicating a high clinical effectiveness of CRC screening were obtained. The presented data indicate that even opportunistic screening (2017), in comparison with the rejection of it, demonstrates a more optimal detection of the disease, both in quantitative and structural terms, as well as the predicted life expectancy of CRC patients at diagnosis. The number of patients with newly diagnosed CRC in 2017 increased by 3.49% (24 people) compared to 2016; the number of the identified at stages I–II of the disease – by 15.53% (118 people), the average predicted life expectancy of patients increased by 11.1 months. The very first year of the screening program implementation in the territory of the subject reinforced these advantages. The introduction of software screening in 2018, compared with its absence in 2016, provided an increase in the indicators under consideration by 11.9% (82 people), 16.81% (155 people) and 12.8 months, respectively (the expected consequence of these advantages will be a natural decrease in the mortality rate from CRC in the territory of the region in subsequent years).

These clinical effects are extrapolated into the cost reduction of expensive anticancer pharmacotherapeutic interventions, the most expensive among which are palliative drug regimens used in the treatment of advanced stages of colorectal cancer [28–30]. This economic advantage is confirmed by performing an analytical calculation of "the impact of medical technology on the budget": a decrease in the level of the economic burden of the disease under study in the territory of the region in 2018 was 23% (367,147,703 rubles/$5,140,009) compared to the period not included in the screening program. Thus, the considered medical technology sig-

[15] Application to the minutes of the meeting of the project committee for the national project "Health". Passport of the federal project "Fight against cancer, 2018. Available from: http://zdrav.tmbreg.ru/assets/files/Gosprogramm/nacionalnyy-proekt-zdravoohranenie/pasporta-fp/пфп-борьба-с-онкологичесиким-заболеваниями-14.12.2018.pdf. Russian

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nificantly reduces the economic burden of CRC, avoiding excessive costs. This is consistent with the results of the screening programs in the United States and most of Europe. To assess the cost-effectiveness of CRC screening and other prevention strategies in these countries, a target willingness-to-pay threshold (WPT) of $30,000–50,000 is used, depending on the national health policy per life year saved (LYS) or per quality-adjusted life year saved (QALYS). The calculated cost-effectiveness ratio (CER) is on average $3,380/LYG, which makes it possible to consider a CRC program screening as the standard of the economic effectiveness [19]. Many countries, including the Russian Federation, comply with the WHO recommendations, adopting the formal threshold value of willingness–to-pay in monetary terms, equal to 1–3 level indicators of the gross domestic product (GDP) per capita. When applied to this criterion, the targeted implementation of CRC screening also demonstrates the results of a highly effective economic investment [17, 19, 23].

The experience of the US specialists also reports on significant long-term effects of screening, several times higher than the corresponding achievements at the initial stage of its implementation [3, 13]. So, after the initial growth in the CRC diagnostics rate, several years after the introduction of the program, there is a consistent decrease in the CRC incidences. At the same time, in the structure of the revealed CRC, the frequency of cases at stages I–II reaches 80%, at stages III–IV – no more than 25% [4]. Alongside with an early detection of malignant pathology, the aspect of premalignant neoplasms diagnostics is no less important in the CRC screening program. In this aspect, CRC screening, has obviously an even wider range of clinical and economic results, since the elimination of potentially malignant CRC neoplasms can reduce the CRC incidence by 20–90% according a number of experts' data [5, 8, 19]. These effects are expressed in an increase in the duration and improvement of patient QoL, as well as in significant savings in health care resources and costs of the population. This makes it possible to confidently expect the emergence of further evidence of a reduction in human and material losses due to the prolongation and mass implementation of screening software.

CONCLUSION

Accelerating progress in the struggle against colorectal cancer can be achieved by ensuring an access to high-quality precision health care for all patients and promoting healthier lifestyles to prevent cancer. Healthy behaviors such as a achieving normal body weight, being physically active, and avoiding excessive alcohol or smoking can reduce the CRC risk by at least one third. However, clinical and economic evidence of the widespread screening effectiveness states that even more cases of colorectal cancer and deaths from this disease could be prevented with its help. The steadily increasing burden of oncological pathology determines the perception of medical interventions aimed at the prevention and early diagnostics of malignant neoplasms as a necessary national strategy. For the full implementation of all the possibilities of CRC screening, the coverage of the population with research should be close to 80%. The principles of its application should be the universal availability and integrity of the health care system. If appropriate management decisions are made, a long-term prolongation of this technology in the territory of each constituent entity of the state can become a part of the practical implementation of the Federal project "Combating Cancer" (2019–2024). That will extrapolate to the implementation of its most important task: reducing the mortality rate from cancer by 6% (no more than 185 cases per 100 thousand people) until 2024.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

Yulia Yu. Petukhova – study planning, data collecting and organizing, study conducting, analyzing and interpreting the results obtained, reviewing literary sources, manuscript writing, forming a list of references;
Ekaterina V. Eliseeva – review of critical intellectual content, final approval of the manuscript for publication;
Antonina G. Petukhova – participation in the development of the concept and design of the study, statistical processing of the results.

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RESEARCH ARTICLE

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ANTITUMOR ACTIVITY *IN VIVO* OF AQUEOUS AND ALCOHOLIC EXTRACTS OF *THYMUS MARSCHALLIANUS* WILLD

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The aim of research is to study the antitumor activity of aqueous and alcoholic extracts of *Thymus marschallianus* Willd. on male outbreed white rats with transplanted liver tumor PC-1.

Materials and methods. The object of study is crushed grass of *Thymus marschallianus* Willd: collected in the Saratov vicinity in the flowering phase. Extracts from the specified plant material were made in two different ways: first way, water was used as an extractant, in the other, ethyl alcohol 95%. 15 male outbreed white laboratory rats weighing 200±50 were used in experiment. The subcutaneous injections of alveolar liver cancer RS-1 were made in scapula area. Animals with transplanted tumor were randomly divided into 3 groups of 5 rats: the first was a control (negative control) that did not receive extract; the second was an experimental one that receiving alcoholic extract of *Thymus marschallianus* Willd.; the third was an experimental one that receiving aqueous extract of *Thymus marschallianus* Willd. To study the pathomorphosis of the tumor, morphological and morphometric methods were used standard histological staining with hematoxylin and eosin.

Results It has been established that alcoholic and aqueous extracts of *Thymus marschallianus* Willd. have antitumor activity. Morphological examination of animal tumors showed a decrease in the number of preserved tumor cells in the view field, pronounced necrobiotic and atrophic changes in tumor cells, absence of mitosis, proliferation of connective tissue fibers corresponding to the II-III degree of tumor pathomorphosis.

Conclusion. *Thymus marschallianus* Willd. aqueous extract showed more potent antitumor activity. Introduction into tumor tissue revealed morphological signs of apoptosis: the appearance of apoptotic bodies, karyopycnosis, and condensation of nuclear chromatin in tumor cells. It can be assumed that the more pronounced antitumor effect of the aqueous extract is due to the higher yield of flavonoids.

Keywords: extract; *Thymus marschallianus* Willd.; antitumor activity

Abbreviations: NCI – nuclear cytoplasmic index; WHO – World Health Organization; MTS – metastases; SP RF XIV^{ed} – State Pharmacopoeia of Russian Federation XIV edition.

ПРОТИВООПУХОЛЕВАЯ АКТИВНОСТЬ *IN VIVO* ВОДНОГО И СПИРТОВОГО ЭКСТРАКТОВ *THYMUS MARSCHALLIANUS* WILLD

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Цель. Изучить противоопухолевую активность водного и спиртового экстрактов тимьяна Маршалла (*Thymus marschallianus* Willd.) на самцах беспородных белых крыс с перевитой опухолью печени РС-1.

Материалы и методы. Объект исследования – измельченная трава тимьяна Маршалла, которая собрана в окрестностях города Саратова в фазе цветения. Экстракты из указанного растительного материала были приготовлены двумя разными способами: в одном, в качестве экстрагента использовали воду, в другом –спирт этиловый 95%-ный. В эксперименте использовано 15 самцов беспородных белых лабораторных крыс массой 200±50 г, которым имплантировали подкожно, в область лопатки, альвеолярный рак печени – РС-1. Животные с перевитой опухолью методом случайной выборки были разделены на 3 группы по 5 крыс: первая, контрольная, не получавшая экстракт; вторая, опытная, получавшая спиртовой экстракт тимьяна Маршалла; третья, опытная, получавшая водный экстракт тимьяна Маршалла. Для изучения патоморфоза опухоли применялись морфологические и морфометрические методы с использованием стандартной гистологической окраски гематоксилином и эозином.

Результаты. Экспериментально установлено, что спиртовой и водный экстракты тимьяна Маршалла обладают противоопухолевой активностью. Морфологическое исследование опухолей животных показало снижение количества сохранных опухолевых клеток в поле зрения, выраженные некробиотические и атрофические изменения клеток опухоли, отсутствие митозов, разрастание соединительнотканных волокон, что соответствует II–III степени патоморфоза опухоли.

Заключение. Водный экстракт тимьяна Маршалла проявил более сильную противоопухолевую активность. При его введении в опухолевую ткань выявлены морфологические признаки апоптоза: появление апоптотических телец, кариопикноз и конденсация ядерного хроматина в опухолевых клетках. Можно предположить, что более выраженный противоопухолевый эффект водного экстракта обусловлен бóльшим выходом флавоноидов.

Ключевые слова: экстракт; Thymus marschallianus Willd.; противоопухолевая активность

Список сокращений: ЯЦИ – ядерно-цитоплазматический индекс; ВОЗ – Всемирная организация здравоохранения; MTS – метастазы; ГФ РФ XIV изд. – Государственная Фармакопея Российской Федерации XIV издания.

INTRODUCTION

According to the World Health Organization (WHO)[1], cancer is the second leading cause of death in the world. In 2018, 9.6 million people died from this disease. Cancer is causing every sixth death in the world. The common methods of cancer treatments are surgery, chemotherapy and radiation therapy[2]. However, the antitumor activity of both individual natural compounds (alkaloids, terpenoids, quinones), and plant extracts, for example, Aco*nitum baicalense* Turcz. ex Rapaics *Ranunculaceae* family [2], *Kalanchoe daigremontiana* Raym.-Hamet & H.Perrier *Crassulaceae* family, *Aloe arborescens* Mill. *Asphodelaceae* family [3], has been proven The drugs of this compounds are prescribed in the complex treatment of cancer [1]. Flavonoids, polysaccharides, etc. are being studied as potential antitumor agents [1]. Herbal extracts are a promising subject of research in the field of cancer therapy. This is possible due for substances with a new mechanism of action that allows targeted action on tumor cells without damaging normal ones. The common chemical compounds of *Thymus marschallianus* Willd. are essential oil, phenolic compounds, triterpene compounds, polysaccharide complexes, mineral elements, amino acids, and organic acids [4–11], that makes this species promising in terms of antitumor activity study.

Previously, the antitumor activity was studied for species of *Thymus* L. genus of different countries – *T. algeriensis* [12–13], *T. vulgaris* [12, 14–17], *T. serpyllum,* [12, 18–19]*, T. caramanicus, T. carnosus* Boiss.*, T. citriodorus, T. mastichina, T. pulegioides, T. satureioides, T. schimperi, T. zygis* [12]. For example, MTT assay *in vitro* is used to show the antiproliferative effects of ethanol lyophilic extract and *Thymus algeriensis* Boiss & Reut. essential oil. The effects were evaluated on five human cancer cell lines: MCF-7 human adenocarcinoma cells and MDA-MB-231, human cervical adenocarcinoma cells HeLa, human prostate cancer cell line PC3 and human leukemia cell line K56S [13]. Essential oil has been shown more effective growth suppression of cancer cells all lines than ethanol extracts. Thus, LD_{50} of ethanol extract was more than 10,000 μg/ml, LD_{50} of essential oil in range 300–1067 μg/ml for various cancer cell lines used in this study. As a positive control, doxorubicin (an anticancer drug) was used, which demonstrated LD_{50} values in the range from 1 to 20 μg/ml [13]. Another experiment [16] showed the antiproliferative effect of *Thymus vulgaris* L. essential oil on the MCF-7 and MDA-MB-231 cell lines using MTS colorimetric assays (to control cytotoxicity) and ELISA (to control cell proliferation). The results showed that thyme essential oil significantly reduced metabolic activity and subsequently cell survival in both tested cell lines: at a concentration of 0.12 μg/ml – MDA-MB-231 cell lines and 0.13 μg/ml – MCF-7 cell lines [16]. It has been established that methanolic extract of *Thymus serpyllum* L. showed anti-cancer activity on the human cervical epithelial carcinoma cell line (HCerEpC) in MTT assay. In the experiment cytotoxicity varied from 50 to 100% respectively in concentration range of 500–2500 μg/ml [18]. In addition, the ability of creeping thyme extract to induce apoptosis of a breast cancer tumor cell line (MCF-7 and MDA-MB-231) has been established. At the same time, it does not exhibit a cytotoxic effect on the human healthy breast cell line (MCF-10A) [19].

[1] World Health Organization. Cancer. 2021. Available from: https://www.who.int/ru/news-room/fact-sheets/detail/cancer.
[2] Ibid.

Thymus serpyllum L. is officinal species. On the territory of the Saratov region occurs as a rare invasive species and has no resource value in the region. [20]. The most common closely related species in the territory of the Saratov region is a *Thymus marschallianus* Willd. Closely related plants species often exhibit a similar pharmacological effect, because they have a similar chemical properties [21]. For example, wild-growing *Thymus marschallianus* Willd, previously showed expectorant, anti-inflammatory, angioprotective, antioxidant activity better then pharmacopoeial species – *Thymus serpyllum* L. The effects of these two types are similar [22]. At the same time, the quantitative content of essential oils in the grass of *Thymus marschallianus* Willd exceeded the content of oils in the grass of *Thymus serpyllum* L. [23].

It should be noted that the most experimental studies of antitumor activity *Thymus* genus representatives were based *in vitro* [12–15, 17–19], while *in vivo* [16] experiments number is very small. There is no information in literature about the effect of extracts from raw *Thymus marschallianus* Willd on tumor cells.

THE AIM of the research is to study the antitumor activity of aqueous and alcoholic extracts of *Thymus marschallianus* Willd on male outbreed white rats with a PC-1 liver tumor.

MATERIALS AND METHODS

The object of research is crushed grass of *Thymus marschallianus* Willd. Collected in flowering phase (June-July 2018) in the vicinity of Saratov. The species was determined according to the key of V. N. Gladkova and Yu. L. Menitsky "Flora of the European part of the USSR"[3] [24]. The raw material was dried in well-ventilated place, then crushed to particles passing through a sieve with holes of 2 mm in size.

Method of extracts preparation

Extracts from the specified plant material were made in two different ways:

1. According to the methodology of the State Pharmacopeia of Russian Federation XIV^{ed} (SP RF XIV) GPhM.1.4.1.0018.15 "Infusions and decoctions" 10 g of raw materials were placed in a glass preheated in a boiling water bath, 100 ml of water was poured at room temperature (the ratio of raw materials and extractant 1:10), closed with a lid and insisted on water bath for 15 minutes, then at room temperature – 45 minutes. The resulting extract was evaporated in a water bath until a thick extract was obtained (product yield – 1.0 ± 0.2 g), then diluted with water for injection to concentration of 100 mg/ml. The technology of this extraction method is regulated by the SP RF XIV, validated and easily reproducible.

2. According to the patented technique [24], a two-time extraction was carried out with ethyl alcohol 95% (10 g of raw materials were poured with 100 ml of ethanol), boiled for 15 minutes. The obtained extract was drained, the remaining raw materials were again poured with 100 ml of alcohol, brought to a boil and drained for the first extraction. The obtained extract was evaporated in a water bath to the state of thick extract, diluted with distilled water, purified with chloroform, centrifuged for 15 minutes, then the purified aqueous fraction was evaporated in a water bath until thick extract was obtained (product yield – 0.4 ± 0.1 g), then diluted with water for injection to concentration of 100 mg/ml. The obtained extract of *Thymus marschallianus* Willd. by this method previously show antimicrobial activity [25]. In addition, the obtained extract of *Gratīola officinālis* by this method previously shown antitumor activity [24, 26].

Work with laboratory animals

The work with laboratory animals was carried out in accordance with the research protocol that does not contradict Directive 2010/63/eu of the European Parliament and of the Council of the European Union of September 22, 2010 on the Vertebrate Animals used in experimental research. The aim and descriptions of the experiments were approved by the Ethical Commission of the Saratov State Medical University named after V.I. Razumovsky (Protocol No. 4, May 3, 2020).

Study design

The experiment was conducted in accordance with the guidelines for the experimental (preclinical) study of new pharmacological substances[4]. 15 male outbreed white laboratory rats weighing 200±50 g were injected subcutaneous in the scapula by 0.5 ml of 25% tumor suspension of Hanks solution of the strain hepatic alveolar cancer RS-1, obtained from the bank of tumor strains of the N.N. Blokhin State Research Center of the Russian Academy of Medical Science. Animals with transplanted tumor were randomly divided into 3 groups of 5 rats: the first was a control (negative control) that did not receive extract; the second was an experimental one that receiving alcoholic extract of *Thymus marschallianus* Willd; the third was an experimental one that receiving aqueous extract of *Thymus marschallianus* Willd. After the tumor reached 1 cm^3 (on the 18th day from the beginning of experiment), the rats in the experimental groups were injected intraperitoneally by extract in dose of 100 mg/kg, once a day for the next 14 days (18-31 day experiment). After extract withdrawal laboratory animals were monitored for 7 days (32-38 day experiment). In connection, animals of all groups were withdrawn from the experiment ahead of schedule on the 32nd

[3] Fedorov AA, Menitskiy YuL. Flora of the European part of the USSR. Leningrad: Nauka, 1978: 259 p. Russian

[4] Mironov A.N., Bunyatyan N.D., Vasiliev A.N., et al. Guidelines for conducting preclinical studies of medicines. Moscow: Grif and K. 2012: 944 p. Russian.

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day, because tumor has disintegrated in groups receiving alcoholic extract of thyme.

The dynamics of tumor growth was estimated by the change of volume according to the formula:

$$V = A \times B \times C,$$

where: *A* is the tumor width; *B* is the thickness; *C* is the height of the tumor.

Measurements were made with caliper every two days from the beginning of the experiment. To analyze the results, the true mass of the animal was calculated by subtracting the theoretical mass of the tumor (multiplying the volume of the tumor by density) from the weighing mass and true mass change:

$$M_{true} = M_{animal.} - V \times p,$$

where: M – is the weighing weight; V – tumor volume; p – the density of transplanted tumor 0,74 g/cm^3 [26].

The change in true weight of the animals (delta) body was determined by subtracting the mass of the animal before the start of experiment from true mass of the animal on experiment day and offered as a percentage. On day 32, the rats were removed from the experiment and samples of organ tissue, tumors, and blood were collected for additional studies.

To study the pathomorphosis of the tumor, morphological and morphometric methods were used standard histological staining with hematoxylin and eosin. In the study of tumor tissue, the presence of dystrophic and necrobiotic changes was assessed, as well as morphometric indicators such as: the diameter of the tumor cell, the ratio of the diameters of the tumor cell and its nucleus, the nuclear cytoplasmic index (NCI). The calculation was carried out on 100 cells in 10 view fields of each micropreparation using medical transmitted light Microvizor μVizo-101 (LOMO, Russia).

Statistical processing of results

Statistical processing of results was performed using the application Software package Statistica 10.0 (StatSoft Inc., USA). The normal distribution of quantitative features and the equality of general variances were checked using the Shapiro-Wilk test and the Fisher's exact test. Descriptive statistics of quantitative traits were presented in the form of central tendency, median (Me), interval (minimum and maximum values of the studied trait), and interquartile range (25 and 75 percentiles). In the text, these indicators were specified as Me, [min-max], (LQ; UQ). The difference of the groups was determined using the Kruskal-Wallis test and also the Mann-Whitney *U*-test. The significance of the null statistical hypothesis was 0.05.

RESULTS AND DISCUSSION

In the middle of the experiment a noticeable growth of the tumor was observed in animals receiving ethanol extract of *Thymus marschallianus* Willd, but significant differences from the control are not found (P = 0.427). The dynamic of changes in the tumor volume of rats receiving aqueous extract, was comparable to the measurements in the control group and did not differ from it (P = 0.919).

Animals of all groups gained maximum weight before the tumor became 1 cm^3 in volume (at 11 day). During the experiment, fluctuation in the dynamic of the animals true mass were observed: in the control group, this change was 4.6%.The dynamic vibration of the true weight of animals were also observed when introducing extracts of *Thymus marschallianus* Willd (18–31 days). In the group receiving ethanol extract, the true body weight of rats increased by 2.7% in the end of the experiment, but these changes in compared with the control were unreliable (P = 0.835). In animal group receiving aqueous extract, it increased by 14.4%, relative to the weight at the beginning of the experiment compared with the control group (P = 0.037) (Table 1).

These changes indicate that in the animal group receiving aqueous thyme extract, body weight loss occurred more slowly than in the control group and the group receiving ethanol extract.

The morphological examination revealed that the transplanted tumor in the control group consisted of cellular structures of different shapes and sizes, separated by thin layers of connective tissue (Fig. 1). Tumor cells of oval or rounded shape, in the cytoplasm are large vacuoles containing mucus and pushing the oval nucleus to the periphery of the cell. The number of mitoses was 6 in one visual field. Single tumor cells of necrosis were noted.

During the morphological and morphometric study of the tumor tissue in the rats group receiving alcoholic thyme extract, attention was paid to the pronounced pathomorphosis mainly in the central parts of the tumor. A large number of "shadow cells", a decrease in the size of tumor cells, and extensive zones of necrosis were noted. The intact tumor cells are represented by small rounded cells with rounded or bean shaped nuclei with single small vacuoles containing mucus. The tumor cells are located in cells formed by thickened connective tissue partitions with a large number of thin-walled blood vessels. Connective tissue fibers are infiltrated by lymphocytes. Mitosis was determined only in one case of observation (Fig. 2).

In the group of rats receiving aqueous extract of thyme, the tumor is represented by small rounded cells with a reduced flattened nucleus located on the periphery of the tumor tissue. The central parts are represented by extensive necrotic foci, a large number of "shadow cells" and thickened connective tissue partitions with a large number of blood vessels, as well as extensive clusters of tumor cells with signs of karyopycnosis, nuclear chromatin condensation and karyorexis, and a large number of apoptotic cells (Fig. 3, 4).

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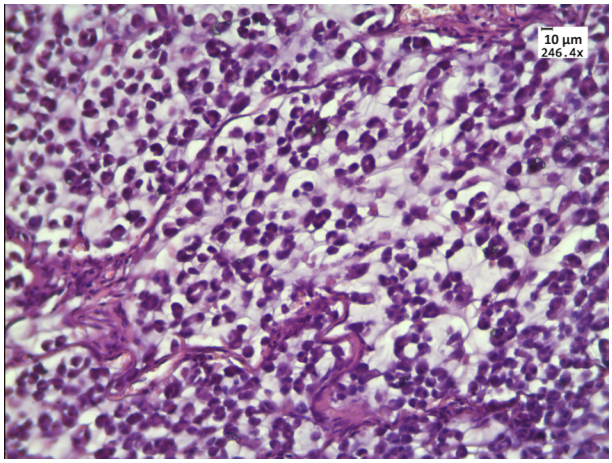

Figure 1 – Histological tumor structure of the control group

Note: the tumor cells are separated by thin layers of connective tissue. Stained with hematoxylin and eosin. Magnification 246.4×.

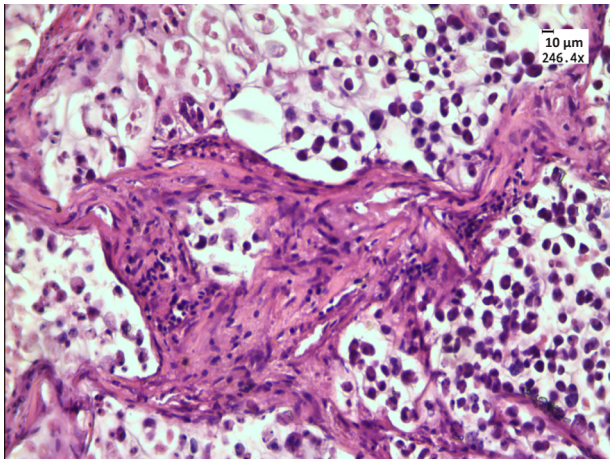

Figure 2 – Histological structure of the transplanted hepatic cancer in the group receiving alcohol extract of *Thymus marschallianus* Willd.

Note: dystrophic and necrotic changes in tumor cells, "shadow cells" (black arrow), thickening of connective tissue partitions (white arrow). Stained with hematoxylin and eosin. Magnification 246.4×.

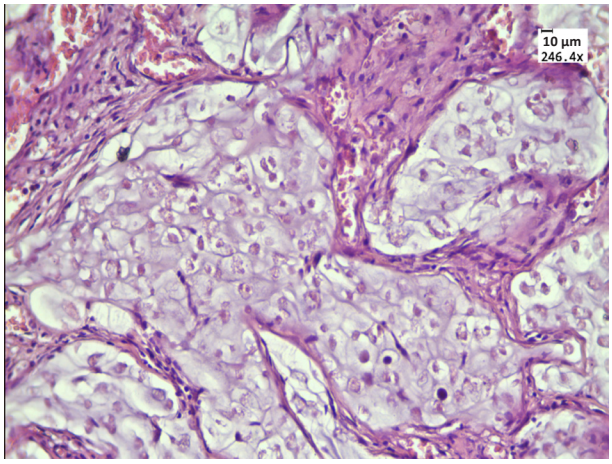

Figure 3 – Histological structure of transplanted hepatic cancer in the group receiving aqueous extract of *Thymus marschallianus* Willd.

Note: necrotic foci of tumor tissue (black arrow), thickening of connective tissue partitions (white arrow). Stained with hematoxylin and eosin. Magnification 246.4×.

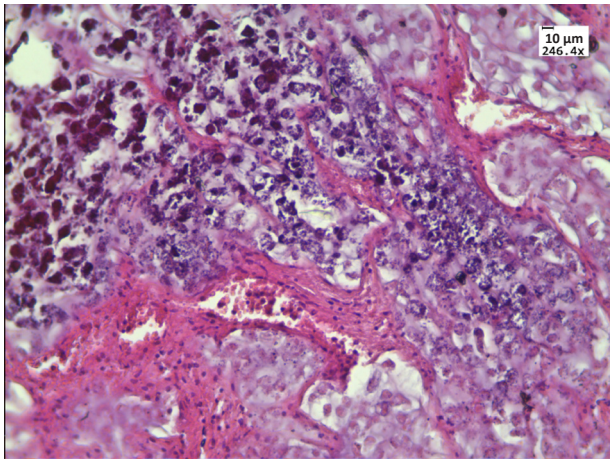

Figure 4 – Histological structure of transplanted hepatic cancer in the group receiving aqueous extract of *Thymus marschallianus* Willd.

Note: nuclear chromatin condensation and karyorexis in tumor cells (white arrow). Stained with hematoxylin and eosin. Magnification 246.4×

Table 1 – Dynamic of changes in the volume of the transplanted tumor of rats RS-1 and true body weight of experimental animals

Group Indicator	Control group	Alcoholic thyme extract Me (LQ; UQ) [min-max]	Aqueous thyme extract	P*	P**
Tumor volume	11087; (3678–22210); [162–62350]	15150; (4863.5–20736); [180–95040]	9948; (3072–19941); [160–49910]	0.583	0.329
Change in the true body weight of animals	6.53; (3.7–6.9); [–5.7–11.5]	0.7; (–2.1–8.7); [–3.1–9.4]	15.4; (11.3–16.4); [8.0–20.7]	0.000	0.037

Note: Me – median, LQ – lower quartile, UQ – upper quartile; [min-max] – the minimum and maximum values of the defined attribute; P* – the significance of the differences between the groups was assessed using the Kruskal-Wallis test; P** – the significance of the differences between the two experimental groups was assessed using the Mann-Whitney test.

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Table 2 – Morphometric parameters of transferable hepatic cancer cells

Group / Indicator	Control group	Alcoholic thyme extract	Aqueous thyme extract	P*	P**
		Me (LQ; UQ) [min-max]			
Cells number in the view field	90 (82–96); [68–123]	39 (34–47); [24–64]	11 (9–15); [6–18]	0.000	0.000
Number of necrotic cells	1 (1–2); [0–4]	20.05 (11–34); [7–47]	42 (38–44); [28–54]	0.000	0.000
Diameter of t tumor cell	14 (13–15); [12–21]	7 (6–8) [6–12]	7 (6–8); [6–10]	0.000	0.065
Diameter of the tumor cell nucleus	8 (7–9); [6–10]	3 (3–4) [2–5]	3 (2–3); [2–3]	0.000	0.000
Nuclear-cytoplasmic ratio	0.6 (0.5–0.6); [0.5–0.8]	0.43 (0.38–0.5); [0.3–0.83]	0.38 (0.33–0.43); [0.2–0.5]	0.001	0.001

Note: Me – median, LQ – lower quartile, UQ – upper quartile; [min-max] – the minimum and maximum values of the defined attribute; P* – the significance of the differences between the groups was assessed using the Kruskal-Wallis test; P** – the significance of the differences between the two experimental groups was assessed using the Mann-Whitney test.

The morphometric study determined the decrease in the average number of intact tumor cells in the field of view. In compared with the control group a decrease was observed 2.2 times in the rat groups receiving ethanol extract of thyme, and 7.8 times in the group receiving aqueous extract.

The reducing of tumor cells size was observed in both experimental groups compared to the control group. Thus, in the rats group receiving alcoholic thyme extract, the average diameter of the tumor cell was 1.86 times, and in the group receiving aqueous extract - 1.99 times less than in the control group. In addition, the reducing of nucleus diameter in the tumor cells and also the values of the nuclear-cytoplasmic ratio was noted in both the animal groups receiving ethanol extract (0.43) and aqueous thyme extract (0.38) compared to the control group (0.6) (Table 2).

Previously Kubatko P. et al. [16] studied the antitumor activity of *Thymus vulgaris* L. on models of breast carcinoma *in vivo*. Chemoprophylaxis (NMU-induced breast cancer model in female rats) and therapeutic use (4T1 adenocarcinoma model in female mice) were studied experimentally. The animals were fed pellets of thyme grass (the grass was crushed to particles of 2 mm in size and processed using a "cold granulation procedure") in two concentrations of 1 g/kg and 10 g/kg. The pellets were given to rats a week before the carcinogen would been administered on and continued for up to 15 weeks of the experiment. For mice – from the day of inoculation of carcinoma cells and up to 15 days. Food intake during the experiment was monitored four times in rats and twice in mice for 24 hours. The average daily dose of thyme for rat was 16.27 mg (thyme 1 g/kg) and 172.00 mg (thyme 10 g/kg), for mouse – 2.06 mg (thyme 1 g/kg) and 15.13 mg (thyme 10 g/kg). Thyme dose of 10 g/kg significantly inhibited the formation of breast cancer by 53% compared with the control, but the latency of the tumor and average volume does not significantly change. The chemoprophylactic efficacy (tumor frequency) observed in this rats group significantly correlated with tumors reduced, i.e. a small number of new tumors that grew longer were noted. Thyme at dose of 1 g/kg doesn't show any significant changes compared with control. The volume of tumors was significantly smaller in the two experimental groups receiving thyme compared with control: 85% in group receiving pellets at concentration of 1 g/kg and 84% in group receiving pellets at concentration of 10 g/kg. Moreover, thyme in both doses significantly reduced the necrosis ratio of entire tumor area –77% (thyme 1 g/kg) and 81% (thyme 10 g/kg) compared with control, as well as the mitotic activity index –31.5% (thyme 1 g/kg) and 25% (thyme 10 g/kg) compared with the control (with adenocarcinomas).

Our *in vivo* experiment conducted on rats, alveolar hepatic cancer PC-1 was used. *Thymus marschallianus* Willd. herb extracts were administered intraperitoneal to animals at dose of 100 mg/kg (0.1 g/kg) for 14 days after the tumor became 1 cm^3. We studied the effectiveness of the therapeutic use of *Thymus marschallianus* Willd. extracts. In the article Kubatko P. et al. [16], the general condition of animals (changes of animal weight, appetite) was not described. This does not allow us to compare the antichectic activity of common thyme granules and studied *Thymus marschallianus* extracts. It should be noted that the rats chemocarcinogen-induced breast carcinogenesis in, thyme granules at dose of 10 g/kg

significantly reduced the incidence of tumors (about 53%). That proves the effectiveness of thyme granules use as a chemoprophylactic agent. it is difficult to assess the key parameters of the therapeutic effect (tumor volume) of this experimental model, since the rats with effective chemoprophylaxis had a relatively small number of new tumors compared to the control group. The model of carcinoma transplantation in mice [16], as in our experiment, makes it possible to evaluate the therapeutic effect of the investigated substances, since their action is directed against existing cancer cells. Our experiment was show that the volume of tumors in experimental groups changed in the same way as in the control group, however, histological tumors analysis of animals receiving extracts at dose of 100 mg/kg We revealed a decrease in the number of intact tumor cells in the field of view, pronounced necrobiotic and atrophic changes in tumor cells, the absence of mitosis, proliferation of connective tissue fibers, due to which, probably, the volume of tumors did not decrease. At the same time, the aqueous extract of *Thymus marschallianus* Willd. showed a more pronounced antitumor effect, since morphological signs of apoptosis were revealed when it was administered.

In the experiment Kubatka P. et al. [16] the volume of tumors in mice receiving thyme granules in two studied doses (1 g/kg and 10 g/kg) was less than in the control group. The results described by the authors are demonstrated several mechanisms of antitumor action studied in the experiment – proapoptotic, antiproliferative, antiangiogenic, antioxidant [16]. Thus, our experiment and research Kubatka P. et al. [16] demonstrated significant anti-cancer activity *in vivo* of species of the *Thymus* L. genus (*Thymus marschallianus* and *Thymus vulgaris*), but despite the fact that these species are closely related, the nature of the effects manifested is different. It can be assumed that the different changes in the volume of tumors and the different nature of the structural tumor changes of the two experiments are due to the different sensitivity of cancer cells *in vivo* to phytochemicals, the variability of the chemical composition of the studied plant species, as well as dose dependence.

CONCLUSION

The growth dynamic of tumor volume under the influence of both alcoholic and aqueous extracts of *Thymus marschallianus* Willd. was similar to the measurements in the control group (no significant differences from the control group were found). At the same time, in both experimental groups receiving thyme extracts, along with necrotic changes, there was an overgrowth of connective tissue fibers, which probably explains the unreliability of changes in the volume of tumor formations in groups.

Morphological analysis of the tumor tissue showed antitumor activity of both alcoholic and aqueous extracts *Thymus marschallianus* Willd. herb, and this is evidenced by a decrease in the number of preserved tumor cells in the view field, pronounced necrobiotic and atrophic changes in tumor cells, the absence of mitoses, and the growth of connective tissue fibers corresponding to the II-III stage of tumor pathomorphosis [27]. It should be noted that the aqueous extract of *Thymus marschallianus* Willd showed stronger antitumor activity, since morphological signs of apoptosis were revealed: the appearance of apoptotic bodies, karyopycnosis and condensation of nuclear chromatin in tumor cells. It can be assumed that the more pronounced antitumor effect of the aqueous extract is due to the high yield of flavonoids.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS CONTRIBUTION

Anna S. Sheremetyeva – collection of plant material for the experiment, conducting the experiment and data collection, analysis and interpretation of the data obtained, statistical processing of the results obtained, literature analysis, writing the manuscript; Aneta M. Napsheva – analysis and interpretation of the data obtained, statistical processing of the results obtained, verification of critical intellectual content, final approval for publication of the manuscript; Natalia A. Durnova – research planning, participation in the development of the concept and design of the study, verification of critical intellectual content, final approval for the publication of the manuscript.

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EFFECT OF GLYPROLINES ON THE LEVEL OF APOPTOTIC AND NEUROTROPHIC FACTORS UNDER CONDITIONS OF "SOCIAL" STRESS

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The aim of the article was to study the effect of glyproline neuropeptide compounds Thr–Lys–Pro–Arg–Pro–Gly–Pro (Selank), Pro–Gly–Pro and Pro–Gly–Pro–Leu, on the level of apoptotic factors (caspase-3, caspase-8, the tumor necrosis factor) and neurotrophic factors (the nerve growth factor and the brain neurotrophic factor) in the blood serum of white rats under the experimental modeling of "social" stress.

Materials and methods. The experimental studies were carried out on 90 nonlinear white male rats aged 6 months. By the type of behavior, in the process of "social" stress modeling, all the rats were divided into "aggressors" and "victims". In the study, the following experimental groups (n=10) were formed: control individuals; groups of the rats exposed to stress for 20 days; groups of the animals treated intraperitoneally at the dose of 100 μg/kg/day, starting from the 1st day of the stress factor exposure, with a course of 20 days of glyproline compounds Thr–Lys–Pro–Arg–Pro–Gly–Pro (Selank), Pro–Gly–Pro and Pro–Gly–Pro–Leu. The effect of the compounds on the level of apoptotic and neurotrophic factors was assessed by determining the level of caspase-3, caspase-8, the tumor necrosis factor, the nerve growth factor and the brain neurotrophic factor of white rat blood serum by enzyme immunoassay.

Results. According to the results of the study, it was found out that under the conditions of "social" stress, there was an increase in the apoptotic processes accompanied by an increase in the level of caspase-3, caspase-8, TNF-α in the blood serum of white rats, as well as a decrease in the concentration of neurotrophic factors – BDNF and NGF. The administration of giproline compounds against the background of stress, contributed to the restoration of the studied indicators level, which is most likely due to the presence of antiapoptotic and neuroprotective effects in giprolines due to the inhibition of the caspase-dependent cascade of apoptosis reactions, as well as the induction of the synthesis of neurotrophic factors with the antiapoptotic activity.

Conclusion. Thus, the administration of glyproline neuropeptide compounds Thr–Lys–Pro–Arg–Pro–Gly–Pro (Selank), Pro–Gly–Pro and Pro–Gly–Pro–Leu under stress conditions, contributes to the restoration of the initiating and effector caspases level, as well as of neurotrophic factors. As a result of the experiment, an anti-apoptotic effect is observed due to the inhibition of the caspase-dependent cascade of reactions, as well as a stress-protective effect is observed due to the restoration of the brain neurotrophic factors level.

Keywords: glyprolins; neuropeptides; "social" stress; apoptosis; caspases; tumor necrosis factor; brain neurotrophic factor; nerve growth factor

Abbreviations: TNF-α – tumor necrosis factor; NGF – nerve growth factor; BDNF – Brain-derived Neurotrophic Factor; CNS – central nervous system; cIAP – cellular inhibitor of apoptosis proteins.

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RESEARCH ARTICLE

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ВЛИЯНИЕ ГЛИПРОЛИНОВ НА УРОВЕНЬ АПОПТОТИЧЕСКИХ И НЕЙРОТРОФИЧЕСКИХ ФАКТОРОВ В УСЛОВИЯХ «СОЦИАЛЬНОГО» СТРЕССА

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Цель. Изучить влияние глипролиновых нейропептидных соединений Thr–Lys–Pro–Arg–Pro–Gly–Pro (Селанк), Pro–Gly–Pro и Pro–Gly–Pro–Leu на уровень апоптотических (каспаза-3, каспаза-8, фактор некроза опухоли) и нейротрофических (фактор роста нервов и нейротрофический фактор головного мозга) факторов в сыворотке крови белых крыс в условиях экспериментального моделирования «социального» стресса.

Материалы и методы. Экспериментальные исследования проводили на 90 нелинейных белых крысах-самцах 6-месячного возраста. В процессе моделирования «социального» стресса все крысы были разделены по типу поведения на «агрессоров» и «жертв». В исследовании формировались экспериментальные группы (n=10): контрольные особи; группы крыс, в течение 20 дней подвергавшиеся воздействию стресса; группы животных, получавших внутрибрюшинно в дозе 100 мкг/кг/сут, начиная с 1-го дня воздействия стресс-фактора, курсом 20 дней глипролиновые соединения Thr–Lys–Pro–Arg–Pro–Gly–Pro (Селанк), Pro–Gly–Pro и Pro–Gly–Pro–Leu. Влияние соединений на уровень апоптотических и нейротрофических факторов оценивали путем определения уровня каспазы-3, каспазы-8, фактора некроза опухоли, фактора роста нервов и нейротрофического фактора головного мозга сыворотки крови белых крыс методом иммуноферментного анализа.

Результаты. По результатам проведенного исследования было установлено, что в условиях «социального» стресса наблюдалось усиление апоптотических процессов, сопровождающихся увеличением уровня каспазы-3, каспазы-8, TNF-α в сыворотке крови белых крыс, а также снижение концентрации нейротрофических факторов: BDNF и NGF. Введение глипролиновых соединений на фоне стресса способствовало восстановлению уровня исследуемых показателей, что, вероятнее всего, связано с наличием у глипролинов антиапоптотического и нейропротекторного действия за счет ингибирования каспаза-зависимого каскада реакций апоптоза, а также индукции синтеза нейротрофических факторов, обладающих антиапоптотической активностью.

Заключение. Таким образом, введение глипролиновых нейропептидных соединений Thr–Lys–Pro–Arg–Pro–Gly–Pro (Селанк), Pro–Gly–Pro и Pro–Gly–Pro–Leu в условиях стрессогенного воздействия способствует восстановлению уровня инициирующей и эффекторной каспаз, а также нейротрофических факторов. По итогу проведенного эксперимента наблюдается антиапоптотический эффект за счет ингибирования каспаза-зависимого каскада реакций, а также стресс-протекторный за счет восстановления уровня нейротрофических факторов мозга.

Ключевые слова: глипролины; нейропептиды; «социальный» стресс; апоптоз; каспазы; фактор некроза опухоли; нейротрофический фактор мозга; фактор роста нервов

Список сокращений: TNF-α – фактор некроза опухоли; NGF – фактор роста нервов; BDNF – нейротрофический фактор головного мозга; ЦНС – центральная нервная система; cIAP – клеточный ингибитор белков апоптоза.

INTRODUCTION

At present, scientific works reflecting the results of studying the pathological influence of stress factors, including “social” stress, on various body systems, are of particular interest [1, 2]. The recent studies prove the fact that a prolonged exposure to stress contributes to the formation of neurological, immune, endocrine, oxidative, metabolic and other types of disorders; that ultimately leads to the development of violations of the molecular and cellular mechanisms of a programmed cell death, including apoptosis of neurons. [3, 4]. To date, close attention is paid to assessing the role of apoptotic and neurotrophic factors in the implementation of the stress response.

The most informative indicators of apoptotic processes assessment are initiator and effector caspases,

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which activate each other and trigger the caspase cascade [5–7]. It has been proven that when the body is exposed to stress factors, the apoptotic processes of neurons are activated due to the initiation of effector caspase-3 by caspase-8 [8–10]. The established initiation process is characteristic of the apoptosis development in lymphoid and endothelial cells, which, in turn, contributes to the development of immune dysfunction, as well as pathology of the cardiovascular, urinary and other systems [11, 12]. It has been proven that as a result of the exposure to stress factors, the development of caspase-dependent apoptosis is observed, which, to an even greater extent, is aggravated by the accumulation of free radicals. A caspase-dependent pathway, or the pathway of "death receptors" located on the cell surface, is characteristic of intact cells; the mitochondrial pathway mediated by the Bcl-2 family of proteins is characteristic of pathologically transformed cells. The pathway of "death receptors" is regulated by cytokines and is shorter than the other pathway – mediated by mitochondria, but functionally both of them are closely related to each other.

A tumor necrosis factor (TNF-α) is an equally important participant in apoptotic processes. It was found out that, as a result of the exposure to stress factors, the formation of a TNF-α complex with Fas receptors is observed. It is followed by triggering of signaling molecules, which activate caspase-3 and 8, leading to irreversible damage to neurons [12]. It has been proven that, alongside with a pronounced pro-inflammatory activity, a tumor necrosis factor contributes to an increase in the secretion of inflammatory mediators and the induction of apoptosis, due to binding to receptors on the target cell membrane, in particular to the membrane receptor TNF-R2 [13, 14]. In this case, TRAF2 molecules are inactivated, and, in their turn, they support the process of triggering cIAP apoptosis inhibitor proteins. In addition, TNF-α causes the cell death by the necrosis mechanism, promoting the formation of reactive oxygen species, which cause the destruction of membranes and death of the target cell. The above mentioned makes it possible to classify this cytokine as one of the important participants in apoptosis. It has been proven that a tumor necrosis factor plays an important role in the pathogenesis of diseases such as myocardial infarction, chronic renal failure, bronchial asthma [14], and reveals its initiating effect on the development of autoimmune pathology [13]. It was found out that the tumor necrosis factor is increased in the patients with neuropsychiatric and neurodegenerative diseases, as well as a traumatic brain injury [15].

When considering the neurotrophin hypothesis of various pathological disorders development (including apoptosis), such neurotrophic factors as the nerve growth factor and the brain neurotrophic factor, which have a pronounced neurospecificity, are of great importance in the manifestation of a neuroprotective effect. This action is realized due to the ability of the neurotrophic factors to induce the synthesis of anti-apoptotic proteins and inhibition of pro-apoptotic ones, thereby influencing the survival and differentiation of individual neurons populations. A number of studies reflect a direct dependence of apoptosis on the balance of NGF and BDNF, which activate the receptors of tyrosine kinases and have a neuroprotective effect [16–18].

It has been established that the nerve growth factor attracts the attention of scientists as a promising kind of treatment for various neuropsychiatric diseases such as Alzheimer's disease and depression [19]. The recent research results indicate that, alongside with a direct effect on the nervous system, NGF has a multifactorial effect on the body [20–22]. The nerve growth factor plays a key role in the regulation of regeneration processes. That is due to its influence on the mechanisms of maintaining homeostasis, inflammation, proliferation and tissue remodeling. The ability of the nerve growth factor to induce the release of immunoactive neuropeptides and neurotransmitters, as well as to influence innate and adaptive immune responses, has been proven [20, 21]. It should be notified that the level of the brain neurotrophic factor expression reflects the treatment effectiveness of hypoxic-ischemic, traumatic and toxic lesions of the central nervous system [22]. It has also been established that the level of BDNF serum has a negative correlation with the severity of anxiety disorders and even determines the development of neurodegenerative processes in some cases [23, 24].

Thus, apoptotic and neurotrophic factors play the role of active participants in the implementation of adaptive mechanisms to stress effects of various origins and determine the prospects of considering them as a target for pharmacological agents with a stress-protective activity [24].

Currently, neuropeptide compounds with a versatile pharmacological activity, including a stress-protective activity, are of particular interest [25]. A large number of highly effective and safe drugs are synthesized based on neuropeptides [26]. It should be notified that neuropeptides are capable of penetrating the blood-brain barrier and exerting a pharmacological effect at minimal concentrations [27]. Being modulators of physiological processes, peptide preparations are able to control the expression of cellular messengers and cytokines, thereby influencing the initiation of apoptotic processes in the nervous system and performing the antiapoptotic protection function [28]. To date, in a series of peptide compounds, regulatory peptides of a glyproline nature have been isolated as a separate group [29]. Its most significant representative is the registered drug Selank (Trademark No. 199370), synthesized by scientists of the Institute of Molecular Genetics of the National Research Center "Kurchatov Institute" by a merger with Pro–Gly–Pro to the C-termini

of tuftsin, originally used as an immunomodulator [30]. In practical medicine, Selank is used to improve mnestic functions [31], providing antiasthenic, adaptogenic, antihypoxic [32] and actoprotective effects [33]. Currently, domestic scientists from leading scientific organizations are studying in detail the pharmacological action of glyprolines [27–35]. It has been established that this class of peptides is able to prevent atherosclerotic processes and reduce thrombus formation by activating the fibrinolytic and anticoagulant mechanisms [33]. The studies have shown that glyproline peptides have a hepatotropic effect [34]. The results of a number of the experimental data have demonstrated hypoglycemic and hypolipidemic effects of these compounds [27]. The Pro-Gly-Pro tripeptide itself, which has a pronounced physiological activity, is of considerable interest from the perspective of a promising therapeutic agent [30]. Numerous works have established that the uniqueness of glyproline neuropeptides lies in their pleiotropy, i. e., in the combination of psycho- [25], neuro- [35], nootropic effects [26]. The presence of the immunotropic activity of glyprolines has been proven [36]. It is confirmed by their participation in the induction of various neurotrophic factors, pro- and anti-inflammatory cytokines, and the regulation of apoptosis processes [34, 35]. The properties described above actualize the need for a detailed study of the pharmacological action of the neuropeptides of the glyproline structure.

THE AIM of the article was to study the effect of glyproline neuropeptide compounds Thr–Lys–Pro–Arg–Pro–Gly–Pro (Selank), Pro–Gly-Pro and Pro–Gly–Pro–Leu, on the level of apoptotic factors (caspase-3, caspase-8, the tumor necrosis factor) and neurotrophic factors (the nerve growth factor and the brain neurotrophic factor) in the blood serum of white rats under experimental modeling of "social" stress.

MATERIALS AND METHODS

Laboratory research

The experimental studies were carried out on 90 white male rats aged 6 months, obtained from the vivarium of the laboratory of physiology, morphology, genetics and biomedicine of Astrakhan State Medical University (Russia, Astrakhan). Keeping laboratory animals met the requirements of regulatory documents[1,2,3]. The experiment was carried out on the basis of the protocol of the Ethics Committee of Astrakhan State Medical University No. 8 dated November 24, 2015.

Experimental model

The model of "social" stress was implemented by providing living arrangements of rats where there is a sensory contact and no physical contact, followed by the formation of aggressive and submissive types of behavior [35]; herewith, the animals were placed in pairs in the cages separated by a transparent partition. In order to observe inter-male confrontations, the partition was removed daily for 10 minutes, according to the results of which the groups of rats "aggressors" and rats "victims" were formed. In the experimental animals, the manifestation of aggression was expressed in the forms of upright and sideways offensive postures – "threat" or attack. In addition, submissiveness was manifested by various acts of individual behavior: immobility, sniffing, autogrooming, and upright defensive postures [37–39].

Experimental groups

In the study, the following experimental groups (n=10) were formed: control individuals; groups of the rats exposed to stress for 20 days; groups of the animals treated intraperitoneally at the dose of 100 μg/kg/day, starting from the 1st day of the stress factor exposure, with a course of 20 days of glyproline compounds Thr–Lys–Pro–Arg–Pro–Gly–Pro (Selank), Pro–Gly–Pro and Pro–Gly–Pro–Leu.

The choice of the glyproline compounds dose was based on a preliminary study of the severity of the psychomodulatory effect by assessing behavioral reactions using psychopharmacological settings. The studies were carried out with the administration of glyproline compounds at the doses of 25, 50, 100, and 200 μg/kg/day. It was found out that glyprolines were most active at the doses of 100 and 200 μg/kg/day. In this connection, subsequently, the lowest experimental dose of 100 μg/kg/day was chosen.

Methods

The effect of neuropeptides of the glyproline structure on the level of caspase-3, caspase-8, the tumor necrosis factor, the brain neurotrophic factor and the nerve growth factor in the blood serum of white rats was assessed by the method of enzyme-linked immunosorbent assay using an immunological analyzer "Multiscan FC" and a highly sensitive ELISA Kit for Caspase -8 (USA); ELISA Kit for Caspase-3 (USA); ELISA Kit for Tumor Necrosis Factor Alpha (TNF-α) (USA), ELISA Kit for Brain Derived Neurotrophic Factor (BDNF) (USA); ELISA Kit for Nerve Growth Factor (NGF) (USA). Before use, the serum was kept at room temperature for two hours, centrifuged for 20 minutes at 1000 rpm, and then immediately subjected to analysis. The choice of this type of biological material was made, based on the analysis of the literature data [40, 41].

[1] Directive of the European Parliament and of the Council of the European Union on the protection of animals used for scientific purposes (2010/63/EU). Saint Petersburg, 2012. – 50 pp. Russian.
[2] The International Convention for the Protection of Vertebrate Animals used for Experimental and Scientific Purposes (Strasbourg, 1986). Russain.
[3] Order of the Ministry of Health of the Russian Federation No. 199n dated 01.04.2016. "On the approval of the Rules of laboratory practice".

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Statistical processing of results

Statistical processing of the research results was carried out using the software packages Microsoft Office Excel 2007, BIOSTAT 2008 Professional 5.8.4.3 taking into account the Mann-Whitney criterion. The differences were considered statistically significant at p≤0.05.

RESULTS AND DISCUSSION

The results reflecting the effect of glyprolines on the level of caspases-3 and 8 in the blood serum of white rats under conditions of "social" stress are presented in Table 1.

While the formation of "social" stress in the animals with an aggressive type of behavior was taking place, the level of caspase-3 increased by 1.8 times (p≤0.01) in relation to the control. While the introduction of glyproline neuropeptide compounds was taking place, a decrease in the studied indicator was notified: with Thr–Lys–Pro–Arg–Pro–Gly–Pro (Selank) – by 1.6 times (p≤0.01), with Pro–Gly–Pro – by 1.5 times (p≤0.01) and with Pro–Gly–Pro–Leu – by 1.3 times (p≤0.01) in comparison with the stress group.

In the group of the stressed animals with a submissive type of behavior, the level of caspase-3 increased by 60% (p≤0.01) in relation to the control group. With the administration of Thr–Lys–Pro–Arg–Pro–Gly–Pro (Selank), there was a decrease in the indicator by 50% (p≤0.01), with Pro–Gly–Pro–by 29% (p≥0.05) and with Pro–Gly–Pro–Leu – by 10% (p≥0.05) in comparison with the "social" stress group.

The formation of "social" stress led to an increase in the level of caspase-8 by 2.6 times (p≤0.01) in comparison with the control group of rats. The administration of glyproline compounds Thr–Lys–Pro–Arg–Pro–Gly–Pro, Pro–Gly–Pro and Pro–Gly–Pro–Leu contributed to a decrease in this indicator by 2.2 times (p≤0.01), by 1, 7 (p≤0.01) and by 1.5 times (p≥0.01), respectively, in relation to the group of the stressed animals.

The level of caspase-8 in the group of the stressed animals with a submissive type of behavior increased by 2.4 times (p≤0.01) in relation to the intact animals. The administration of glyproline neuropeptide compounds Thr–Lys–Pro–Arg–Pro–Gly–Pro (Selank), Pro–Gly–Pro and Pro–Gly–Pro–Leu contributed to a decrease in the level of the studied indicator by 1.9 (p≤0.01), by 2.4 (p≤0.01) and by 1.3 times (p≥0.01), respectively, compared with the "social" stress group.

Table 2 presents the results reflecting the effect of neuropeptides of the glyproline structure on the level of TNF-α in the serum of white rats under conditions of "social" stress.

The formation of "social" stress in the animals with an aggressive type of behavior led to an increase in the level of the tumor necrosis factor by 45% (p≤0.01) in comparison with the control group. Against the background of the administration of glyproline compounds Thr–Lys–Pro–Arg–Pro–Gly–Pro (Selank), Pro–Gly–Pro and Pro–Gly–Pro–Leu, a decrease by 30% (p≤0.01), by 25% (p≤0.01) and by 22% (p≤0.05) was observed, respectively, in relation to the group of stressed individuals.

In the group of rats with a submissive type of behavior, during the stress formation, the level of TNF-α increased by 52% (p≤0.01) in comparison with the control. The compounds Thr–Lys–Pro–Arg–Pro–Gly–Pro (Selank), Pro–Gly–Pro and Pro–Gly–Pro–Leu caused a decrease in this indicator by 33% (p≤0.01), by 22% (p≤0.05) and by 23% (p≤0.05) in relation to the "social" stress group.

Fig. 1 shows the results reflecting the effect of glyprolines on the NGF serum level of white rats under "social" stress.

In the group of animals with a "social" stress type of behavior and aggressive ones, a decrease in the NGF level by 40% (p≤0.01) was observed in comparison with intact rats. The administration of glyprolines Thr–Lys–Pro–Arg–Pro–Gly–Pro (Selank), Pro–Gly–Pro and Pro–Gly–Pro–Leu increased the level of the studied factor by 40% (p≤0.01); by 20% (p≤0.05) and by 17% (p≤0.05), respectively, compared with the "social" stress group.

The formation of "social" stress in the group of animals with a submissive type of behavior led to a decrease in the NGF level by more than 30% (p≤0.01) in comparison with the control group. Against the background of the glyprolines administration (Thr–Lys–Pro–Arg–Pro–Gly–Pro (Selank), Pro–Gly–Pro and Pro–Gly–Pro–Leu), an increase in the level of the nerve growth factor by 56% (p≤0,01), by 36% (p≤0.01) and by 29% (p≤0.01), respectively, compared with the "social" stress group, was notified.

Fig. 2 shows the results reflecting the effect of glyprolines on the level of the neurotrophic BDNF factor in the blood serum of white rats under the conditions of "social" stress.

In the group of stressed animals with an aggressive type of behavior, a decrease in the level of the brain neurotrophic factor by 40% (p≤0.01) in comparison with the control group was notified. The Thr–Lys–Pro–Arg–Pro–Gly–Pro (Selank), Pro–Gly–Pro and Pro–Gly–Pro–Leu compounds increased the level of the brain neurotrophic factor relative to the stressed group of the animals by 45% (p≤0, 01); by 26% (p≤0.05) and by 24% (p≤0.05), respectively.

In the group of stressed rats with a submissive type of behavior, a 45% decrease in the BDNF level (p≤0.01) was notified in comparison with control animals.

With the administration of glyproline compounds, the changes in the level of the studied neurotrophic factor were also notified in the form of a statistically significant increase (p≤0.01): against the background of Thr–Lys–Pro–Arg–Pro–Gly–Pro (Selank) – by 52%, Pro–Gly–Pro – by 35% and Pro–Gly–Pro–Leu – by 32% in relation to the group of the animals exposed to "social" stress.

Table 1 – The level of caspase-3 and caspase-8 in the blood serum of white rats under conditions of experimental "social" stress influenced by neuropeptides of the glyproline structure

Groups of experimental animals	Caspase 3 (pg/ml)	Caspase 8 (pg/ml)
Control	17,41±1,22	2,33±0,91
Animals with aggressive behavior		
"Social" stress	30,62±2,13**	6,14±1,21**
"Social" stress + Thr–Lys–Pro–Arg–Pro–Gly–Pro (Selank)	19,22±2,01##	2,78±0,76##
"Social" stress + Pro–Gly–Pro	20,61±2,23##	3,65±0,56#
"Social" stress + Pro–Gly–Pro–Leu	23,76±2,14##	3,98±0,82
Animals with submissive behavior		
"Social" stress	27,83±2,21**	5,64±0,87**
"Social" stress + Thr–Lys–Pro–Arg–Pro–Gly–Pro (Selank)	18,67±1,21##	2,96±0,89#
"Social" stress + Pro–Gly–Pro	22,57±2,13	2,36±0,81##
"Social" stress + Pro–Gly–Pro–Leu	25,43±2,87	4,36±0,99

Note: ** – p≤0.01 – relative to control; #; ## – p≤0.05; p≤0.01 – relative to the "social" stress group.

Table 2 – The level of TNF-α in the blood serum of white rats under conditions of experimental "social" stress influenced by neuropeptides of the glyproline structure

Experimental groups of animals	TNF-α (pg/ml)
Control	78.65±6.8
Animals with aggressive behavior	
"Social" stress	113.83±8.2**
"Social" stress + Thr–Lys–Pro–Arg–Pro–Gly–Pro (Selank)	79.32±6.8##
"Social" stress + Pro–Gly–Pro	85.60±8.1##
"Social" stress + Pro–Gly–Pro–Leu	88.77±7.4#
Animals with submissive behavior	
"Social" stress	119.35±7.8**
"Social" stress + Thr–Lys–Pro–Arg–Pro–Gly–Pro (Selank)	79.87±6.5##
"Social" stress + Pro–Gly–Pro	93.15±8.6#
"Social" stress + Pro–Gly–Pro–Leu	91.77±8.2#

Note: *; ** – p≤0.05; p≤0.01 – relative to control; #; ## – p≤0.05; p≤0.01 – relative to the "social" stress group.

DISCUSSION

In the course of this study it was established that "social" stress is accompanied by a decrease in the level of the brain neurotrophic factor and the nerve growth factor, which is associated with a change in neuroplasticity with a subsequent inhibition of neurogenesis. A number of experimental studies have shown that BDNF has pronounced neuroprotective properties, contributing to the inhibition of cell apoptosis, preventing, in turn, the neuronal death and stimulating the growth of cholinergic nerve fibers [42, 43]. It has been found out that under the conditions of "social" stress, alongside with a decrease in the levels of neurotrophic factors, there is an increase in the levels of caspase-3 and caspase-8, as well as the tumor necrosis factor in the blood serum of white rats. According to the literature data [44, 45], such a variability of these indicators shows an increase in apoptotic processes.

The essential role of neurotrophic factors in the induction or inhibition of apoptosis has been proven in other experimental studies. It has been established that NGF inhibits apoptosis in a number of neurodegenerative diseases [20]. In addition, it has been proven that the nerve growth factor and the brain neurotrophic factor implement their action both directly and through the genetic mechanisms of induction of apoptotic processes [5]. In contrast, a number of cytokines, in particular human interferons and a tumor necrosis factor, presumably have a stimulating effect on apoptosis [9]. That had also been confirmed in the authors' experiments before [36].

A decrease in the expression of neurotrophic fac-

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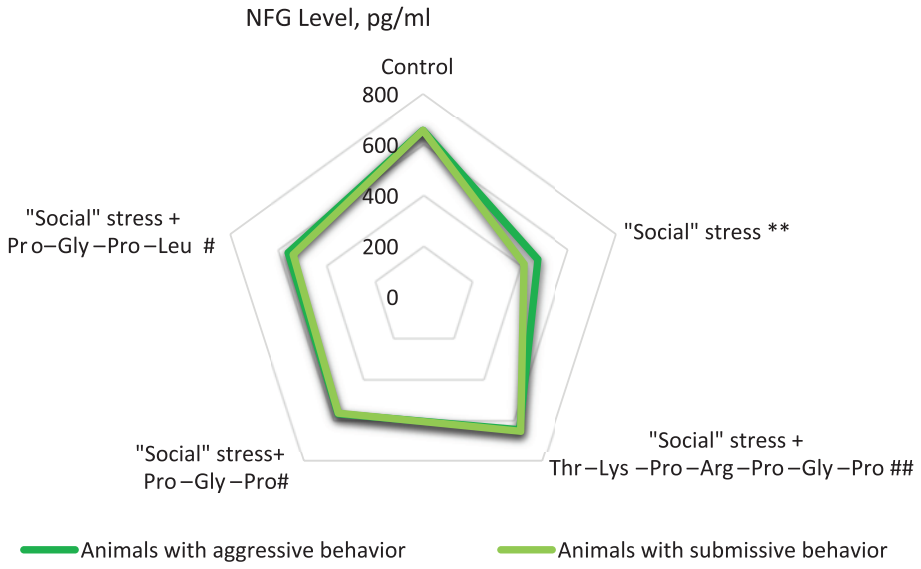

Figure 1 – The nerve growth factor level in the blood serum of white rats under conditions of experimental "social" stress influenced by neuropeptides of the glyproline structure

Note: ** – p≤0.01 – relative to control; #; ## – p≤0.05; p≤0.01 – relative to the "social" stress group.

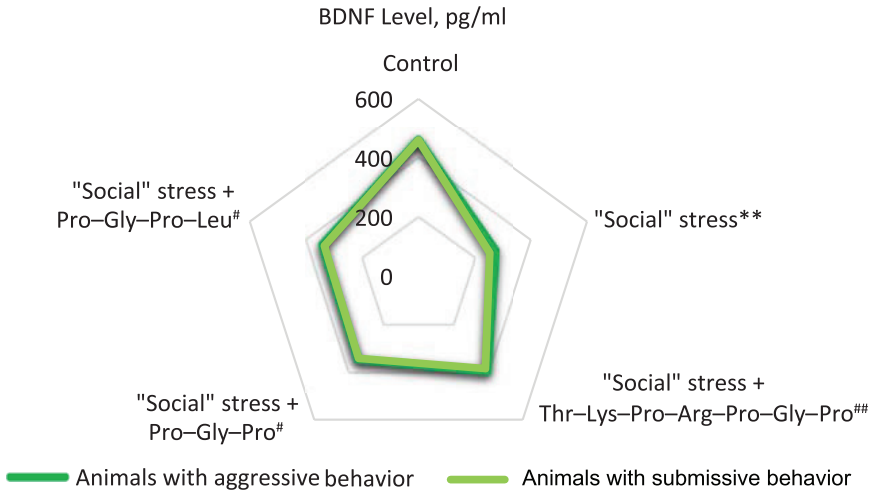

Figure 2 – The brain neurotrophic factor level in the blood serum of white rats under conditions of experimental "social" stress influenced by neuropeptides of the glyproline structure

Note: ** – p≤0.01 – relative to control; # – p≤0.05 relative to the "social stress" group; ## – p≤0.01 – relative to the "social stress" group.

tors as a result of stressful influences of various kinds of nature and the restoration of its level by a prolonged administration of corrective agents, led to the creation of a neurotrophic hypothesis for the development of stress-induced depression. According to this concept, a change in the level of neurotrophic factors is a key mechanism for the formation and development of approaches to the treatment of such disorders [17]. This fact is confirmed by the established decrease in the level of the nerve growth factor and the brain neurotrophic factor during the formation of a depressive state, and by an increase in the process of pharmacotherapy, as well as a positive correlation of the levels with the degree of the state improvement [7]. It has been proven that the effectiveness of antidepressant and stress-protective therapy is achieved due to the effect of drugs on the intensity of neurogenesis and neuronal plasticity [24]. According to the literature data [25], the established corrective activity of glyproline neuropeptides in relation to the level of neurotrophic factors under a "social" stress, indicates the manifestation of Thr–Lys–Pro–Arg–Pro–Gly–Pro (Selank), Pro–Gly–Pro and Pro–Gly–Pro–Leu expressed anti-stress and neuroprotective effects.

Alongside with this, it has been established that the administration of glyproline neuropeptide compounds Thr–Lys–Pro–Arg–Pro–Gly–Pro (Selank), Pro–Gly–Pro and Pro–Gly–Pro–Leu against the background of "social" stress contributes to a decrease in the level of apoptotic indicators - capsase-3, caspase-8 and the tumor necrosis factor. This is mediated by the possible inhibition of the caspase-dependent cascade of reactions to the destruction of cellular structures by

hydrolysis of the nuclear lamina, cleavage of adhesive proteins and a destruction of the cytoskeleton. In the case of a caspase-dependent pathway, the signal for the onset of the programmed death of neurocytes is pathogenetic pathways formed upon the exposure to hypoxia, the agents of various kinds of nature (stressful, physical or chemical, etc.) [10, 11]. This pathway, alongside with caspases, is realized due to the binding of the tumor necrosis factor to receptors on the target cell membrane. Previously, the presence of the antioxidant action and the ability of neuropeptides to influence the level of pro- and anti-inflammatory cytokines have been proven [46]. It has been established that under the conditions of "social" stress, neuropeptide compounds induce a pronounced inhibition of free radical oxidation processes and reduce the concentration of proinflammatory cytokines such as IL-1β, IL-6, and TNF-α [46, 47]. Based on the results obtained, it can be concluded that glyprolines have an anti-apoptotic action due to the effect on the level of caspases, the concentration of pro-inflammatory cytokines and the inhibition of lipid peroxidation processes.

The regulation of apoptotic and neurotrophic processes is complex; it involves various cytokines within a large number of signaling cascades, which requires a further detailed study [48, 49].

CONCLUSION

Thus, at present, focused attention is paid to assessing the role of apoptotic and neurotrophic factors in the implementation of the stress response. In this connection, an effector caspase-3 and an initiating caspase-8, a tumor necrosis factor, as well as neurotrophic factors (the nerve growth factor and the brain neurotrophic factor), are actively studied as targets for the action of stress-protective drugs of a neuropeptide structure in various pathological conditions, including those caused by a prolonged exposure to stress factors. The carried-out study has established the presence of an antiapoptotic activity in Thr–Lys–Pro–Arg–Pro–Gly–Pro (Selank), Pro–Gly–Pro and Pro–Gly–Pro–Leu due to the inhibition of the caspase-dependent cascade of apoptosis reactions. Alongside with this, a pronounced stress-protective effect has been determined due to the restoration of the level of brain neurotrophic factors. The obtained results actualize a further detailed study of the caspase-dependent and neurotrophic factors-mediated mechanism of the anti-stress effect of glyprolines.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

Anna L. Yasenyavskaya – collecting literature data, text writing, experiment setting up, the results obtained analysis, preparing a draft manuscript; Alexandra A. Tsibizova – collecting literature data, experiment setting up, the results evaluating, substantiating and statistically processing of the data obtained, preparing a draft manuscript; Liudmila A. Andreeva – synthesis of compounds, research planning, manuscript editing, results evaluating; Nikolai F. Myasoedov – synthesis of compounds, research planning, manuscript editing, results evaluating; final approval for manuscript publication; Ol'ga A. Bashkina – research planning, manuscript editing, results evaluating, final approval for the manuscript publication; Marina A. Samotrueva – concept development and study design, study planning, experiment setting up, critical intellectual content reviewing, and final approval for manuscript publication.

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ОРИГИНАЛЬНАЯ СТАТЬЯ

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ANALYSIS OF THE PERSONNEL'S CONSISTENT READINESS FOR CHANGES AS ILLUSTRATED BY THE EXAMPLE OF AN EXPORT-ORIENTED BIOTECHNOLOGICAL ENTERPRISE

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The biotechnology industry is currently one of the most dynamically developing sectors of the pharmaceutical industry, that is why it requires improvement in the personnel management system aimed at increasing the flexibility and adaptability of the organization.

The aim of the research is to determine the degree of readiness of the organization's employees for innovations as illustrated by the example of an export-oriented enterprise.

Materials and methods. The source information was collected from the employees of the biotechnological enterprise through a questionnaire survey. The representative sample included 588 respondents. The statistical processing of data was carried out using the specialized software IBM SPSS STATISTICS (IBM, USA, 2017). The consistent readiness of the organization's employees for innovations was determined using I.O. Zagashev's methods. To assess the reliability of the psychological test, an internal consistency model with Cronbach's alpha was applied. Statistical hypotheses were tested by comparing the central tendencies of two independent samples using Student's t-test and the Mann–Whitney nonparametric test.

Results. The distribution results of key motivating factors for personnel showed that motivating factors such as an adequate salary and sustainable employment took the leading positions. However, the assessment of the employees' consistent readiness for innovations according to I.O. Zagashev's methods shows a high degree of the personnel's readiness for changes due to positive emotional perception of any innovations.

Conclusion. The results obtained make it possible to arrive at the conclusion that the established team favorably responds to all innovations, and is ready to support them in the future being aware of the organization's desire for innovations. In the future, the results will be used to determine the required management functions and goals and to develop the personnel management strategy in the context of the knowledge transfer, technology and export policy of the pharmaceutical enterprise.

Keywords: personnel management; motivation; biotechnological company; export; immunobiologicals

Abbreviations: VHI – voluntary health insurance

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АНАЛИЗ СИСТЕМНОЙ ГОТОВНОСТИ ПЕРСОНАЛА К ИЗМЕНЕНИЯМ НА ПРИМЕРЕ ЭКСПОРТНО ОРИЕНТИРОВАННОГО БИОТЕХНОЛОГИЧЕСКОГО ПРЕДПРИЯТИЯ

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Биотехнологическая промышленность на сегодняшний день является одним из наиболее динамично развивающихся секторов фармацевтической отрасли, поэтому требует совершенствования системы управления персоналом, направленной на повышение гибкости и адаптивности организации.

Цель. Определение степени готовности сотрудников организации к инновациям на примере экспортно ориентированного биотехнологического предприятия.

Материалы и методы. Первичная информация была собрана методом анкетирования сотрудников биотехнологического предприятия. Репрезентативная выборка составила 588 респондентов. Статистическая обработка данных проводилась с использованием специализированной программы IBM SPSS STATISTICS (IBM, USA, 2017). В определении системной готовности сотрудников организации к инновациям использовалась методика И.О. Загашева. С целью оценки надежности психологического теста применялась модель внутренней согласованности с использованием коэффициента Альфа Кронбаха. Проверка статистических гипотез в рамках сравнения центральных тенденций двух независимых выборок проводилась с использованием критерия Стьюдента и непараметрического метода Манна-Уитни.

Результаты. Результаты распределения ключевых мотивирующих факторов для персонала показали, что лидирующие позиции заняли такие мотивационные факторы, как достойная заработная плата и стабильная работа, однако оценка системной готовности сотрудников к инновациям по методике И.О. Загашева указывает на высокую степень готовности персонала к изменениям на основе позитивного эмоционального восприятия всего нового.

Заключение. Полученные результаты позволяют сделать вывод о том, что сложившийся коллектив хорошо воспринимает все нововведения и готов поддерживать их в дальнейшем в условиях осознания стремления организации к новшествам. Полученные результаты в дальнейшем позволят определить требуемые управленческие функции и цели, а также разработать стратегию управления персоналом в условиях трансфера знаний, технологий и экспортной политики фармацевтического предприятия.

Ключевые слова: управление персоналом; мотивация; биотехнологическая компания; экспорт; иммунобиологические лекарственные препараты

INTRODUCTION

As a part of the state policy of the Russian Federation for the development of the pharmaceutical industry, one of the key tasks is to create an export-oriented potential and increase the volume of exports of pharmaceutical products to foreign markets [1, 2]. Today, many Russian pharmaceutical companies are already focused on the international cooperation, Russian companies are increasingly opening their branches and subdivisions abroad, while the J07 group “Vaccines” occupies a leading position of finished medicinal products in export volumes from Russia [3]. It should be notified that since 2013, the Saint Petersburg Research Institute of Vaccines and Sera and Manufacturer of Bacterial Preparations has been actively developing a project to introduce Russian immunobiological products and relevant production technologies in the markets of Central and Latin America. And since 2015, the Russian Federation has been implementing a project to create a joint Russian-Nicaraguan venture – Instituto Latinoamericano de Biotecnología MECHNIKOV, S.A., which will meet the needs of the Latin American region in vaccines, currently being acquired by the Pan American Health Organization Revolving Fund [1, 4].

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However, at present, many Russian companies with foreign subdivisions face a number of problems associated not only with mastering improved technology, creating a new product required by the market in the region of presence, but also with improving organizational structures. According to the statistics, 70-80% of innovations are not implemented in organizations due to a failure to improve the organization's Management of Change System, including personnel recruitment and management. As illustrated by the example of Saint Petersburg Research Institute of Vaccines and Sera and Manufacturer of Bacterial Preparations, which is a parent company under the project above, a number of problems arise. The analysis of the Nicaraguan labor market shows a lack of specialists with necessary qualifications, therefore, in addition to the technology transfer, another key task is the transfer of knowledge [4]. Lack of professional competencies in a subsidiary's evolving team requires maximum strength from the parent company in performing its key management functions – planning, organization and control in the host environment and motivating the personnel by Saint Petersburg Research Institute of Vaccines and Sera and Manufacturer of Bacterial Preparations. A high level of the employees' resistance to changes due to assignment of new job functions should be taken into account. In this context, the personnel management needs a specific approach and adaptation of existing experience to the organizational changes. Considering the foregoing, the improvement of the personnel management system aimed at increasing the organization's flexibility and adaptability is a key vector in such projects.

For the purpose of competent planning of management mechanisms, at the first stage, it is advisable to timely assess the current state and determine the degree of readiness of the organization's employees for changes as illustrated by the example of an export-oriented biotechnological enterprise Saint Petersburg-Research Institute of Vaccines and Sera and Manufacturer of Bacterial Preparations, which was **THE AIM** of this study**.**

MATERIALS AND METHODS

The source information was collected from the employees of Saint Petersburg–Research Institute of Vaccines and Sera and Manufacturer of Bacterial Preparations through a questionnaire survey in March 2021. The representative sample included 588 respondents.

The worked out questionnaire consisted of an introduction, personal details that would depict a respondent's personality, and the main part structured in 2 blocks. In the first block of 14 proposed factors motivating an employee to work, the respondent was asked to rate them in the order of increasing importance. The second block of questions was aimed at determining the employees' consistent readiness for innovation using I.O. Zagashev's methods, which included 56 statements encouraging the respondents to express their attitudes on a 4-point scale. The methods analyzes 7 aspects, such as readiness to follow the leader, readiness due to a material reward, readiness due to an opportunity to take responsibility for innovation, readiness in the context of personal and professional self-realization, readiness in the absence of major changes, readiness based on the past experience, readiness due to positive emotional perception of novelties [5].

Statistical data analysis

The statistical processing of data was carried out using the specialized software IBM SPSS STATISTICS (IBM, USA, 2017). In order to assess the reliability of the psychological test according to I.O. Zagashev's methods an internal consistency model using Cronbach's alpha was applied, which was based on the scale homogeneity and calculated as the sum of correlations between respondents' answers to the questions within the same aspect [6].

To test statistical hypotheses, within the framework of comparing central tendencies of two independent samples, Student's test (*t*-test) and the Mann-Whitney nonparametric test (*U*-test) were used. The differences were considered significant at $p<0.05$.

RESULTS

The survey involved respondents aged from 21 to 73, the average age was 38 years, they were mostly women (63%). According to the survey results, the distribution of respondents burdened and not burdened with family obligations, was 57% and 43%, respectively, which may indicate a sufficient contingent of employees with a stable approach to work and those who are ready to assume additional job responsibilities, are interested in business trips and mastering new competencies. The sample of respondents was represented by specialists (46%), workers (36%) and managers (20%). The income level is mostly represented by the categories of 30,000-50,000 RUB. and 50,000–75,000 RUB. The distribution of respondents by divisions showed that, according to the current organizational structure, 65% of the employees belong to the production and technical division, 20% – to the support division, 10% – to the development division, and 5% represent other areas. The distribution of respondents by work experience indicates the prevalence of those with 1 to 6 years of experience (49%), emphasizing a significant share of employees with work experience of up to 1 year (20%), which is explained by

a high pace of the organization's development and, consequently, the need for new employees. It should be notified that the company has employees with experience of more than 15 years, who may be involved in guidance and mentorship. It is also important-to notify that at the time of the survey, a significant proportion (57%) of the employees were actively engaged in the implementation of the above said project. Herewith, it is noteworthy that almost half of the personnel (49%) not involved in the project, showed interest in the innovations implemented in the organization and expressed their desire to take part in them.

Given the fact that the development of innovative processes in the organization requires an employee to acquire additional new competencies and skills and be ready for changes. The second stage of the study was the analysis of personnel's motivation, the results of which are advisable to be applied by the management in the process of organizing activities, so that each process participant strives to perform their professional duties as well as possible [7]. The incentive and motivation system in an organization is presently an important and efficient tool of personnel management, the efficiency of which depends on a multitude of factors including the communication system and the style of work of the organization's management. Modern incentive systems require proper development and correct use in practice [8].

The data analysis showed (Fig. 1) that the leading positions were taken by such motivating factors as an adequate salary, stable employment, interest in work, relationships in the team, comfortable conditions and opportunities for professional growth. The intangible motivation (a labor union activity, efficiency of VHI (voluntary health insurance) programs, corporate culture, team atmosphere, relationships with colleagues), training opportunities (improving and expanding the range of professional skills, learning foreign languages) were rated lower. Other factors (opportunities to work with foreign colleagues, abroad, working with professionals and the best scientists, relative discretion in performing duties, participation in the implementation of the company's strategy, development of leadership qualities, and achievement of leadership positions) did not overcome the 5% barrier. At the same time, attention should be paid to the low extent of participation in innovation projects, which points out at low staff awareness in terms of benefits for each participant of the innovation process. The results of the distribution of key motivating factors for personnel, depending on their participation in the project, indicate the prevalence of the same criteria that had been identified in the analysis by personnel categories (Fig. 2).

The development and implementation of readiness formation mechanisms of an organization to change, make it possible to enhance the efficiency of implementing innovative processes, and the precise assessment tools make it possible to identify the employees involved in the change, actively supporting, resisting or conservatively inclined employees, and then to develop action plans with various groups of employees [8, 9].

The analysis results of Saint Petersburg Research Institute of Vaccines and Sera and Manufacturer of Bacterial Preparations employees' consistent readiness for innovations, indicate a high degree of readiness due positive emotional perception of novelties and make it possible to arrive at the conclusion that the existing team favorably responds to all innovations and is ready to support them in the future being aware of the organization's desire for innovations (Fig. 3, 4). In principle, the employees perceive innovations well, and at the early stages of introducing an innovation, they will support it if they feel its novelty.

Moreover, the aspect of readiness in the context of personal and professional self-realization, especially among specialists, was a significant share. The employees are ready to support novelties and innovations if they are perceived as being associated with personal and professional self-realization and make them feel more competent at work.

Readiness due to a material reward occupies an insignificant share, in contrast to direct questionnaires, which showed a high share of an adequate salary as a motivating factor. Thus, an intermediate conclusion can be made that the methods discovers hidden motivational components of the staff's readiness for innovation.

The factor of readiness to follow the leader was better manifested among the working employees: an employee supports an organizational novelty, provided there is a leader who will offer explanations, accept responsibility and take control.

The aspect of readiness under the condition of being capable of accepting responsibility for an innovation is predominant among leaders, which speaks of highly manifested leadership qualities of the leaders who are able to support novelties that help them to fulfil themselves as leaders, to take responsibility for a matter. Readiness under the condition of there being no serious change is characteristic of the employees who are ready to support an innovation, if it does not, in their opinion, bring noticeable change in customary activities. Readiness on the basis of past experience, is characteristic of the leaders who support innovations, since they are related to the success of participating in innovations in the past.

The next stage of the study was to assess the reli-

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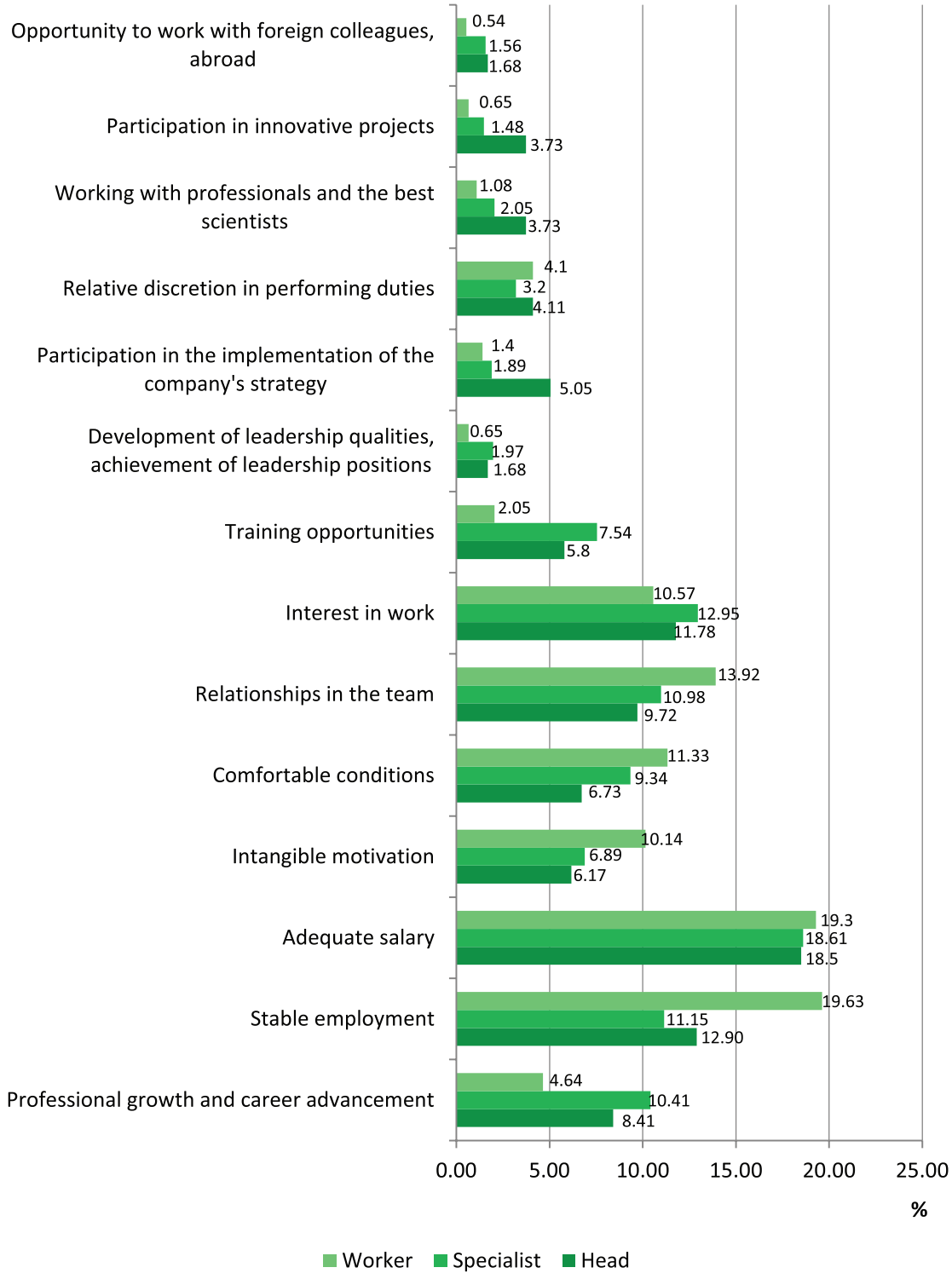

Figure 1 – Analysis of the personnel motivation system
Note: breakdown by personnel categories

ability of the psychological test using Cronbach's alpha test, which increases as the mutual correlations of variables grow, and reflects the internal consistency of the reliability assessment of test results.

As the obtained data show, for most aspects, the internal consistency coefficient has values close to "1", which indicates an acceptable and high reliability of the internal consistency for aspects No. 2, 3, 4, 5, 6 and the leading aspect No. 7 (Table 1). The comparison of central tendencies of two independent samples using Student's t test (t test) and the nonparametric Mann-Whitney test in most cases showed $p<0.05$, which indicates significant differences between distributions in the corresponding samples (specialist, manager, worker).

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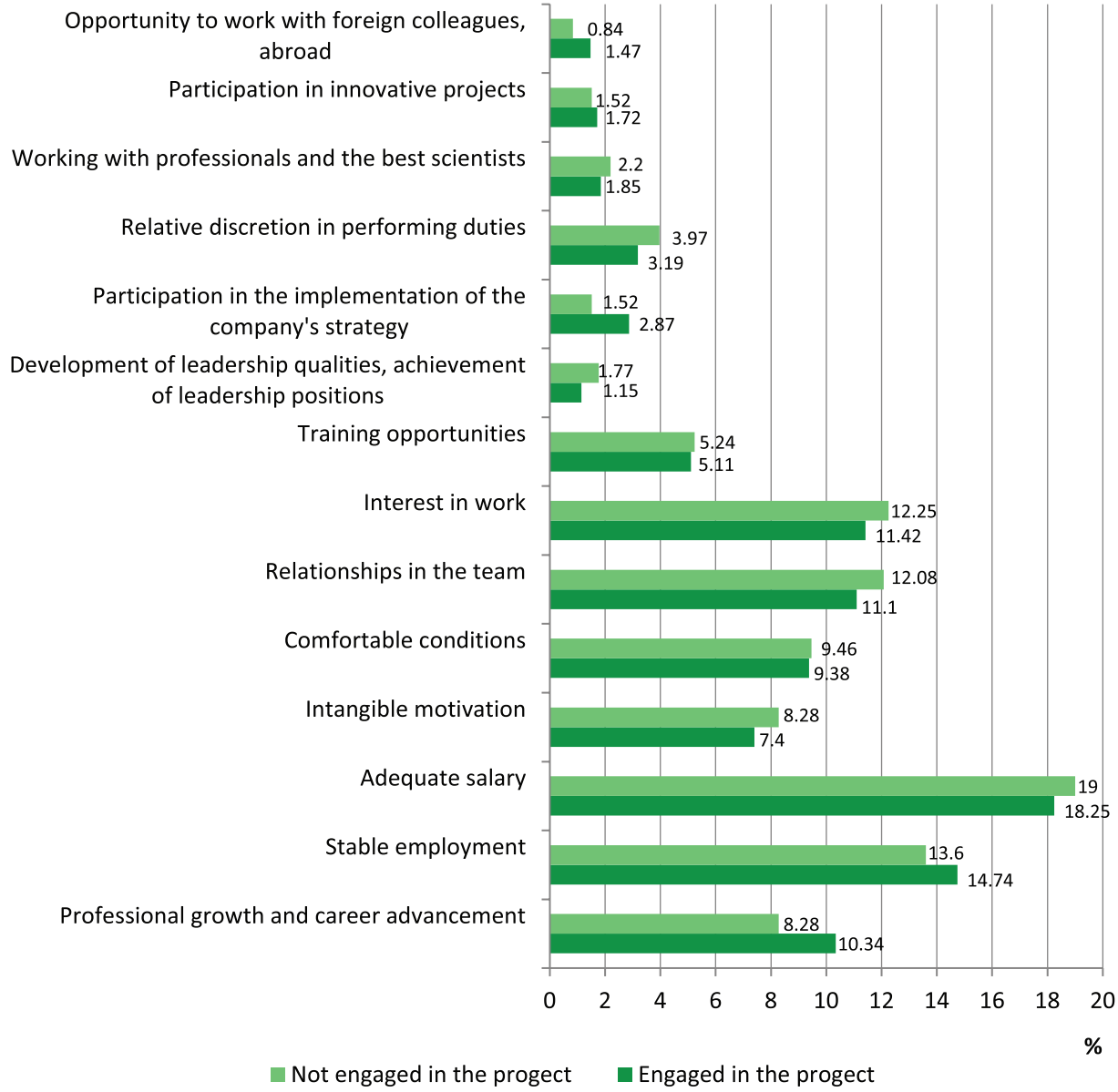

Figure 2 – Analysis of the personnel motivation system
Note: breakdown by project partition

DISCUSSION

At present, all pharmaceutical companies, including biotechnology ones, are subject to changes. In view of the above, the development of innovation activities and the executive managers' ability to use them efficiently in the administration process is relevant. According to a number of authors, almost all large companies are required to apply "auxiliary" projects in change management, with a mandatory assessment use of rational components of organisation's readiness and development of a change implementation plan [10].

Under the conditions of organisational changes, the key management functions include planning. This fact involves development of a future vision and preparation of necessary resources, the organisation providing for the establishment of a structured approach to transformation of systems and strategies, staffing aimed at providing the organisation with necessary human resources, leadership and control. It is important to notify that changes in the company management should be implemented at individual, group and system levels. Organisations should pay particular attention to human and organisational development measures in order to support their employees and create the organisational culture focused on development [11–14]. In order to maximise the changes acceptance at the individual level, models are widely used in practice today. Such a model presupposes a company employee's awareness of the need for change, their willingness to participate in it and support the change, their knowledge of ensuing changes as well

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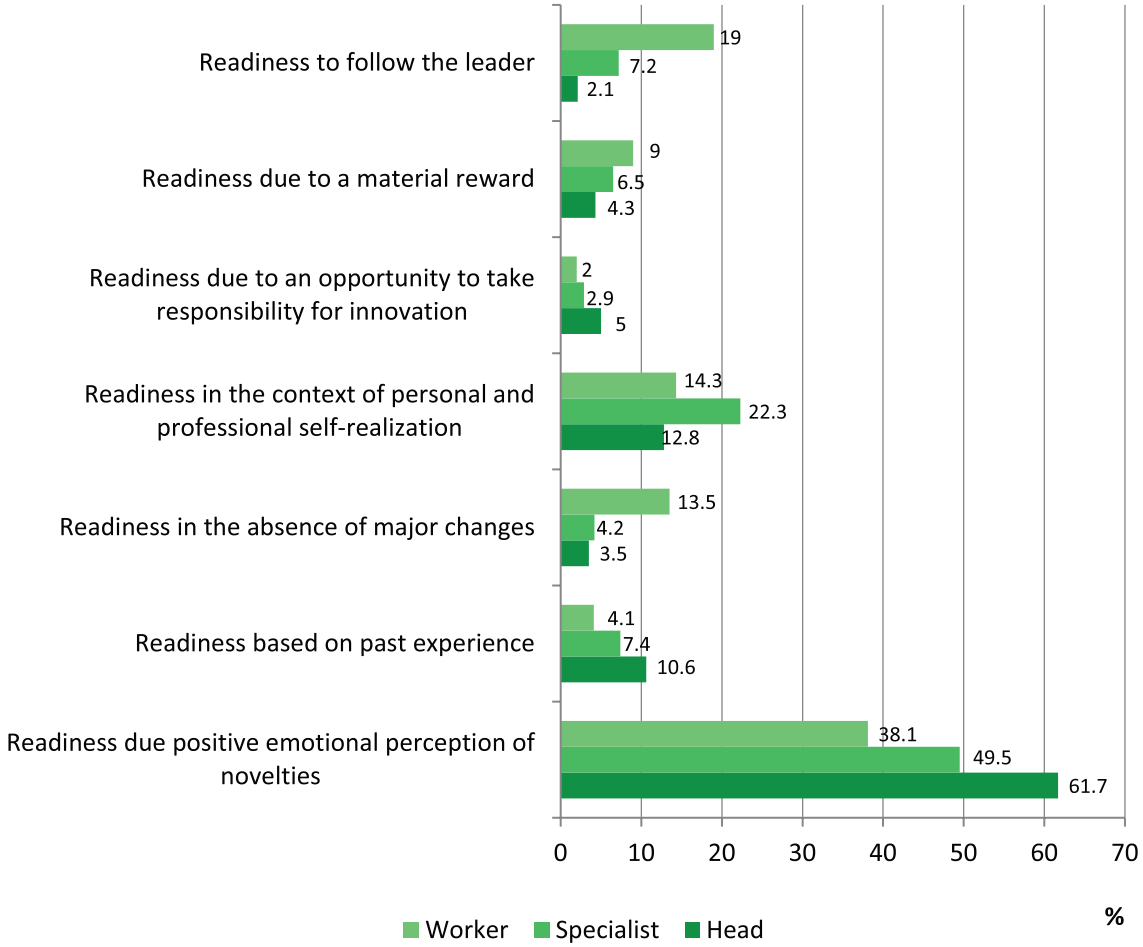

Figure 3 – The staff's consistent readiness for innovation
Note: breakdown by personnel categories

as subsequent benefits, risks and consequences of the changes implementation, as well as the ability to implement these changes. Managing innovations implementation requires modifying the management style, which needs to be adapted, defining the types of mechanisms to be involved, distributing roles and job responsibilities.

Today, innovation activities are understood, inter alia, as targeted changes in the system of interaction within an organisation, which promotes its performance. The management of changes and innovations is becoming an important administration objective, not only to ensure the organisation survival, but also to achieve sustainable success [15]. In order to achieve this goal, the primary objective is change implementation diagnostics; its results help to assess the company's readiness for changes, to identify resources and develop a strategy. The inability to assess readiness for organisational and individual changes may result in expenditures of the organisation, including time expenditures on combating resistance to change. When assessing a company's readiness for changes, it is necessary to consider the human factor, to maximise the use of the situational approach and a system factor analysis, to actively develop the training organisation concept, to apply mechanisms of self-organisation in management [10]. The company's readiness for changes depends on the degree the company's employees appreciate the changes, and whether they positively evaluate (and to what extent) the three key factors accounting for the possibility of realisation: goal-specific requirements, availability of resources and situational factors.

However, it should be notified that despite a rather high level of the development in this area, for the most part, the scholarly sources to date provide discrete practical methods for assessing organisational readiness for changes [16]. In addition, it is important to emphasise that there is not any coordinated concept of organisations' readiness for changes, while the readiness of company employees to innovations in terms of export-oriented biotechnology enterprises has not been studied so far.

In the framework of the research, it has been shown that at all levels, the role of managers is significant in the context of an increased risk in the course of realisation of

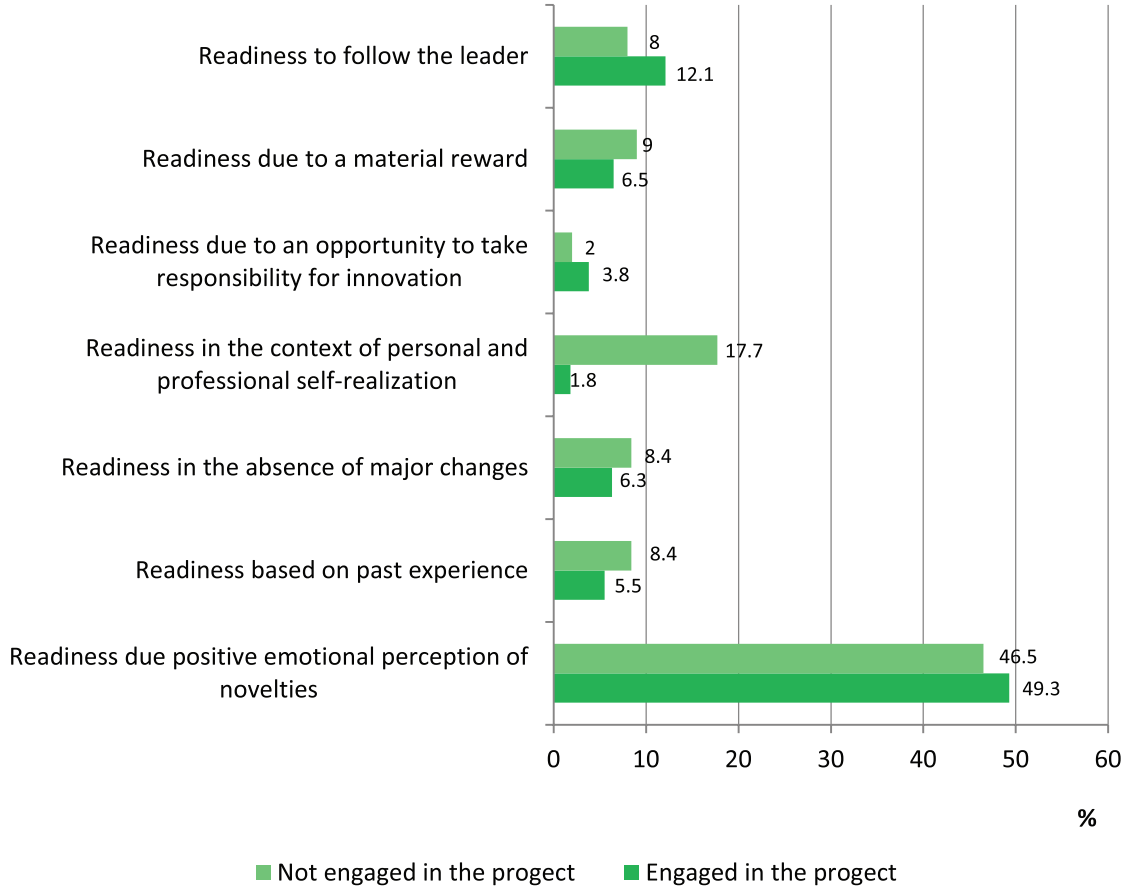

Figure 4 – The staff's consistent readiness for innovation
Note: breakdown by project partition

innovation processes that are usually related to change processes. These can be, for instance, increased uncertainty, an inflated level of stress, a high probability of failures. The obtained results confirm the idea expressed by a number of authors on the key role of leaders in the development of employees' creativity and innovations [15, 17]. It has been established that in order to implement any planned changes successfully, true leaders need to create readiness for changes in their employees, which, in turn, will develop their commitment to change and behavioural support for changes [18]. The leaders' role presupposes creation of readiness and strengthening of institutionalisation in respect of relatively large-scale organisational changes towards corporate sustainability [19]. Considering that the leadership behaviour contributes to the organisational identity at periods of uncertainty, it is recommended to shift the vector of managers' activities to such competencies as organisational change management, team management, development of communication skills [20, 21]. In addition, it should be notified that the manager's special attention should be aimed at the identification and subsequent motivation of change agents that serve as an organisation's transformation catalysts through support, development and control of practical innovation, take part in team building, provide intergroup interaction [22].

Under the conditions of organisational changes, human resource policy requires mandatory research of psychological readiness of the staff, which goes through specific emotional stages in the context of innovations implementation, such as denial and anger, bargaining, depression, revision and acceptance [23]. It has been proven in practice that the carried out organisational changes contribute to the development of psychosocial risks. As a consequence of it, many initiatives do not lead to the desired results [24]. Furthermore, when implementing a labour protection management system, the administration should consider possible risks [25].

A staff motivation commitment is defined as a perceived need for a change or its inevitability. The more the company members value the change, the more they wish to realise it. The labour motivation system study results obtained in the course of work over the subject in question, lead to an intermediate conclusion that both intrinsic and extrinsic employee motivation methods can be applied in the organisation, with the use of both persuasive and persevering management strategies. It should be notified that the given strategies are feasible

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Table 1 – Assessment of reliability of the psychological test according to I.O. Zagashev's methods

Aspect	Personnel category	X	p_t	p_u	σ	α	α_{gen}	N
No. 1 Readiness to follow the leader	worker	31.55	**/***	**/***	5.68	0.43	0.44	8
	specialist	30.27	*	*	4.74	0.46		
	manager	29.97	*	*	4.58	0.40		
No. 2 Readiness due to a material reward	worker	28.75	**/***	**/***	6.39	0.60	0.62	8
	specialist	27.25	*	*/***	6.02	0.66		
	manager	26.03	*	*/**	5.21	0.63		
No. 3 Readiness due to an opportunity to take responsibility for innovations	worker	25.66	**/***	**/***	8.14	0.79	0.78	8
	specialist	30.56	*/***	*/***	6.61	0.76		
	manager	34.66	*/**	*/**	5.17	0.74		
No. 4 Readiness in the context of personal and professional self-realization	worker	30.73	**/***	**/***	7.02	0.69	0.71	8
	specialist	35.25	*	*	5.74	0.72		
	manager	36.06	*	*	5.27	0.68		
No. 5 Readiness in the absence of major changes	worker	29.92	**/***	**/***	6.64	0.65	0.69	8
	specialist	26.25	*/***	*/***	6.18	0.65		
	manager	22.92	*/**	*/**	6.57	0.74		
No. 6 Readiness based on past experience	worker	24.53	**/***	**/***	7.94	0.83	0.84	8
	specialist	30.26	*/***	*/***	7.35	0.83		
	manager	34.95	*/**	*/**	6.38	0.84		
No. 7 Readiness due positive emotional perception of novelties	worker	33.86	**/***	**/***	7.35	0.77	0.79	8
	specialist	37.06	*/***	*/***	6.11	0.80		
	manager	39.94	*/**	*/**	5.12	0.77		

Note: X – arithmetical mean responder score; σ – mean square deviation; α – Cronbach's alpha; n – number of observations; p_t – Student's *t*-test; p_u – Mann-Whitney *U*-test; * – <0.05 in comparison with worker; ** – <0,05 in comparison with specialist; *** – < in comparison with manager

to use in formation of internal corporate social responsibility. The psychological climate factors include transparency of mission and goals, team cohesion, communication, openness to changes. The analysis of scholarly sources shows that well-being of employees is a fundamental factor in efficiency of an organisation, including pharmaceutical companies. Organisations with a high level of corporate social responsibility can achieve success through employees' innovative behaviour [26–28].

The change process evaluation results showed that a current participation in innovative projects is not a significant factor for organisation employees. Such results of the research revealing a staff attitude towards a participation in organisational change, require development of a modern approach to management aimed at changing staff beliefs, appropriate assessment of opinions and attitudes of organisation employees, development and inculcation of relevant marketing strategy focused on reduction of risks and subsequent enhancement of viability and competitiveness of the organisation [10, 16].

Thus, the study conducted and presented in the essay, as to analysing organisational readiness for changes, makes it possible to predict the ability of employees to participate in proposed innovations, and subsequently to apply appropriate incentive methods and reward systems with respect to certain staff categories. In addition, similar studies show how far the proposed changes are applicable for a given organisation, reveal the leaders' commitment to ongoing activities and help to develop due corporate culture [8].

The results obtained in this paper, will form a basis for the development of an adaptive model of human resource management in the conditions of organisational changes. It was found out that incorporating the principles of motivational interviewing for change readiness, can help individual employees to accept the change process, increasing achievements in terms of initiatives on changes. The forehanded analysis shows that the anticipatory phase before the introduction of innovations represents not only a passive waiting stage for company members, but also an active process of comprehension and positioning of the company's future [13, 29–31].

CONCLUSION

The degree of the organization employees' readiness for changes has been analyzed. It included the analysis of motivating factors, and the factors that impede or contribute to the development of innovative processes. They have been identified as illustrated by the example of an export-oriented biotechnological enterprise. The results can be further used to determine the required management functions and goals, and to develop the personnel management strategy in the context of the knowledge transfer, technology and export policy of the pharmaceutical enterprise.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

Elena V. Kazakova – selection of the research area, idea, development of the research algorithm, literature review, conducting all research stages, processing results, writing the article; Viktor P. Trukhin – formulation of the aim and objectives of the research; Igor A. Narkevich – consulting on the conduct of certain research stages; Irina I. Basakina – consulting on the conduct of certain research stages.

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USE OF SEQUENCING METHODS FOR SPECIES IDENTIFICATION EXEMPLIFIED BY PHYLOGENETIC RELATIONSHIPS WITHIN GENUS *HEDYSARUM* L.

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At the moment, a relevant objective in pharmacognosy, is the use of all kinds of the DNA analysis methods for identifying plant materials, detecting counterfeits, genetically modified crops and products.

The aim of the research is to study the possibility of using molecular genetic research methods in the analysis of the genus *Hedysarum* L., for the identification of medicinal plant materials. This article presents the results of the application of molecular genetic research methods in the analysis of the genus *Hedysarum* L. in the flora of the North Caucasus.

Materials and methods. The study material was the samples of the genus *Hedysarum* L. species collected in the North Caucasus: *Hedysarum caucasicum* M. Bieb. (in the fruiting phase in the territory of the Karachay-Cherkess Republic); *Hedysarum grandiflorum* Pall. (in the fruiting phase in the Volgograd region); *Hedysarum daghestanicum* Rupr. ex Boiss. (in the flowering phase in the Republic of Dagestan). Sequencing of the ITS1-5.8S-ITS2 marker region of gene 5.8S by the RNA ribosome was carried out according to the Sanger method on the AbiPrism 3130 genetic analyzer at the laboratory of biosystematics and cytology of Komarov Botanical Institute of the Russian Academy of Sciences.

Results. Based on a comparative study of the marker region of the nuclear ribosomal gene 5.8S rRNA, marker nucleotide substitutions of *Hedysarum caucasicum* M. Bieb., *Hedysarum daghestanicum* Rupr. ex Boiss., *Hedysarum grandiflorum* Pall, have been identified. The most probable secondary structure of 5.8S rRNA has been constructed. It has been shown that based on the analysis performed, it is possible to predict additional raw material sources of mangiferin and other groups of xanthones using the molecular data exemplified by the *Obscura* section.

Conclusion. Based on the data obtained, it can be concluded that the morphological classification of the genus *Hedysarum* L. can be confirmed within the *Obscura* section.

Keywords: *Hedysarum; Hedysarum caucasicum; Hedysarum daghestanicum; Hedysarum grandiflorum;* sequencing; 5.8S rRNA gene

Abbreviations: rRNA – ribosomal ribonucleic acid; tRNA – transfer ribonucleic acid; RNase – ribonuclease; CTAB – cetyltrimethylammonium bromide; DNA – deoxyribonucleic acid.

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ИСПОЛЬЗОВАНИЕ МЕТОДОВ СЕКВЕНИРОВАНИЯ ДЛЯ ИДЕНТИФИКАЦИИ ВИДОВ НА ПРИМЕРЕ ФИЛОГЕНЕТИЧЕСКИХ СВЯЗЕЙ В ПРЕДЕЛАХ РОДА *HEDYSARUM* L.

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Актуальной задачей в фармакогнозии на данный момент является применение всевозможных методов ДНК-анализа для идентификации растительного сырья, выявления фальсификатов, генетически модифицированных сельскохозяйственных культур и продуктов.

Цель. Изучить возможность применения молекулярно-генетических методов исследований при анализе рода *Hedysarum* L. для идентификации лекарственного растительного сырья. В данной статье представлены результаты применения молекулярно-генетических методов исследования при анализе рода *Hedysarum* L. флоры Северного Кавказа.

Материалы и методы. Материалом для исследования послужили образцы видов рода *Hedysarum* L., собранные на территории Северного Кавказа: *Hedysarum caucasicum* M.Bieb. (фаза плодоношения на территории Карачаево-Черкесской Республики); *Hedysarum grandiflorum* Pall. (фаза плодоношения в Волгоградской области); *Hedysarum daghestanicum* Rupr. ex Boiss. (фаза цветения в Республике Дагестан). Секвенирование маркерного участка ITS1-5.8S-ITS2 гена 5.8S рибосомой РНК проводили по методу Сэнгера на генетическом анализаторе AbiPrism 3130 на базе лаборатории биосистематики и цитологии Ботанического института имени В.Л. Комарова РАН.

Результаты. На основе сравнительного изучения маркерного участка ядерного рибосомного гена 5.8S рРНК были идентифицированы маркерные нуклеотидные замены *Hedysarum caucasicum* M. Bieb., *Hedysarum daghestanicum* Rupr. ex Boiss., *Hedysarum grandiflorum* Pall. Была построена наиболее вероятная вторичная структура 5.8S рРНК. Показано, что на основании проведенного анализа можно составить прогноз дополнительных сырьевых источников мангиферина и других групп ксантонов при использовании молекулярных данных, на примере секции *Obscura*.

Заключение. На основании полученных данных можно сделать заключение о том, что в пределах секции *Obscura* можно подтвердить морфологическую классификацию рода *Hedysarum* L.

Ключевые слова: *Hedysarum; Hedysarum caucasicum; Hedysarum daghestanicum; Hedysarum grandiflorum;* секвенирование; ген 5.8S рРНК

Список сокращений: рРНК – рибосомная рибонуклеиновая кислота; тРНК – транскрипционная рибонуклеиновая кислота; РНКаза – Рибонуклеаза; СТАВ – гексадецилтриметиламмониум бромид; ДНК – дезоксирибонуклеиновая кислота.

INTRODUCTION

Alongside with the known methods of the pharmacognostic analysis, lately, a promising molecular genetic research method has been introduced [1–5, 20]. Molecular pharmacognosy methods occupy a fairly stable position in the preparation of regulatory documents for medicinal plant raw materials, as well as in the study of phylogenetic relationships among closely related species and within varieties[1]. For example, some taxa of the legume family, complete chloroplast genomes have been identified, including 115 genes consisting of 79 protein-coding genes (68.7%), 31 tRNA genes (26.96%), and 5 rRNA (4.35%) [6]. Chloroplast genomes are potential sources of genetic markers for phylogenetic studies, genetic diversity, and molecular identification. [7]. Molecular research methods are widely used to study representatives of the Fabaceae family, among them, there are: Glycine max [L.] Merr., V. radiata var. radiate, *V. angularis var. angulari*, *Phaseolus vulgaris* L., *Cicer arietinum, Arachis hypogaea. Astragalus membranaceus var. mongholicus, Hedysarum polybotrys* [8–11].

As for the study of the genus *Hedysarum* L., the works by Chennaoui H., Marghali S., Marrakchi M., Trifi-Farah N.

[1] Lu-qi H. Molecular Pharmacognosy. Second Ed.- Springer Nature Singapore Pte Ltd. & Shanghai Scientif &Technic Publish. 2019: 303 p. DOI: 10.1007/978-981-32-9034-1.

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should be notified. The phylogenetic relationships within the genus *Hedysarum* L. have been studied on the basis of its morphological and biochemical characters[2] [12–14]. Nafisi H., Ranjbar M., Wojciechowski M. et al. have carried out molecular genetic studies based on a comparative analysis of ribosomal genes of the genus *Hedysarum* L. species growing in Southeast Asia [15, 16].

The *H. chaiyrakanicum* and *H. theinum* species, for which the authors notified a pronounced polymorphism of the internal transcribed ITS spacers of the 5.8S rRNA gene and the traces of a phylogenetic relationship with Mediterranean species of the genus, have been studied. A relationship is notified between the Asian and European species H. *chaiyrakanicum* and H. *gmelinii*, which belong to the related *Subacaulia* and *Multicaulia* sections [17–19].

In addition to well-known foreign authors, a significant contribution to the genus *Hedysarum* L. was made by domestic authors, including I.A. Shantser and Suprun N.A., who had studied the genetic variation of *H. grandiflorum* Pall., *H. biebersteinii* and *H. argyrophyllum* [4, 20–23]. To study the genetic polymorphism of *Hedysarum* L., the analysis of ISSR markers had been used, which made it possible to analyze more than 100 DNA fragments [24–27].

THE AIM of the research is to study the possibility of using molecular genetic research methods in carrying out complex pharmacognostic studies, to study the intra- and interpopulation variability of three species of the genus *Hedysarum* L. collected in the North Caucasus (*Hedysarum caucasicum* Bieb.(H_1), *Hedysarum grandiflorum* Pall. (H_2), *Hedysarum daghestanicum* Rupr. ex Boiss.(H_0)), to determine possible phylogenetic relationships between the species of the genus *Hedysarum* L.

MATERIALS AND METHODS

The material for the study was the samples of the genus *Hedysarum* L. species gathered in the territory of the North Caucasus: *H. caucasicum* Bieb., collected in the fruiting phase in 2017 in the Alibek gorge, the Dombai section in the territory of the KChR; *H. grandiflorum* Pall., collected in the fruiting phase in 2018 in the village of Kondrashi, the Ilovlinsky district of the Volgograd region; H. *daghestanicum Rupr.* ex *Boiss,* collected in the flowering phase in 2015 in the village of Andi in the Republic of Dagestan [28]. In this work, sequencing was performed according to the Sanger method determining the sequence of ITS1-5.8S-ITS2 rRNA [29]. The DNA sequencing was performed on an AbiPrism 3130 genetic analyzer (Applied Biosystems, USA) at the Biosystematics and Cytology Laboratory in *Komarov Botanical Institute* of the Russian Academy of Sciences.

The DNA sequence analysis was performed using the MEGA 10.0 software, USA. The isolation of genomic DNA was carried out by the CTAB method from the leaves of the herbarium samples [14]. For the amplification, Dream Taq PCR Master Mix reagents (Thermo Scientific, USA) were used. The polymerase chain reaction was carried out on a C1000 Thermal Cycler (Bio-Red, USA). The amplification cycle parameterswere: 3 min 98°C; 35 cycles: (1 min 98°C; 30 seconds 54°C; 30 seconds 72°C); 10 min 72°C.

The Big Dye Terminator Kit v. 2.0 (Perkin Elmer Life Sciences, Inc., USA) and the ABI Prizm 3130 sequencer (Applied Biosystems, UK) were used for sequencing. The DNA isolation from the leaves or herbarium samples was performed using the CTAB technique and included the following stages: the leaves of the studied samples were ground into a fine powder for 10 seconds using a TissueLyser hemogenizer (QIAGEN, USA). Then 700 µl of prewarmed extraction buffer EB was added and vigorously shaken; it was incubated at 65°C for 1, 2 or more hours; purified with an equal volume of a chloroform mixture: isoamyl alcohol (24:1) was shaken for 5 minutes, the samples were centrifuged for 10 minutes at room temperature at 14000 rpm. The upper phase was transferred into a new 1.5 ml tube, the DNA was precipitated with 2/3 of the isopropanol volume (5 min at room temperature), centrifuged at room temperature for 10 minutes at 14,000 rpm; the supernantant was removed and the supernantant was washed twice with Wash Buffer (WB). The precipitate was air dried and dissolved in 300 µl of TE buffer; 3 µl of RNase L (10 mg/ml) was added and incubated for 30 min at 37°C. The concentration was adjusted with 2M sodium chloride solution; precipitated again by adding 2 volumes of 96% ethyl alcohol, washed with 500 µl of 70% ethyl alcohol, then the granules were air dried and dissolved in TE buffer. For a direct PCR analysis, the Phire Plant Direct PCR Master Mix (Thermo Scientific, USA) was used, which was directly intended for plant leaves and seeds without any preliminary DNA purification.

The amplified DNA fragments were purified using a standard agarose electrophoresis method. The control was carried out visually using a UV transilluminator, since the bands of the DNA stained with fluorescent dyes, formed by molecules of the same size when moving through the pores of the gel, are visible in the UV light. Ethidium bromide (λ_{max} = 590 nm) which intercalates into DNA molecules (embedded between adjacent pairs of nucleotides), was used as a DNA dye. The intensity of this fluorescence is 20 times higher. The gel strip containing the necessary DNA fragment, was excised. To isolate DNA from the gel, a QIAquick Gel Extraction Kit (QIAGEN, USA) was used.

The ITS1-5.8S-ITS2 marker region of the 5.8S rRNA gene was sequenced in the representatives of the genus *Hedysarum*. The phylogenetic reconstruction was based on a comparison of this marker region from the sequenced samples of different geographic origins and the data from the Genbank NCBI[3]. To construct phylogenetic trees, the maximum likelihood method was used in the MEGA 10.0 program.

RESULTS AND DISCUSSION

A comparative molecular study of *Hedysarum caucasicum* M. Bieb., *Hedysarum daghestanicum* Rupr. ex Boiss, *Hedysarum grandiflorum* Pall. samples represented in the flora of the Caucasus, were carried out. The ITS1-5.8S-ITS2 sequences of the 5.8S rRNA gene in *Hedysarum caucasicum* were compared with the data presented in Genbank[4] [30–34]. The resulting phylogenetic tree is shown in Fig. 2.

[2] Bojnanský V, Fargašová A. Atlas of Seeds and Fruits of Central and East-European Flora: The Carpathian Mountains Region. Dordrecht: Springer. 2007: 1046 p.

[3] GenBank Overview. Available from: http://www.ncbi.nlm.nih.gov/Genbank.

[4] Ibid.

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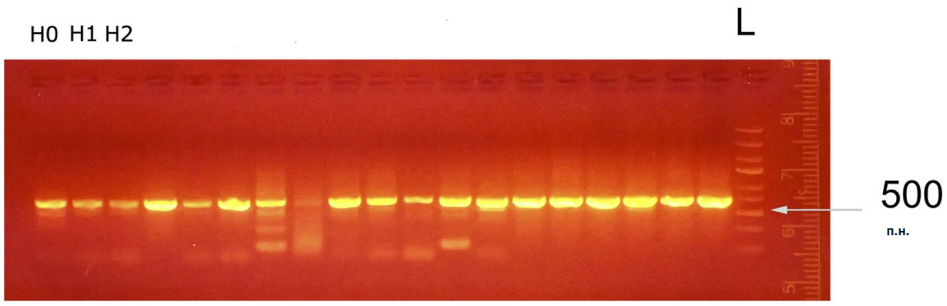

Figure 1 – Results of DNA staining in UV light (ethidium bromide dye)

Note: H_0 – *Hedysarum daghestanicum* Rupr. ex. Boiss; H_1 – *Hedysarum caucasicum* M. Bieb.; H_2 – *Hedysarum grandiflorum* Pall.

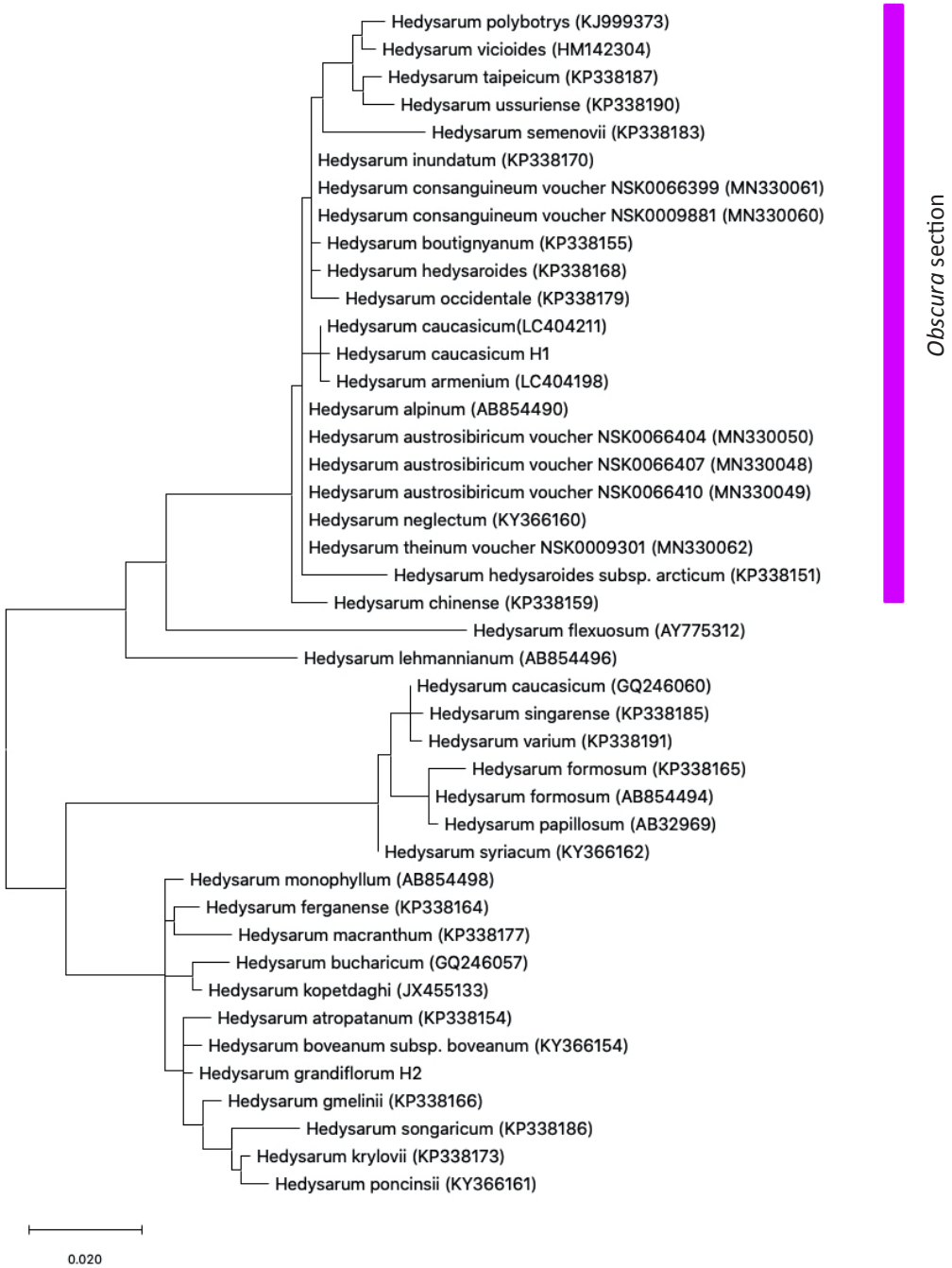

Figure 2 – Phylogenetic relationships of the genus *Hedysarum* L. species

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Table 1 – Comparative characteristics of morphological and ecological-phytocenotic features of the genus *Hedysarum* L. species based on the phylogenetic tree growing in the territory of the Russian Federation

SeqNo	Species name	Life form, leaves	Inflorescence elements, flower structure	Phytocenotic type
		Section 2. *Spinosissima*		
1	*Hedysarum flexuosum* L.[5,6]	An anual plant; the stem is strong, brachiate, 50–120 cm high with 2–5-paired leaves, the stipules are large, free; the leaflets are 2–5-paired, almost round or oblong-ovate, scatteredly fluffy below, 15–22 mm long.	The flower spikes are shortly appressed hairy; the inflorescences are elongated; the corolla is purple or pink, 10–12 mm long, the ovary is hairy.	Slopes, meadows, deep clay soils; up to 100 m.
		Section 3. *Obscura*		
2	*Hedysarum arcticum* B.Fedtsch.[7]	A perennial plant; the stem is strong, glabrous, 20–35 cm high; the stipules are a lot, fused together; the leaflets are 4–9 in number, paired, oblong-elliptical, glabrous above, pubescent below along veins and edges, or almost glabrous, 12–20 mm long.	The flower spikes are axillary, at the beginning of flowering the racemes are compressed, 4–7 cm long; the flowers are 5–30 in the amount, somewhat drooping, purple, violet-red.	In the arctic and adjacent forest zone in the tundra, on sandy islands, in larch sparse forests, in riverside shrubs; in the alpine zone.
3	*Hedysarum inundatum* Turez.[8,9,10]	A perennial plant; the stems are upright, glabrous, 20–40 cm high; the leaves are compound, the leaflets are 5–9 paired, oblong-elliptical, the peduncles are light-brown, 8–12 cm long.	The flower spikes are apical; the racemes are 4–6 cm long; the flowers are 24–35 in the amount, slightly drooping; the floral bracts are light brown, linear-lanceolate, the corolla is 16–20 mm long, mauve.	Along gravels and cliffs in the subalpine and alpine zones, in the zones of mountain ranges.
4	*Hedysarum consanguineum* DC.[11,12]	A perennial plant; the stems are upright, short fluffy or almost glabrous, 20–45 cm high; the leaves are green, forming 2–4 internodes; the stipules are numerous, accrete, short, brown; the leaflets are 4–8-paired, almost glabrous or appressed short hairy.	The flower spikes are apical; the racemes are 10–18 cm long; the flowers are 27–30 in the amount, horizontal or drooping; the calyx is short-campanulate, fluffy, the teeth are lanceolate-subulate; the corolla is 17–19 mm long, mauve.	Through river valleys and swampy forests. In the alpine zone in alpine, subalpine meadows, stony placers, in the tundra.
5	*Hedysarum austrosibiricum* B.Fedtsch.[13,14]	A perennial plant; the stems are upright, strong, glabrous, 20–40 cm high, the stipules are accreted at the base of the stems; the leaflets are 4–9 pairs in number, oblong-elliptical, glabrous above, barely pubescent below along the midrib and edges, 15–25 mm long, 7–10 mm wide.	The flowers are lilac, violet-lilac, 15–30 flowers in dense racemes, elongating with fruits up to 10–15 cm; the bracts are lanceolate, yellow-brown, almost reaching the teeth of the calyx.	In the alpine zone on forest, alpine and subalpine meadows, in the tundra, on rubble and lichenaceous barrens.

[5] Fedchenko BA. Flora of the USSR. Family *Leguminosae*. M.-L.: Publishing house of the Academy of Sciences of the USSR. 1948; 3:259–319. Russian
[6] Castroviejo S, et al. Flora Iberica. Plantas vasculares de la Península Ibérica e Islas Baleares / *Leguminosae* VII(II) (partim). Madrid: Real Jardín Botánico. 2000: 1121 p.
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[8] Ibid.
[9] Stepanov NV. Vascular Plants of the Yenisei Sayan Mountains. Krasnoyarsk: Siberian Federal University 2016: 593 p. Russian
[10] Flora of Siberia *Fabaceae (Leguminosae).* Ed. Polozhiya A.V., Malysheva L.I. Novosibirsk: Science. 1994;9: 280 p. Russian
[11] Ibid.
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[13] Ibid.
[14] Flora of Siberia *Fabaceae (Leguminosae),* 1994. Russian

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SeqNo	Species name	Life form, leaves	Inflorescence elements, flower structure	Phytocenotic type
6	*Hedysarum hedysaroides* L.[15]	A perennial plant; the stems are upright, glabrous, 30–60 cm high; the stipules are brown, the lower ones are almost completely fused together, the upper ones are partially fused, with lanceolate-elongated free ends; the leaflets are 6–9 paired, with sparse hairs 12–20 mm long.	The flower spikes are longer than the leaves; the racemes are thin, elongated; the flowers are numerous, 30–40, drooping, dark purple; the calyx is with an oblique limb, the teeth are lanceolate or subulate.	Along mountain meadows in the upper parts of the forest zone.
7	*Hedysarum neglectum* Ldb.[16,17,18]	A perennial plant; the stems are upright, glabrous or short fluffy 25–60 cm high, the stipules are numerous, short, brown, accrete; the leaves are petiolate, the leaves are 4–10-paired, oblong-elliptical, appressedly pubescent on both sides, 17–22 mm long.	The flower spikes are apical, the racemes are loose, the floral bracts are brown, linear-lanceolate, glabrous or slightly pubescent; calyx is short-campanulate, short-fluffy; the corolla is lilac, purple-lilac.	In the alpine zone, in descends into the forests; on subalpine, alpine, forest meadows, rocky slopes, in sparse larch forests.
8	*Hedysarum caucasicum* M. Bieb.[19,20]	A perennial plant; the stems are upright, foliate, 30–60 cm long, the leaflets are 7–12-paired, elliptical or ovate-oblong, with a pointed apex, 12–16 mm long [13].	The flower spikes are longer than the leaves; the racemes have long stems, 1.5–2 times longer than the leaves, not very dense; the corolla is dark purple or crimson [13].	Alpine meadows 1500–3500 m above the sea level, on subalpine and alpine meadows, on moraines, on slide rocks, in crooked forests, on rock ledges.
9	*Hedysarum Semenovii* Rgl. et Herd.[21,22,23]	A perennial plant; the stems are solitary, rarely 2–3 in number, upright, grooved, glabrous or sparsely hairy, 50–120 cm high; the stipules are large, fused to each other, brown, the lowest ones are leafless, amplexicaul; the leaves are shortly petiolate; their petioles, like the leaf axis, are appressed-hairy; the leaves are 4–8-paired, round or orbicular-ovate, tender, glabrous above, scatteredly appressed-hairy below, 15–35 mm long.	The flower spikes are axile, accumbent-hairy, the racemes are oblong, multi-flowered, dense; the floral bracts are lanceolate, brown, 4–6 mm long; the calyx is wide campanulate-shaped, 5–6 mm long, slightly oblique, glabrous or slightly hairy; the corolla is yellow.	On grassy slopes, in the spruce forest belt.
10	*Hedysarum alpinum* L.[24,25,26]	A perennial plant; the stems are upright, glabrous or pubescent in the upper part with short and slightly curly hairs, 40–120 cm high; the leaves are compound, odd-pinnate; the leaflets are 6–11-paired, oblong-ovate, 15–30 mm long.	The inflorescences are long, the racemes are dense; the flowers are in the amount of 20–30, pink, mauve, purple when dried, 5–15 cm long in the racemes; the calyx is 3.5–4.5 mm long, covered with short, slightly curly hairs; the corolla is 13–15 mm long, the ovary is glabrous or pubescent.	On forest meadows, in birch and pine forests, on slopes and on sea cliffs.

[15] Fedchenko BA. Flora of the USSR. Family *Leguminosae*, 1948. Russian
[16] Ibid.
[17] Wu ZY, Raven PH, Hong DY. Flora of China (*Fabaceae*). Beijing: Science Press. 2010; 10: 577 p.
[18] Flora of Siberia *Fabaceae (Leguminosae)*, 1994. Russian
[19] Ibid.
[20] Grossheim AA. Flora of the Caucasus. M.L.: Publishing house of the Academy of Sciences of the USSR. 1952;5: 454 p. Russian
[21] Fedchenko BA. Flora of the USSR. Family *Leguminosae*, 1948. Russian
[22] Stepanov NV. Vascular Plants of the Yenisei Sayan Mountains, 2016. Russian
[23] Wu ZY, Raven PH, Hong DY. Flora of China (*Fabaceae*), 2010.
[24] Ibid.
[25] Fedchenko BA. Flora of the USSR. Family *Leguminosae*, 1948. Russian
[26] Flora of Siberia *Fabaceae (Leguminosae)*, 1994. Russian

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SeqNo	Species name	Life form, leaves	Inflorescence elements, flower structure	Phytocenotic type
11	*Hedysarum vicioides* Turez.[27,28]	A perennial plant; the stem is glabrous, 50–120 cm high; the stipules are accreted in the form of a vagina; the leaflets are 8–9-paired, linear-oblong, glabrous above, scarcely pubescent along the mid-rib and edges below.	The racemes are loose, 15–20 cm long; the flowers are in the amount of 10–30; the calyx is glabrous; the corolla is pale yellow, 10–12 mm long.	In river valleys, on the meadows, on riverine gravels, in larch and pine forests.
12	*Hedysarum ussuriense* I.Schischk&-Kom.[29,30]	A perennial plant with a thick, woody, with a long tap root; the stems are numerous, up to 50 cm high; the leaves are odd-pinnate; the leaflets are 3–5-paired, oblong-ovate, almost glabrous, 10–20 mm long.	The flowers are whitish-yellow, numerous (up to 20), in a loose, unilateral, racemose inflorescence, on a long flower spike exceeding the leaves.	On limestone rocks and open rocky slopes up to 700 m above the sea level. In some places it forms thickets. Obligate calcephile.
13	*Hedysarum theinum* Krasnob.31[31,32]	A perennial herb; the stems are grooved, glabrous or slightly appressed-hairy, 80 cm high, the stipules are brown, fused together, large, lepidodendroid; the leaflets are 2–5-paired, short-petiolate, elliptical or ovate-elliptical, glabrous above.	Purple-lilac flowers are organized in loose racemes of 15–30 pcs.	Hedysarum L. grows on alpine and subalpine meadows, on grassy and rocky slopes, in calciphile forests and on river gravels.
14	*Hedysarum armenium* Boiss. et Tchih.[33]	A perennial herb; the stems are upright, shortened, 10–30 cm high, the stipules are light brown, wide; the leaflets are 10–13-paired, elliptical or oblong-elliptical, dark green, 8–12 mm long [13].	The racemes are dense, 20–30 flowers in each; the corolla is 15–16 mm long, dark purple, the ovary is pubescent [13].	On mountain meadows, at an altitude of 2100–3000 m [13].
		Section 4. *Multicaulia*		
15	*Hedysarum formosum* Fisch. et Mey.[34,35]	A perennial herb; the stems are coriaceous, up to 5 mm in diameter, upright, slightly flexuose, brachiate, 40–70 cm high; the stipules are large, lanceolate, the lower ones are fused, the upper ones are usually free; the leaflets are 6–10 paired, elliptical, glabrous above, pubescent below [13].	The racemes are elongated; the calyx teeth are subulate from the lanceolate base; the corolla is yellow, 13–15 mm long, pale [13].	Solonized steppes [13].
16	*Hedysarum atropatanum* Bge. ex Boiss.[36,37]	A perennial herb; the stems are white, sparsely silvery-hairy, 30–40 cm high; the stipules are white-membranous, appressed-hairy, short-triangular; the leaflets are 6–10 paired, rounded-elliptical or oblong, smothered with appressed silvery hairs on both sides, 10–12 mm long [13].	The racemes are elongated; 10–15 flowers in each; the calyx is campanulate-shaped, its teeth are subulate from the narrow-lanceolate base, glabrous upwards; the corolla is pink-violet, 15–18 mm long [13].	On clay-slates [13].

[27] Ibid.
[28] Fedchenko BA. Flora of the USSR. Family *Leguminosae*, 1948. Russian
[29] Ibid.
[30] Stepanov NV. Vascular Plants of the Yenisei Sayan Mountains, 2016. Russian
[31] Flora of Siberia *Fabaceae (Leguminosae)*, 1994. Russian
[32] Fedchenko BA. Flora of the USSR. Family *Leguminosae*, 1948. Russian
[33] Ibid.
[34] Ibid.
[35] Grossheim AA. Flora of the Caucasus, 1952. Russian
[36] Ibid.
[37] Fedchenko BA. Flora of the USSR. Family *Leguminosae*, 1948. Russian

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SeqNo	Species name	Life form, leaves	Inflorescence elements, flower structure	Phytocenotic type
17	*Hedysarum kopetdaghi* Boriss.[38]	A perennial multicaulis herb; 25–35 cm high, the stipules are membranous, 5–7-paired leaflets, which are oblong-elliptical, obtuse at the apex, with a short mucro, rounded-wedge-shaped to the base, appressed hairy on both sides, 15–30 mm long.	The inflorescences are racemes, the flowers have long stems; the floral bracts are filiform, membranous, up to 5 mm long, the calyx is wide campanulate-shaped, the corolla is purple.	On grassy mountain slopes, at an altitude of 1800-2000 m.
18	*Hedysarum varium* Willd.[39,40]	A perennial plant; the stems are ascending, numerous, brachiate, 20-40 cm high, the stipules are accrete, appressed-hairy; the leaflets are 3-5-paired, oblong or elliptical, glabrous above, fluffy below [13].	The racemes are dense, equal to or longer than the leaves; the corolla yellow; its purple carina is at the apex [13].	On dry slopes [13].
19	*Hedysarum Gmelinii* Ldb.[41,42]	A perennial plant; the stems are few, almost upright,, pubescent, with appressed hairs, below 2–3 mm in diameter, the stipules are membranous, accrete, the leaflets are 5–11-paired, oblong, hairy below, sometimes glabrous above, 7–30 mm long.	The flower spike is longer than leaves; the racemes, 15-30 in number, are flowering, dense, elongated at the end; the floral bracts are lanceolate, the calyx is appressed-hairy; the corolla is pink-purple.	On meadows and in stony steppes.
20	*Hedysarum songaricum* Bong.[43,44]	A perennial plant; the stems are well developed, numerous, almost glabrous, 25–60 cm high; the stipules are membranous, the lower ones are fused at the base, slightly colored, hairy; the leaflets are 5–8 paired, lanceolate or oblong-elliptical, glabrous above, about 20 mm long	The racemes are multi-flowered, oblong; the floral bracts are scarious, lanceolate; the calyx is campanulate-shaped, with lanceolate-subulate teeth, the corolla is pink-violet	In steppes, on rank soil and finely earthy slopes in the middle and lower mountain belts[17,20]
21	*Hedysarum atropatanum* Bge. ex Boiss.[45]	A perennial plant; the stems are brachiate, silvery hairy; the stipules are white-membranous, appressed-hairy; the leaves short-petiolized; leaflets 4–6-paired, rounded-elliptical or oblong, 10–12 mm long.	The racemes are elongated, 10–15 flowers in each; the calyx is campanulate-shaped, the corolla is pink-violet, 14–15 mm long.	On clay-slates.
		Section 5. *Subacaulia*		
22	*Hedysarum Lehmannianum* Bge.[46]	A perennial acaulous plant; the stems are appressed hairy, 15–35 cm high; the stipules are oblong-lanceolate, brown, fused to each other, appressed-hairy; the leaflets are 7–12-paired, oblong-elliptic, smoothly appressed-hairy below, pubescent above, 10–18 mm long.	The racemes are pretty compressed, multi-flowered; the flowers are 12–20 in the amount, the calyx is divaricately-hairy, the teeth are lanceolate-subulate; the corolla is purple.	On rocky mountain slopes, through bushes in subalpine steppes
23	*Hedysarum monophyllym* Boriss.[47]	A perennial sericeous hairy plant 5–12 cm high; the stipules are imbricate at the base of leaves, the stipules are sericeous with strigose; the leaflets are simple, bottom sand, with a rounded-ovoid plate, sericeous with strigose, rounded or acute leaf apexes, sometimes sinuated, rounded at the base, 10–40 mm long.	The inflorescence is rounded-cephalanthium, compact, the floral bracts are ovoid, white-hairy outside, the calyx is wide campanulate-shaped, the corolla is dry, yellowish.	On stony and clay slopes of mountains, at an altitude of up to 2500 m.

[38] Ibid.
[39] Ibid.
[40] Grossheim AA. Flora of the Caucasus, 1952. Russian
[41] Flora of Siberia *Fabaceae (Leguminosae)*, 1994. Russian
[42] Fedchenko BA. Flora of the USSR. Family *Leguminosae*, 1948. Russian
[43] Ibid.
[44] Wu ZY, Raven PH, Hong DY. Flora of China (*Fabaceae*), 2010.
[45] Fedchenko BA. Flora of the USSR. Family *Leguminosae*, 1948. Russian
[46] Ibid.
[47] Ibid.

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SeqNo	Species name	Life form, leaves	Inflorescence elements, flower structure	Phytocenotic type
24	*Hedysarum grandiflorum* Pall.[48]	A perennial acaulous plant with a tigellum (rarer); реже стебли сильно укороченные, the stipules are large, leatherly, membranous, accrete, brown, sparsely hairy; the leaflets are 1–4 paired, ovate or wide elliptic, smoothly silvery-sericeous beneath, 20–30 mm long [13].	The racemes are multi-flowered, with deviated flowers; the floral bracts are lanceolate, light brown, hairy, the calyx is campanulate-shaped; the corolla is yellow or purple-violet [13].	Forest-steppe, stony and thyme steppes, rubble and clay-limestone slopes, limestones, chalk steppes, on outcrops of chalk and marl [13].
25	*Hedysarum ferganense* Korsh.[49,50]	A perennial acaulous plant; 10-30 cm high, the leaves are 4–7-paired, oblong or elliptical, covered with short white, appressed hairs, on the upper surface of the leaves the pubescence is not so dense, 10–18 mm long.	The floral bracts are brownish, lanceolate; the raceme is dense; the calyx is campanulate-shaped, its teeth are linear, 2–3 times longer than the tube, covered with adpressed or somewhat divaricately hairs; the corolla is lilac-violet.	On mountain meadows, on stony and gravelly slopes.
26	*Hedysarum Poncinsii* B.Franchet.[51]	A perennial acaulous plant; densely white-tomentose, 5–10 cm high, the stipules are white-membranous, with a slight admixture of brownish color; the leaflets are 3–4-paired, oblong-obovate, 5–7 mm long.	The raceme is compressed, cephalanthium; the calyx is campanulate-shaped, its teeth are linear-filiform, covered with detached hairs; the corolla is dark purple.	Steppes, at an altitude of 2800–3200 m.
27	*Hedysarum Krylovii* Sumn.[52,53]	A perennial plant; the stipules are white, scarious, appressed hairy; the leaflets are 3–7-paired, oblong-lanceolate, glabrous or with slightly appressed hairs, silvery beneath, 10–23 mm long.	The inflorescence is multi-flowered, oblong, less often ovate, the floral bracts are nearly equal to the calyx tube; the flowers are purple.	In solonized steppes and gravelly terraces.
28	*Hedysarum daghestanicum* Rupr. ex Boiss.[54,55,56,57]	A perennial plant; gray in color because of appressed pubescence; the stipules accreted; the leaves are covered with sericeous pubescence on both sides; the leaflets are 2–3-paired, oblong or ovate-lanceolate, acute, the apical leaflet is larger, up to 18 mm long.	The raceme is pauciflorous, dense; the flowers are big, creamy-white or purple [13].	Calcareous and dry grassy slopes, rocky places, at an altitude of from 800 to 1500 m [13].
		Section 6. *Crinifera*		
29	*Hedysarum macranthum* Freyn. et Sint.[58]	A stemless or almost stemless perennial; 20-30 cm high; the stipules are sericeous, fused together, lanceolate; the leaves are 5–7 paired.	The racemes are longer than leaves, with acute apexes; the floral bracts are hairy; the calyx is wide campanulate.	On stony and gravelly slopes.
30	*Hedysarum bucharicum* B.Fedtsch.[59]	A perennial plant; the stems are thin, ascending, appressed-hairy; the stipules are pale, fused at the bases, triangular-lanceolate; the leaflets are 5–8-paired, oblong-lanceolate, 14–18 mm long.	The racemes are not dense; the flowers are 12–25 in the amount; the floral bracts are nondeciduous; the corolla is bright purple.	Gypsum and limestone, among juniper woodlands and absinthial mixed herbs communities.

[48] Ibid.
[49] Ibid.
[50] Wu ZY, Raven PH, Hong DY. Flora of China (*Fabaceae*), 2010.
[51] Stepanov NV. Vascular Plants of the Yenisei Sayan Mountains, 2016. Russian
[52] Ibid.
[53] Flora of Siberia F*abaceae (Leguminosae),* 1994. Russian
[54] Stepanov NV. Vascular Plants of the Yenisei Sayan Mountains, 2016. Russian
[55] Litvinskaya SA, Murtazaliev RA. Flora of the North Caucasus: Key Atlas. Moscow: Fiton XXI. 2013: 688 p. Russian
[56] Grossheim AA. Flora of the Caucasus, 1952. Russian
[57] Galushko AI. Flora of the North Caucasus: key in 3 vol. Publishing house of Rostov University. 1980; 2: 352 p. Russian
[58] Stepanov NV. Vascular Plants of the Yenisei Sayan Mountains, 2016. Russian
[59] Ibid.

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Table 2 – Chromosome numbers of the genus *Hedysarum* L. species

Seq No.	Species name[60,61,62,63]	GenBank number[64]	Chromosomal complement
	Section 1. *Fruticosa*		
1.	*Hedysarum fruticosum* Pall. [13]	–	2n=16
	Section 2. *Spinosissima*		
2.	*Hedysarum flexuosum* L.	AY775312	2n=16
	Section 3. *Obscura*		
3.	*Hedysarum arcticum* B. Fedtsch.	KP338151	2n=14
4.	*Hedysarum inundatum* Turez.	KP338170	2n=8, 2n=28
5.	*Hedysarum consanguineum* DC.	NSK0066399 (MN330061); NSK0009881 (MN330060)	2n=14
6.	*Hedysarum austrosibiricum* B. Fedtsch.	NSK0066407 (MN330048); NSK0066410 (MN330049); NSK0066404 (MN330050)	2n=14
7.	*Hedysarum hedysaroides* L. [16]	KP338168	2n=14
8.	*Hedysarum neglectum* Ldb.	KY366160	2n=14
9.	*Hedysarum caucasicum* M. Bieb.	GQ246060; LC404211; RC – The object under study	2n=14
10.	*Hedysarum Semenovii* Rgl. et Herd.	KP338183	–
11.	*Hedysarum alpinum* L.	AB854490	2n=14
12.	*Hedysarum vicioides* Turez.	HM142304	2n=14
13.	*Hedysarum ussuriense* I. Schischk & Kom.	KP338190	2n=16
14.	*Hedysarum theinum* Krasnob.	NSK0009301 (MN330062)	–
	Section 4. *Multicaulia*		
15.	*Hedysarum formosum* Fisch. et Mey.	AB854494; KP338165	–
16.	*Hedysarum atropatanum* Bge. ex Boiss.	KP338154	–
17.	*Hedysarum kopetdaghi* Boriss.	JX455133	–
18.	*Hedysarum varium* Willd.	KP338191	–
19.	*Hedysarum Gmelinii* Ldb.[15]	KP338166	2n=28
20.	*Hedysarum songaricum* Bong.	KP338186	–
	Section 5. *Subacaulia*		
21.	*Hedysarum Lehmannianum* Bge.	AB854496	–
22.	*Hedysarum monophyllym* Boriss.	AB854498	–
23.	*Hedysarum grandiflorum* Pall.	The object under study	–
24.	*Hedysarum ferganense* Korsh.	KP338164	–
25.	*Hedysarum setigerum* Turcz.	–	2n=28, 32, 48
26.	*Hedysarum Poncinsii* B. Franchet.	KY366161	–
27.	*Hedysarum Krylovii* Sumn.	KP338173	–
	Section 6. *Crinifera*		
28.	*Hedysarum macranthum* Freyn. Et Sint.	KP338177	2n=16
29.	*Hedysarum bucharicum* B. Fedtsch.	GQ246057	–

Table 3 – The content of xanthones in various species of the genus *Hedysarum* L.

Seq No.	Species name	The sum of the xanthones
	Section 3. *Obscura*	
1.	*Hedysarum austrosibiricum* B.Fedtsch.	3.0
2.	*Hedysarum neglectum* Ldb.	3.6
3.	*Hedysarum flavescens* Rgl. et Schmalh.	5.5
4.	*Hedysarum caucasicum* M.Bieb.	4.0
5.	*Hedysarum alpinum* L.	6.0
6.	*Hedysarum theinum* Krasnob.	3.1
	Section 4. *Multicaulia*	
7.	*Hedysarum Gmelinii* Ldb.	1.2

Some of the studied species form well-maintained clades. That can be said about the species assigned to the *Obscura* section. As a result of the research, a comprehensive ecomorphology and molecular genetic analysis has been carried out, which makes it possible to carry out a correlation between morphological, ecological-phytocenotic, molecular-genetic indicators. 29 species of 309 species of the genus *Hedysarum* L., which belongs to the Fabaceae family, grow in the territory of the Russian Federation, 17 species are found in the flora of the Caucasus.

The genus *Hedysarum* L. includes 6 sections: *Fruticosa, Spinosissima, Obscura, Multicaulia, Subacaulia,*

[60] Stepanov NV. Vascular Plants of the Yenisei Sayan Mountains, 2016. Russian
[61] Flora of Siberia *Fabaceae (Leguminosae)*, 1994. Russian
[62] Bolkhovskikh ZV, Grif VG, Zakharieva OI, Matveeva TS. Chromosome numbers of flowering plants. L.: Nauka. 1969 : 927 p. Russian
[63] Castroviejo S, et al. Flora Iberica. Plantas vasculares de la Península Ibérica e Islas Baleares / *Leguminosae* VII(II) (partim), 2000.
[64] GenBank Overview. Available from: http://www.ncbi.nlm.nih.gov/Genbank.

Crinifera. Such morphological indicators as a life form, peculiarities of the leaves structure, elements of the generative organs structure (flowers and fruits) are depicted. In addition, for each species, ecological and phytocenotic characteristics have been indicated, including the peculiarities of growth and altitude level (Table 1).

When analyzing the data obtained, it should be notified the assumed correlation between the habitat of alpine species living mainly on rocks, gravels, rockslides, in the subalpine and alpine belts with moist soil and purple or pink-violet color of the corolla petals. The examples are the species such as: *Hedysarum flexuosum* L., *Hedysarum arcticum* B. Fedtsch., *Hedysarum inundatum* Turez., *Hedysarum austrosibiricum* B. Fedtsch., *Hedysarum hedysaroides* L., *Hedysarum neglectum* Ldb., *Hedysarum caucasicum* M. Bieb., *Hedysarum alpinum* L., *Hedysarum theinum* Krasnob., *Hedysarum kopetdaghi* Boriss., *Hedysarum songaricum* Bong.

Herewith, a group of yellow-flowered *Hedysarum* L. growing mainly on solonized, dry limestone slopes with a low level of soil moisture, on gravels, on clay-slates, can be distinguished. These species can be classified as calcephiles, among them there are: *Hedysarum Semenovii* Rgl. et Herd., *Hedysarum vicioides* Turez., *Hedysarum ussuriense* I. Schischk. & Kom., *Hedysarum formosum* Fisch. et Mey., *Hedysarum varium* Willd., *Hedysarum grandiflorum* Pall. The analysis of chromosome numbers of species of the genus *Hedysarum* has been carried out [35, 36]. The results are shown in Table 2.

The chemical composition of the genus *Hedysarum* L. species has been studied for the accumulation of the main marker group of biologically active substances – xanthone glycoside mangiferin [37–40]. The results are shown in Table 3.

Based on the foregoing, it can be concluded that the species of the section *Obscura* are characterized by a maximum accumulation of xanthone glycosides, mainly mangiferin. Thus, for *Hedysarum alpinum* L. the content was 6.0%; for *Hedysarum flavescens* Rgl. et Schmalh it was 5.5%, and for *Hedysarum theinum* Krasnob. – 3.1%.

Based on the studies carried out, it is possible to suggest several directions of phylogenetic regularities concerning the correlation between the chemical composition of the main groups of biologically active substances involved in the metabolism of this genus, including the marker xanthone glycoside mangiferin, and a change in the color of the corolla petals. A separate direction can be considered a possible correlation between the ecological and phytocenotic characteristics of the growth of species and the accumulation of the main groups of xanthones. As a result of the molecular analysis, it can be assumed that it is possible to make a forecast of additional raw materials sources of mangiferin and other groups of xanthones from the representatives of the *Obscura* section. The analysis of phylogenetic relationships of this section representatives of the genus *Hedysarum* L. species confirms the morphological classification of this genus.

The section *Obscura* which comprises more than 20 species, including the Hedysarum caucasicum studied in this article, is characterized by similar morphological and ecological-phytocenotic parameters. According to the GenBank[65] data, almost all of these species belong to this section, which completely confirms the correlation between the classical taxonomic and molecular genetic methods. This section is characterized by a significant accumulation of mangiferin – from 3.0 to 6.0%. In the aerial part of *Hedysarum caucasicum*, up to 4.0% of the amount of xanthones in terms of mangiferin has been found. It should be notified that this group of species grows mainly in the alpine and subalpine zones and has a characteristic violet-purple color of the corolla petals.

The *Subcaulia* section, which includes 22 species, is characterized by arid habitats, dry rocky limestone slopes. This group is calciphilic, with a predominantly yellow color of the corolla petals. The representatives of this section, including *Hedysarum* L. large-flowered, accumulate a small amount of xanthones – from 0.05 to 0.1%.

When analyzing the data obtained, it should be to notified that the observed correlation exemplified by the representatives of the genus *Hedysarum*, makes it possible not only to establish the degree of phylogenetic relationships of various taxa [8, 9, 40–45], but also to represent the totality of chemotaxonomic characteristics of certain species using the example of the *Fabaceae* family. In the future, the data can be used in pharmacognostic studies and the development of regulatory documents for medicinal plant raw materials.

CONCLUSION

Preliminary results have been obtained. This fact makes it possible to carry out a comprehensive identification of the studied samples of the genus *Hedysarum* L. species (*Hedysarum caucasicum* M. Bieb., *Hedysarum daghestanicum* Rupr. ex Boiss., *Hedysarum grandiflorum* Pall.), growing in various high-mountainous areas of the North Caucasus. Preliminary results of the application of molecular genetic research methods in the analysis of the genus *Hedysarum* L. are presented in this article. For the first time, nucleotide substitutions in the 5.8S rRNA gene sequence of the genus *Hedysarum* L. three species have been identified, and the phylogenetic tree of the genus *Hedysarum* L. is presented.

It should be notified that in the future, the observed correlation between the accumulation of secondary metabolites of the xanthone series and the ecological and phytocenotic characteristics, can serve to isolate the species of the *Obscura* section to search for additional raw material sources of mangiferin and its derivatives with a pronounced antiviral activity.

Taking into account the development of modern molecular pharmacognosy, the data obtained can be further used in the preparation of regulatory documents for medicinal plant raw materials.

[65] Ibid.

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CONFLICT OF INTEREST

The authors declare they have no conflict of interest.

AUTHORS' CONTRIBUTION

Javgarat R. Imachueva – setting up the experiment, discussing the results; Fatima K. Serebryanaya – project management, collection of plant material, discussion of the results; Eduard M. Machs – setting up an experiment, discussing the results; Violetta V. Kotseruba – project management, discussing the results.

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