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MEMBERS OF THE FAMILY *LAMIACEAE* LINDL. AS SOURCES OF MEDICINAL PLANT RAW MATERIALS TO OBTAIN NEUROTROPIC DRUGS

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The aim of this work is to review and analyze the data published in the modern scientific literature obtained in pharmacological, pharmacognostic and pharmacotechnological studies of various types of raw materials obtained from members of the family *Lamiaceae* L., which were sources of biologically active substances, pharmaceutical substances, total extracts and the drugs – with a neurotropic activity.

Materials and methods. For the review, we used the information of scientific literature from open and accessible sources of the last twenty years, located in the scientific and technical libraries of institutions, as well as in electronic databases: Elibrary, PubMed, Scopus, Cyberleninka, GoogleAcademy, J-Stage. The search inquiries were: the species of the family *Lamiaceae* (Russian and Latin), the samples of medicinal plant materials based on them as well as the names of the drugs and biologically active substances obtained from these raw materials.

Results. When working with the sources of scientific information, the main attention was paid to pharmacologic tests performed during the studies on laboratory animals and proving the presence of neurotropic activity in the studied objects – essential oils and extracts from plant raw materials: aqueous, aqueous alcoholic, and methanol ones. It has been established that the potential of the therapeutic and preventive application of pharmaceutical substances and drugs based on the medicinal plant materials obtained from 30 genera members of the Lamiaceae family, remains unrealized despite the close attention of various researchers.

Conclusion. This review comprised 71 species from 30 genera. Despite the significant level of the previous study presented in the analysis of this publication, an enormous potential of this family's species remains unexplored. In the future, they can be of both – pharmacognostic and practical interest, in particular, in creation of new medicinal preparations of the neurotropic action based on them.

Keywords: Literature review, Lamiaceae L., herbal medicine, medicinal plant materials, extract, herbal formulation фитопрепарат, medicinal preparation, pharmacognosy, pharmacology, stress, neurotropic activity, anxiolytic effect, sedative action, antidepressant action, GABA-α-receptors, benzodiazepine receptors

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ПРЕДСТАВИТЕЛИ СЕМЕЙСТВА *LAMIACEAE* LINDL. КАК ИСТОЧНИКИ ЛЕКАРСТВЕННОГО РАСТИТЕЛЬНОГО СЫРЬЯ ДЛЯ ПОЛУЧЕНИЯ НЕЙРОТРОПНЫХ СРЕДСТВ (ОБЗОР)

Е.В. Звездина[1], Ж.В. Дайронас[2], И.И. Бочкарева[3], И.Н. Зилфикаров[1,3], Е.Ю. Бабаева[1], Е.В. Ферубко[1], З.А. Гусейнова[4], Ф.К. Серебряная[2], С.Р. Каибова[5], Т.А. Ибрагимов[5,6]

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Цель работы – обзор и анализ опубликованных в современной научной литературе данных, полученных в ходе фармакологических, фармакогностических и фармако-технологических исследований образцов лекарственного растительного сырья (ЛРС), заготавливаемого от различных представителей сем. *Lamiaceae* Lindl. (яснотковые), из которых получены биологически активные вещества (БАВ), фармацевтические субстанции, суммарные извлечения и лекарственные препараты, обладающие нейротропной активностью.

Материалы и методы. Для обзора использовали сведения научной литературы из открытых и доступных источников последних двадцати лет, размещенных в научно-технических библиотеках учреждений, а также в электронных базах данных: *Elibrary*, *PubMed*, *Scopus*, Киберленинка, *Google*-академия, *J-stage*. Поисковые запросы – названия видов растений сем. *Lamiaceae* (русские и латинские), заготавливаемых от них образцов ЛРС, а также наименования фармацевтических субстанций, лекарственных препаратов и БАВ.

Результаты. При работе с источниками научной информации основное внимание уделено фармакологическим тестам, проведенным в ходе специализированных исследований на лабораторных животных, подтверждающим наличие нейротропной активности у исследуемых объектов – эфирных масел и извлечений из ЛРС (водных, водно-спиртовых, метанольных). Установлено, что потенциал лечебного и лечебно-профилактического применения фармацевтических субстанций и лекарственных препаратов, получаемых из ЛРС представителей 30 родов семейства *Lamiaceae*, остается нереализованным, несмотря на пристальное внимание исследователей.

Заключение. Данный обзор охватил 71 вид из 30 родов. При значительном уровне изученности, который можно отметить при анализе данной публикации, остается не затронутым огромный пласт ресурсных видов данного семейства. В дальнейшем они могут представлять фармакогностический интерес и иметь практическое использование, в частности, для создания на их основе новых лекарственных препаратов нейротропного действия.

Ключевые слова: обзор литературы, яснотковые, *Lamiaceae,* фитотерапия, лекарственное растительное сырье (ЛРС), экстракт, фитопрепарат, лекарственный препарат, фармакогнозия, фармакология, стресс, нейротропная активность, анксиолитический эффект, седативное действие, антидепрессивное действие, ГАМК-α-рецепторы, бензодиазепиновые рецепторы

INTRODUCTION

In the modern world, in conditions of excessive intense and inadequately prolonged stressful effects of various external factors, a human body needs therapeutic and preventive agents that have a protective neurotropic, or neuroprotective, effect. Stress, especially chronic, is justifiably considered one of the main factors causing the development of a lot of pathologies [1–3]. Psychoemotional stress and constant overwork lead to the appearance of various symptoms, urging a human being to seek treatment. In the stressful situation, adaptive changes are observed on the physiological, mental and behavioral levels. In particular, a Canadian scientist Hans Selye described a triad of changes characteristic for ~~of~~ any severe stress: adrenal cortical hypertrophy, thymus involution, ulceration in the gastrointestinal tract [4]. Stress is characterized by an extreme complexity associated, interalia, with the individual char-

acteristics of a person, and can only be corrected by combining etiotropic treatment with prolonged pharmacotherapy with herbal formulations. All types of stress, which is considered a combination of exogenous and endogenous negative factors that create tension in the human body, are characterized by unspecific reactions of the hypothalamic-pituitary-adrenocortical system гипоталамо-гипофизарно-адренокортикальной системы and disorders of vegetal functions нарушения вегетативных функций of the cardiovascular and hematopoietic systems. In the modern world, an increase in the level of the emotional tension combined with сопутствующими negative factors (strokes, myocardial infarction, atherosclerotic cardiosclerosis, and others), has brought cardiovascular diseases to the first place among the causes of mortality. According to the World Health Organization, every year 17.5 million people die of diseases of the cardiovascular system [5]. Medicines correcting a person's perception of exogenous factors, as well as having a regulatory effect on endogenous stress mechanisms, can help overcome the devastating consequences. The advantage of herbal drugs created on the basis of modern scientific achievements, is the presence of a wide range of biologically active substances with multi-directional therapeutic and preventive agents in their composition. In most cases, these properties are combined with safety and the possibility of a long-term use. Extractive (total) drugs, biologically active food additives, separate fractions of biologically active substances and standardized medicinal preparations obtained from the medicinal plant materials of the members of the family *Valerianaceae* (rhizomes with valerian roots), *Paeoniaceae* (*Paeónia anómala* herb, rhizomes and roots), *Hypericaceae* (*Hypericum perforatum* herb), *Passifloraceae* (*Passiflora incarnata* herb), *Polemoniaceae* (*Polemonium coeruleum* rhizomes with roots), *Lamiaceae* (*Leonurum cardiaca* herb), which have pronounced neurotropic, mainly sedative, activities, are widely known [6]. Meanwhile, the analysis of the results of numerous scientific studies of medicinal plant materials carried out in order to expand the range of drugs with sedative and anxiolytic activites, shows that the greatest attention is paid to the members of the family Lamiaceae. The possibilities of creating new drugs and biologically active additives, therapeutic and prophylactic agents containing biologically active substances from raw materials of plant species of the family Lamiaceae, remain unrealized and need further comprehensive researches.

THE AIM of this work is to review and analyze the data published in the modern scientific literature obtained during pharmacological, pharmacognostic and pharmacotechnological studies of various types of raw materials obtained from members of the family *Lamiaceae*, which were sources of biologically active substances, pharmaceutical substances, total extracts and the drugs with a neurotropic activity.

MATERIALS AND METHODS

For the review, we used the information of the scientific literature from open and accessible sources of the last twenty years, located in the scientific and technical libraries of institutions, as well as in electronic databases: Elibrary, PubMed, Scopus, Cyberleninka, GoogleAcademy, J-Stage. The search inquiries were: the samples of medicinal plant materials based on them as well as the names of the drugs and biologically active substances obtained from these raw materials.

RESULTS AND DISCUSSION

The analysis of the publications on plant raw materials obtained from members of the family Lamiaceae, extracts or separate classes of biologically active substances, makes it possible to conclude that they comprise about 30 genera.

The data of pharmacological tests on the presence of the neurotropic effect of medicinal plant materials of the family Lamiaceae species obtained by researchers mainly in the process of the study of essential oils, their components, aqueous and aqueous-alcohol extracts using the following tests: "The open field", "Light-dark chamber", "Elevated plus maze", "Staircase», "The tail suspension test", "The forced swim test", "The hole-board test", "The *rotarod* test" [7–10].

In addition to conducting standard tests, the locomotor activity of the animals and the duration of the barbiturates' effect against the background of taking the investigated pharmaceutical substances, extracts, medicinal preparations, etc., have been studied. During these studies, neurotropic effects in varying degrees have been experimentally found out: anxiolytic, sedative, antidepressant, hypnotic, or the property of sleep prolongation. It has been established, that the drugs obtained from the medicinal plant materials from the members of the family Lamiaceae L. and having a neurotropic effect, enhance the affinity of gamma-aminobutyric acid (GABA) for GABA-receptors in the subcortical formations, primarily in the reticular formation, weakening its stimulating effect onto the cerebral cortex [11].

In the course of tests for the presence of the neutropic activity, the objects of the investigation were not only the crude total extracts, separate classes of biologically active substances, but also compounds isolated выделенные в чистом виде and obtained by synthetic and semi-synthetic methods. Flavonoids, triterpenic acids (ursolic and oleanolic), phenylpropanoids (rosmarinic and caffeic acids), terpenoids and aromatic compounds as components of the essential oil (linalool, linalyl acetate, thymol, carvacrol, etc.), alkaloids, alkaloid-like compounds and iridoids, are most often studied in this context.

Flavonoids, in most cases flavones, are able to interact with different zones of the GABA-α-receptors and, due to this, affect their functioning.

Neurotropic properties, expressed to a vary-

ОРИГИНАЛЬНАЯ СТАТЬЯ
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ing degree, were found out in the following flavones: hispidulin (5,7,4'-trihydroxy-6-methoxyflavone), apigenin (5,7,4'-trihydroxyflavone), chrysoeriol (5,7,4'-trihydroxy-3'-methoxyflavone), luteolin (5,7,3',4'-tetrahydroxyflavone), scutellarein (5,6,7,4'-tetrahydroxyflavone), baicalin (7-O-glucuronide 5,6,7-trihydroxyflavone), baicalein (5,6,7-trihydroxyflavone), etc.

Flavones interact with the GABA-α receptors, as well as benzodiazepines; these are ones of the most commonly used drugs. It is known that when interacting with allosteric sites of GABA-α, also so-called benzodiazepine sites, the influx of chloride ions into the cytoplasm increases, the inhibitory postsynaptic potential increases, and the excitability of neurons decreases. According to this mechanism, benzodiazepines and flavones act as anticonvulsants, providing sedative, hypnotic and anxiolytic effects [12].

In the experiments on Wistar rats, caffeic acid (3,4-dihydroxycinnamic acid) at the doses of 0.5 and 1.0 mg/kg, has an anxiolytic effect without changes in the locomotor activity in "The open field" and «Elevated plus maze» tests. It has also a protective effect in cases of the damage to the brain tissue by hydrogen peroxide [13].

Rosmarinic acid (caffeic acid dimer) at the doses of 2–4 mg/kg, has an anxiolytic effect, which, when the dose is increased to 8 mg/kg, is replaced by a stimulating effect. No effects on long-term and short-term kinds of memory, have been found out [14].

A neurochemical study showed that caffeic and rosmarinic acids did not affect the absorption of monoamines or the activity of monoamine oxidase; but none of the studies have revealed the possibility of these compounds to alter the transmission of monoamines on their receptor directly [15].

A neurotropic activity has also been established for essential oils components. When administered intraperitoneally, citral has a sedative (100 and 200 mg/kg) and muscle relaxant (200 mg/kg) effects at the doses of 100 and 200 mg/kg and increases the duration of barbiturate sleep [16]. In the experiment on mice, cineol has an anti-anxiety (400 mg/kg), antidepressant effect (200 and 400 mg/kg), does not affect the motor activity and reduces the latency of sleep caused by the administration of pentobarbital [17].

Overview of genera and species – sources of medicinal plant materials with neurotropic properties

Genus *Agastache* J.Clayton ex Gronov

The genus *Agastache* J. Clayton ex Gronov. comprises 22 species of perennial medicinal aromatic plants [18] that live mainly in the North America [19]. Some of these species are used as spicy aromatic, ornamental and nectareous plants, others are used as raw materials for obtaining essential oils and drugs [20].

In the aerial part of *Agastache mexicana* (Kunth. Link.et Epling), the fllowing substances were found out: malic acid, 7-O-β-D-glucoside, luteolin flavonoids, 7-O-β-D-luteolin (6"-O-malonyl)-glucoside, 7-O-β-D-glucoside diosmethine, 7-O-β-D-(6"-O-malonyl)-glucoside diosmetin, 7-O-β-D-glucoside acacetin, 7-O-β acacetin-D-(6"-O-malonyl)-glucoside, acacetin 7-O-β-glucoside-D-(2"-acetyl-6"-malonyl), acacetin, diosmetin, gardenin, 5,6,7,8,3-pentahydroxy-4-methoxyflavone, 8-hydroxy-salvigenin [21].

An aqueous extract from *A. mexicana* leaves of exhibits the antidepressant activity [22]. The results of three different tests ("Elevated plus maze", "The forced swim test", and "The open field") showed an anxiogen-like activity. In the "Elevated plus maze" test, the extract reduced the time spent by animals in the open sleeves. The results of "The forced swim test» did not show any antidepressant effect of the extraction (at the dose of 12.0 mg/kg) in comparison with the results obtained when using pentylenetetrazole (at the dose of 15 mg/kg) and desipramine (at the dose of 32 mg/kg) as reference substances. The extract enhanced the antidepressant effect of desipramine similarly to the effect of the sumaltenious administration of pentylenetetrazole and desipramine. "The open field test" did not reveal any sedative effect of the aqueous extraction from *A. mexicana* leaves in the used doses [23].

Genus *Ajuga* Benth L.

Genus *Ajuga* Benth. comprises ca. 80 species of annual and perennial herbaceous plants, common mainly in temperate zones [18].

Ajuga reptans L. and *A. remota* Benth. – syn. A. *integrifolia* Buch.-Ham, are also of great interest to researchers. In the aqueous-alcohol extract from *A. remota* aerial parts, the following substances were found out: iridoids (harpagide, 8-O-acetylgarpagide, 2,3-diacetylgarpagide, 6,8-acetylgarpagid-O-2,3-diacetylglycoside, 6-ramnosyl-garpagide, 6-halogen-7,8 dehydrogarpagide) and steroids (ciasterone, ergosterone-5.8-endoperoxide) [24].

The presence of the anxiolytic effect was established for cisteron steroids and ergosterone-5.8-endoperoxide from the roots of *A. remota* [25].

The oral administration of cisterone and ergosterone-5,8-endoperoxide (at the doses of 5 mg/kg) isolated from methanol extracts of the underground parts of *A. remota,* led to an increase in the duration of the study in the open sleeves by the animals in the «Elevated plus maze» test, and in the number of head dives in «The hole-board test» ($P<0.05$) compared with the control group. The same compounds (at the doses of 25 and 50 mg/kg, respectively) showed a dose-dependent increase ($P<0.01$) in the number and duration of immersion of the head in the holes, which is comparable to the anxiolytic effect of diazepam and indicates a potential use for relief of anxiety [24].

Genus *Anisomeles* L.

Anisomeles L. .is a genus of herbaceous plants that are outwardly similar to the members of the genus *Nepeta* L., growing mainly in the countries of Southeast Asia, in particular, China, India, New Guinea, Australia, etc.

Anisomeles indica (L.) Kuntze. (syn. Indian catnip, Indian catnip) is of the greatest interest for researches. Its chemical composition is represented by the following compounds: pedalitin, apigenin, methyl gallate, 3,4-dihydroxybenzoic acid, calceolarioside, betonioside A, campneoside II, acteoside, isoacteoside and terniflorin [25].

The methanol extract obtained from *A. indica* aerial part, was tested on Swiss albino mice for sedative properties in "The open field" and "The hole-board" tests, and anxiolytic properties - in the «Elevated plus maze» test. In "The open field" and "The hole-board" tests, a dose-dependent decrease in the locomotor activity was notified. In the «Elevated plus maze» test, the animals under the influence of the investigated extract showed an increase in the percentage of time spent in the open sleeves. The studied extract potentiated thiopental sleep to a lesser extent than diazepam [25].

Genus *Ballota* L.

The members of the genus Ballota L. (about 30 species) are perennial herbaceous plants found mainly in the Mediterranean region [26]. The most studied of them is *Ballota nigra* L., common in the European part of Russia. Flavonoids (rutin, dihydroquercetin), phenylpropanoids (verbascoside, forsitoside B, arenarioside, ballotetroside, isoferulic, ferulic, chicoric, cinnamic, caffeic, chlorogenic acids), coumarins and tannins (epicatechin, epigallocatechin gallate, catechin, gallic acid) were found in the composition of the aqueous-alcohol extracts from *B. nigra* herb. [27–30]. The herb *B. nigra* is officinal and is included in the pharmacopoeias of Britain, France, and Europe [31].

The antidepressant activity of the aqueous alcohol extract from *B. nigra* aerial parts is is associated with phenylpropanoids [29]. A mixture of phenylpropanoid glycosides significantly prolonged the sleep induced by pentobarbital; reduced the locomotor activity in mice and produced a slowing-down of the electroencephalographic trace [32]. The antidepressant activity of the extracts from *B. nigra* aerial parts has been proven by behavioral tests on albinos rats: «The forced swim» and «Elevated plus maze») [33]. Affinity tests with rat striates, the whole brain and the receptor-rich drugs, were used in order to study the ability of phenylpropanoids obtained from the aqueous-alcoholic extract of *B. nigra* aerial parts, to bind to связываться с benzodiazepine, dopaminergic and morphine receptors. The results showed that four out of five phenylpropanoids identified (verbascoside, forsitoside B, arenarioside, ballotetroside and caffeic acid) are able to bind to the studied receptors having a neurosedative effect at the doses from 0.4 to 4.7 mg/ml [29].

Phenylpropanoid derivatives isolated from *B. nigra subsp. Anatolica* aerial parts, are of a therapeutic interest as having also an antioxidant activity [34, 35].

Neurosedative properties of aqueous and aqueous-alcoholic extracts from aerial parts of *B. saxatilis* Sieber ex C. Presl. Species, are widely used in European medicine [36].

The aqueous extract from the aerial part of *B. larendana* Boiss. et Heldr., administered intraperitoneally to albino male rats, showed its anxiolytic activity, and its antidepressant activity was comparable to amitriptyline and *Passiflora incarnate L.* extract [33].

Genus *Clerodendrum* L.

The genus of plants is the subfamily *Ajugoide,* fam. Lamiaceae, comprising approx. 300 species – deciduous shrubs, small trees, sometimes vines, which grow in the tropics and subtropics, mainly in Africa, Central America, Southeast Asia. Some species are grown as ornamental plants [37].

The ethanol extract from *Clerodendrum serratum* L. leaves has an antidepressant effect, without reducing a motor activity, in an acute stress and an induced depressive behavior in mice.

The antidepressant and anxiolytic activity of the extract was investigated in "The forced swim" and «The tail suspension» test. The oxidative effects of the acute stress and biochemical changes in the brain tissue, were also evaluated. A preliminary use of the extract for 7 days, can reduce the damaging oxidative effect of the acute stress and quickly restore the level of norepinephrine and 5-hydroxytryptamine in the brain tissue. Flavonoids, apigenin and luteolin derivatives, were found out by HPLC in the butanol and ethyl acetate fractions of the extract from *C. serratum* leaves of [37].

Genus *Clinopodium* L.

Clinopodium L., the genus of herbaceous plants up to 100 cm high, has about 150 species. The scientific literature mentions *Clinopodium mexicanum* Benth Govaerts (Mexican fragrance). Its medicinal raw material is used as anesthetic and sedative remedies in the traditional medicine of Mexico [38].

Flavone glycoside 2S-neopincirin [(2S)-5-hydroxy-4'methoxyflavonone-7-O-{β-glucopyranosyl-(1→6)-β-ramnoside}] was found out in aqueous and methanol extracts from *C. mexicanum* leaves. In the experiments on mice, it had an anxiolytic effect associated with an effect on GABA receptors [38].

Genus *Dracocephalum* L.

The genus of herbaceous plants *Dracocephalum* L. has about 60 species occuring in the temperate climatic zone of the northern hemisphere [39]. The most studied one is *Dracocephalum moldavica* L., which is a promising

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medicinal plant. The species grows everywhere in the Black Sea region, the European part of Russia, in Siberia, Central Asia, the Far East, China, Mongolia, etc. It was introduced into the culture as a spicy-aromatic, ornamental and medicinal plant. The aerial part of the plant accumulates up to 0.15% essential oil, which contains up to 70% citral, as well as geraniol, thymol, nerol [40].

The aqueous extract from *D. moldavica* aerial part, dose-dependently reduced a number of transitions in the avoidance test. This effect can be considered anxiolytic; however, the same doses also induced a significant reduction in the total activity of the mice in "The open field" test compared to the control group. This behavior cannot be considered an anxiogenic effect, since it is a consequence of a decrease in the activity of the animals due to the sedative effect of the drug. The obtained results are similar to those observed at a high dose of diazepam; in them, diazepam also induced a decrease in the number of transitions between the light and dark compartments in the avoidance test and overall activity in "The open field" test.

The aqueous extract from *D. moldavica* aerial part, has sedative and muscle relaxant activities, reduces a locomotor activity and leads to the general inhibition of the neuron activity in the central nervous system of the experimental animals. Most likely, flavone glycosides present in the extraction, contribute to the sedative effect [41].

Genus *Eremostachys* Bunge (*Phlomoides* Bunge)

The genus of *Eremostachys* Bunge herbaceous plants includes about 140 species, growing mainly in Central Asia. The most studied species is *Eremostachys laciniata* (L) Bunge –syn. *Phlomoides laciniata* (L.) Kamelin & Makhm. Pronounced sedative properties were found out in its aqueous-alcoholic extraction from the aerial part, in which flavonoids were identified (luteolin, apigenin, 5,8-dihydroxy-6,7-dimethoxyflavone, 5,7-dihydroxy-6,8-dimethoxyflavone, luteolin 7- O-β-glucoside) [42].

In «The forced swim test», the aqueous extract from *E. laciniata* aerial parts *in vivo* at the low doses showed an antidepressant effect, and at the higher doses - a depressive one.

The authors of the study consider that the antidepressant property is associated with the presence of apigenin derivatives in the extraction of flavonoids. And a depressive property, expressed in an increase in the duration of immobility and observed at higher doses of the extraction, is due to the sedative effect of luteolin. *E. laciniata* medicinal raw material can be a potential source for obtaining antidepressant medicinal preparations [42].

Genus *Hyptis* Jacq.

Members of the numerous genus *Hyptis* Jacq. (up to 300 species) are represented by annual and perennial herbs, shrubs and small trees prevalent mainly in the tropical and temperate zones of North and South America [43]

The aqueous extract from *Hyptis spisigera* Lam. leaves has a sedative effect, increases the duration of sleep induced by diazepam. The sedative activity can be associated with the presence of components that potentiate benzodiazepine and / or activate GABA receptors [43].

Genus *Hyssopus* L.

The genus *Hyssopus* L. has at least 7 species of perennial plants, among which there are herbs and shrubs growing in the Mediterranean, Asia Minor, Middle Asia, the Caucasus, southern Siberia [40]. Some members were introduced into the culture as sources of spicy aromatic raw materials and essential oils. The most studied is *Hyssopus officinalis* L. (Hyssop officinalis), shrub up to 80 cm tall, growing mainly in Africa, Western Asia. It is cultivated as an essential oil and spicy aromatic plant. The herb contains an essential oil (up to 2%), flavonoids (hesperidin, hyssopine, etc.), triterpene acids, bitter substances, etc. [40].

H. officinalis is official in several European countries. For a long time, the extracts and essential oil have been used in diseases of the upper respiratory tract and gastrointestinal tract, as well as an antiseptic. Currently, *H. officinalis* is the subject of numerous pharmacological studies. It was established, in particular, that the extract from the herb obtained by the extraction with 70% alcohol in the intragastric administration to white rats, leads to a significant increase in sleep duration – by 55% relative to the control (nembutal) and by 52% relative to the comparison object (alcohol + nembutal) [40].

Genus *Lagochilus* Bunge

A member of this genus is *Lagochilus inebrians* Bunge – a medicinal plant well-known in folk and official medicine. *L. inebrians* is a shrub growing in Central Asia, its areal is very limited. *L. inebrians* flowers and leaves contain a typical tetrahydric alcohol lagohillin, tannins (up to 14%), vitamins, organic acids, essential oil (about 0.03%), etc. *L. inebrians* aqueous extracts have adaptogenic, hypotensive and sedative activities, an anticonvulsant property, they reduce pain sensitivity, have an antispasmodic effect. Its infusion and tincture enhance blood coagulation [45].

The use of medicinal preparations from *L. inebrians* herbal raw materials gave positive results when obtained in the treatment of neuroses. It was determined that they normalize the balance between inhibition and excitation in the nervous system, inhibit the vestibular analyzer, which has been successfully used in the treatment of Meniere's disease. Thanks to the sedative effect of *L. inebrians,* reduce blood pressure [45]. Previously, *L. inebrians* medicinal preparations were used in medical practice; nowadays the State Register of the Russian

Federation does not include these drugs from *L. inebrians* medicinal plant materials [6].

Genus *Lallemantia* L.

The genus *Lallemantia* L. comprises several species; the most famous of them are: *Lallemantia iberica* F. et M., *L. royleana* (Benth. In Wall.) Benth. (l. Royle) and *L. canescens* L. (l. grayish) [46].

Asia Minor, Transcaucasia, Iran and the mountainous regions of Turkmenistan are considered the *habitats* of *Lallemantia* where it has long been widespread as a wild-growing and a weed-field plant, most often in flax crops. In the wildlife species, В диком виде *Lallemantia* is found in Syria, Mesopotamia, Afghanistan, as well as in the Crimea, in the south of Ukraine, along the eastern coast of the Caspian Sea and in the North Caucasus [46]. *Lallemantia* fruits contain fatty oil, therefore *l. Iberian's* oilseed is cultivated in the countries of the Middle East.

Sugars (mannitol 14.78%, saccharose сахароза 9.36%), fatty oil and fatty acids, essential oil, coumarins, flavonoids, alkaloids were found in the aqueous-methanol extract from *L. royleana* fruits [47]. Fatty oil contains the following acids: linoleic (up to 26%), palmitic (up to 10%), oleic (up to 60%), stearic (about 3%), etc. Aqueous and aqueous-alcohol extracts from fruits are used with insomnia, increased nervous excitement, as well as diseases of the gastrointestinal tract [47].

In the study by Hyder et al., the anxiolytic and sedative effects of the aqueous-methanol extract from *L. royleana* fruits were studied (after the removal of the extractant) in mice. To test the anxiolytic activity, the following tests were used: "The open field test», "The hole-board test", "Elevated plus maze", "Light-dark chamber" and "Stairscase", the reference drug was diazepam. The results showed that the test extract has an anxiolytic effect, maximally expressed at the dose of 250 mg/kg [47].

Genus *Lavandula* L.

The genus Lavandula L. is represented by perennials, mainly semi-shrubs, has about 50 species, distributed mainly in the Mediterranean [48].

The most common and actively studied species is *Lavandula angustifolia* Mill., syn. *L. officinalis* Chaix. This is a perennial, evergreen, highly branched shrub, 60–70 cm high, widely grown as an aromatic and medicinal crop. Shoots, leaves and inflorescences contain essential oil up to 2%, in the composition of which linalool (up to 80%) and its esters, linalyl acetate, terpinen-4-ol, lavenderulol acetate, octimene, cineole have been found out; anthocyanins, phytosterols, tannins have been detected in the aqueous-alcoholic extract [48]. *L. angustifolia* extracts from flowers and essential oil are used in the traditional medicine for migraines, neurasthenia, as an anticonvulsant and a sedative drug. In the study carried out on pigs, the anxiolytic activity has been confirmed. A significant decrease in the motion sickness and stress in the animals during transportation (measured by the concentration of cortisol in saliva) was observed in the animals when the floor of the vehicle was covered by lavender (*L. angustifolia)* [49].

Aqueous and aqueous-alcoholic extracts from *L. angustifolia* aerial parts (at the doses of 100–400 mg/kg), which were studied in comparison with fluoxetine, had an antidepressant effect. In the mice, the duration of immobility was significantly reduced in «The forced swim test» and «The tail suspension test». The inhalation of *L. angustifolia* flowers essential oil, induced an increase in the level of serotonin and its metabolites in pregnant women's plasma during childbirth [50].

Clinical trials on the study of the hypnotic properties of *L. angustifolia* flowers essential oil have shown an increase in sleep time with its use. A clinical study of a group of 245 people was conducted. 72% of patients inhaling lavender oil, experienced a healthy sleep, unlike 11% in the control group. About 80% of the study participants reported overall well-being, in contrast to 25% of those in the control group [49].

Besides *L. angustifolia, L. spica* L. (syn. *L. latifolia* Medik.) extracts from the aerial parts are also widely used in traditional medicine for the treatment of asthenia and depression. *L. spica* flowers contain essential oil with a higher content of camphor and cineole, in comparison to the content of *L. angustifolia* flowers essential oil. A liquid extract obtained from *L. spica* flowers (the extractant was 40% ethyl alcohol), has an established sedative activity. It was notifed that the activity of the extract is associated with the presence of lavenderoside phenylpropanoid (4-O-β-D-glucopyranoside 4-hydroxy-3-methoxy cinnamic acid) in its composition. In addition to these substances, flavonoids cinaroside and cosmosiin were also found in *L. spica* flowers [51–53].

Another species studied as a promising medicinal plant is *L. stoechas* L., which is common and cultivated in the Mediterranean countries. A sedative and hypnotic effect was established in water-methanol extraction (after removal of the extractant) from *L. stoechas* flowers – it contributed to an increase in the duration of pentabarbital sleep in mice by analogy with diazepam [54].

Genus *Leonotis* L.

This genus of perennial herbaceous plants amounts to 9 species, most of which grows in tropics, mainly in South Africa [55]. The most studied species *is Leonotis nepetifolia* L., which is common in tropical Africa and South India. Extracts from its stems are used in traditional medicine as a sedative drug. Methanol extract (after removal of the extractant) obtained from *L. nepetifolia* stems, in intraperitoneal administrationat to the mice at the doses of 37.5 mg/kg, 75 mg/kg and 150 mg/kg was studied. It was established that the mass content of LD_{50} is 3.8 g/kg. The results showed that in all the studied doses, the extract did not have a noticeable effect on the research activity and coordination of the animals' movements. However, at a dose of 150 mg/kg, it induced a

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significant decrease in the number of collected grains in the "Staircase" test, which was also observed with the administrationat of an anxiolytic dose of diazepam, and also significantly increased the duration of sleep induced by diazepam. Preliminary phytochemical analysis showed the presence of alkaloids, saponins, glycosides and triterpenoids in the extract. The results obtained indicate that the crude methanol extract from *L. nepetifolia* stems has an anxiolytic activity, which explains the traditional use of the decoction of this plant as a sedative and tranquilizing agent [55].

Genus *Leonurus* L.

The Leonurus L. genus of perennial, or biennial plants, includes about 25 species growing around the world: in Europe, Asia, Africa and America; 13 species are found in Russia [56]. The most famous European members of the genus are *Leonurus cardiaca* L. and *L. quinquelobatus* Gilib., and in East Asia it is *L. japonicus* Houtt.

For a long time, the drugs obtained from the herbs of these species, have been widely used in traditional and official medicine for anxiety, neurosis, insomnia, as a sedative remedy, for epilepsy, and for the treatment and prevention of cardiovascular diseases [57]. In *Leonurus* L. aqueous-alcohol extracts, iridoids responsible for sedative and hypnotic properties were discovered: monoterpene compounds with partially hydrated cyclopenta / s / pyran system (ayugol, ayugozid, harpagide, harpagide acetate), phenylpropanoids (coffee, ferricoric acids), flavonoids (rutin, hyperoside, quercetrin), nitrogenous bases (leonurine and stachidrin or leonuricardin), tannins [58–60].

The State Register of Medicines of Russia includes *Leonurus* L. medicinal plant material for the preparation of the following infusions and medicines: *Leonurus* L. tincture, *Leonurus* L. extract, Lily of the valley- *Leonurus* L. t drops, Corvalol Neo (diphenhydramine + peppermint oil, oil + *Leonurus* L. tincture + ethyl bromisovalerianate), «Corvalol Fito» (peppermint leaf oil + *Leonurus* L. tincture + ethyl bromizovalerianate), «*Leonurus* L. Forte Evalar» (*Leonurus* L. + [magnesium asparaginate + pyridoxine]), «Calming collection No. 3» (Valerianae officinalis rhizomata cum radicibus+meliloti herba+Origani vulgaris herba+leonuri herba+Thymi serpyllum herba) [6]. Medicinal plant preparations obtained from *L. quinquelobatus,* have sedative properties, regulate the functional state of the central nervous system, lower blood pressure, slow down the rhythm and increase heart rate [61]. The mechanism of neurological action of *L. cardiaca* and *L. japonicus* extracts is based on the interaction with the GABA-α receptor [62]. Neuromodulating and neuroprotective effects of *L. japonicus* extract are associated with the presence of (leonurine, stachydrin) and triterpenoids (leonuruzoleanolide A) in their composition, and a sedative effect is associated with iridoids (stegioside). *L. japonicus* tincture also inhibits 5-HT3A receptors, the antagonist of which is Leonurin with an IC50 of 2.17 ± 0.15 mM. Since this receptor is involved in gastrointestinal motility disorder, *L. Japonicas* medications can be used to treat vomiting and nausea. [63].

Due to the ability to reduce mental stress, *L. Japonicas* drugs are used as sedative remedies for increased nervous excitability, in the early stages of hypertension and sleep disorders. Like other sedatives, they are able to facilitate the onset of natural sleep.

The neuroprotective effect of the synthesized alkaloid-like nitrogenous base of leonurin on nerve cells in the model of an ischemic stroke in rats, is mainly due to a decrease in the formation of the active oxygen forms, which supports a correct functioning of mitochondria and, therefore, apoptosis is inhibited. It is supposed that leonurin can be used to prevent and treat ischemic strokes due to its antioxidant properties and participation in the mechanism of apoptosis [64].

L. quinquelobatus drugs have not only a sedative but also antidepressant effect [61, 65]. In the experiments on rabbits and mice, the sedative effect of *L. Japonicas* tinctures was confirmed. Phenolpropanoid lavandulifolioside was isolated from *L. cardiaca* var. vulgaris Briquet butanol fraction. It has a pronounced negative chronotropic activity (reduces heart rate), the ability to change the parameters of the electrocardiogram (ECG), namely, to extend the intervals of the P-Q and Q-T QRS complex (ventricular complex) and reduce blood pressure. In his study, it was also established that it was not responsible for the sedative effect, since even at the doses of 800 and 1600 mg/kg only slightly reduced the mobility of mice [64]. Unlike the total butanol extract, lavandulifolioside does not reduce spontaneous locomotor activity, therefore its properties do not reflect all the pharmacological effects of *L. cardiaca* drugs [66]. Significant sedative effect was observed under the influence of *L. cardiaca* extract obtained with 30% ethanol. In the «Elevated plus maze» test, the extract increased the time spent by mice in open sleeves by 4 times, decreased a spontaneous activity twice, and increased the duration of sleep induced by barbiturates by 3 times. Similarly, the aqueous extraction of *L. cardiaca* was studied, which, when administered intraperitoneally, induced a decrease in motor activity in mice [64]. In the study [67], the authors compared the sedative activity of motherwort and valerian tinctures on rabbits with electrodes on their hind legs.

The values of the direct current intensity required to contract flexor muscles after the administration of tinctures were measured. It was found out that under the influence of the motherwort tincture there is an increase in the measured values to a greater extent than under the influence of the valerian tincture, which is associated with its strong inhibitory effect on the central nervous system [67].

The Iridol oil extract was worked out from *L. cardiaca*, *L. quinquelobatus,* then standardized by the content of iridoids in it and packaged in soft gelatin capsules.

In the experiments on the outbred male rats, the anxiolytic activity of Iridol was established, comparable with the effect of diazepam. During clinical observations it was found out that the studied drug increases the effectiveness of complex therapy in the treatment of arterial hypertension accompanied by psychoemotional disorders, reduces the dose of antihypertensive drugs, while the activity of the new drug exceeds that of *L. cardiaca*, *L. quinquelobatus* tincture [59].

L. cardiaca oleoresin was administered to 50 patients (300 mg 4 times a day for 28 days) with the first (22 patients) and second (28 patients) degrees of hypertension and symptoms such as anxiety and sleep disturbances.

In the patients with the first degree of hypertension, a reduction in symptoms of anxiety, emotional instability, headaches and sleep disturbances was achieved. After 21 days, there was a significant decrease in the level of blood pressure and its normalization (from 145/96 to 130/87), the patients' state of health and mood were improved, their activity increased and the fatigue decreased. A decrease in heart rate (from 81.7 to 75.4) was not statistically significant. A significant lowering of blood pressure (from 153/103 to 142/92) in patients with the second degree of hypertension, occurred a week later than in the first group. The psycho-emotional state of patients (anxiety, emotional pain, headaches and sleep disturbances) improved seven days before lowering blood pressure. Antihypertensive, anxiolytic and soothing effects could be induced by *Leonurus cardiaca* iridoid extracts [64].

Clinical researches. Arushanyan et al. studied the effect of *Leonurus* L. tincture and benzodiazepine anxiolytic grandaxin on anxiety and light perception in clinical trials in 26 volunteers with increased anxiety. The volunteers were divided into three groups, and a control group consisted of 12 patients without emotional disorders. It was established that in its activity, grandaxin slightly exceeded *Leonurus* L. tincture [68].

In randomized clinical trials, the sedative effect of *Leonurus* L. drugs was established. It was expressed in improving the quality of sleep, reducing the frequency of awakenings and nightmares, as well as in the general psycho-emotional state. Neurotropic effects were accompanied by a decrease in blood pressure [64].

In a double-blind randomised clinical trial, the sedative effects of tablets containing *Leonurus* L. (50 mg), valerian rhizomes with roots (170 mg), мелиссы balm lemon leaves (50 mg) and hop fruit systems (50 mg) were compared with a placebo. The study group consisted of 50 males (the average age was 45.6 years) suffering from alcohol withdrawal syndrome with sleep disorders (from mild to severe insomnia), anxiety and irritability. The patients, divided into two groups, received the preparation an hour before bedtime, once a day, and the next day they were given a placebo. Compared with the placebo, a significant improvement in sleep quality and a decrease in the frequency of awakenings and nightmares proved a mild sedative effect of the product used; however, it caused drowsiness the following day. Motherwort could, therefore, be helpful, to some extent, in disorders associated with alcohol abstinence.

A study to determine if the administration of sedatives, including *Leonurus* L. tincture, decreases the limitation of the retina ability to distinguish colours caused by the state of anxiety, was also carried out. The experiment involved 26 healthy volunteers with a diagnosed state of nervous anxiety. They were divided into three groups, and a control group consisting of 12 patients without emotional disorders. A decrease in anxiety and an improved ability to distinguish colours, both after the application of tofizopam (benzodiazepine derivative) (for 10 days) and *Leonurus* L. tincture, were registered, yet the anxiolytic effect of tofizopam persisted longer (up to one month after cessation) in comparison with the tincture. The positive effect of the treatment on vision, may have resulted from an impact on the GABAergic system in the retina and the brain structures connected with it.

In another experiment, 21 young patients (divided into three groups) with mild symptoms of anxiety and depression, were given melatonin, *Leonurus* L. tincture or placebo for 10 days (the control group was made up of 10 healthy volunteers). The quality of sleep and the emotional state of the patients, as well as the function of their retina, i. e., the threshold of excitability of light stimuli and the time of the sensorimotor reaction of the vision process, were evaluated before and after the use of the drugs. The administration of melatonin led to the increased sensitivity of the retina to light and an accelerated sensorimotor reaction. The effect of *Leonurus* L. tincture on the vision process after the administration of *Leonurus* L., was statistically insignificant, and the sleep quality improved only in some patients. The anxiolytic activity of *Leonurus* L. tincture was confirmed, but it was weaker compared to melatonin.

Genus *Leucas* L.

The genus *Leucas* L. has more than 130 species of herbaceous plants that are widely distributed in Africa, South and East Asia, India, China, Japan, and the islands of the Indian Ocean. In the scientific literature, studies of *Leucas lavandulifolia* Sm. methanol extract in mice and rats using models of psychopharmacological profiles, are referred to. The extract contains alkaloids, flavonoids, phenols, tannins, carbohydrates, proteins and amino acids, and in the experiments it showed a decrease in the animals' spontaneous motor, search and muscle kinds of activity, as well as potentiation of pentabarbital sleep in the mice [69].

Genus *Lycopus* L.

The genus *Lycopus* L. has 21 species of perennial herbaceous plants. The most famous and studied of them is *Lycopus europaeus* L., which is found throughout Europe, the European part of Russia and Siberia [70].

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In "The hole-board test" the methanol extract from *L. europaeus* aerial parts (after the removal of the extractant) containing flavonoids, terpenes, saponins, has a pronounced sedative effect at the doses of 200, 400 and 600 mg/kg (p.o.). In that extract, diazepam was used as a reference drug. At the doses of 800 and 1000 mg/kg, the extract increases the duration of thiopental sleep. Thus, it was found out that methanol extract has pronounced sedative and hypnotic effects, which confirms the possibility of its therapeutic use for insomnia [70].

Genus *Melissa* L.

The genus *Melissa* L. includes, according to various authors, from 2 to 10 species [71, 72]. The greatest application as a spicy aromatic, food and medicinal culture is *Melissa officinalis* L. The place of *M. officinalis* origin is the eastern Mediterranean region, where it is found in wild nature [73–76]. It is also cultivated in many countries of the world, where it is included in the registers of pharmacopoeial and aromatic plants [73, 77–80]. In Russia, it is officially included in the State Pharmacopoeia (14-th ed.) [81].

In medicine, *M. officinalis* aqueous extracts have been used for thousands of years as they have sedative, anxiolytic, antidepressant, antispasmodic, immunomodulating, antiviral, antimicrobial, antioxidant and antiallergic properties [82, 83].

M. officinalis aerial part contains essential oil (0.02–0.20%). Its main components are: citral, geranial, citronellal, neral, geraniol [85]. In addition to the essential oil, the aerial part of the plant contains phenylpropanoids (rosmarinic and caffeic acids, etc.), flavonoids (apigenin, cosmociin, luteolin, cinaroside, etc.), tannins and coumarins [72, 75, 86–92].

In the experiments on mice, the sedative effect of *M. officinalis* extract of the aerial parts, was discovered by French scientists in 1889 [92]. The total crude extract obtained from *M. officinalis* extract induced sleep when a prehypnotic dose of pentobarbital was administered, and lengthened sleep after a hypnotic dose of pentobarbital [78, 79, 93]. In Lin et al [94], the antidepressant activity of *M. officinalis* aqueous extract was established by its effect on the behavior of rats under the conditions of «The forced swim test».

The behavioral effects, activ and subactive, p.o. administration of *M. officinalis* ethanol extract (after the removal of the extractant), were evaluated in Wistar male rats in the «Elevated plus maze», «The forced swim « and «The open field» tests. Diazepam and fluoxetine were used as reference preparations. As a result, it was established that the psychoactive properties of *M. officinalis* extracts can provide a unique pharmacological alternative for the treatment of certain mental disorders; however, the efficacy appears to depend on both gender and duration of the administration [95].

In a double-blind, randomized, placebo-controlled clinical trial, the efficacy and safety of lyophilized dried aqueous extract from *M. officinalis* leaves in adults suffering from mild tachycardia, were evaluated. The results showed that a 14-day course of treatment with the study drug, reduces heart rate and significantly reduces anxiety in patients, compared with placebo. It has been established that a continuous use of such an extract relieves stress. In addition, it does not have any pronounced side effects [96].

In another study, the effect of a continuous administration of the same extract was studied. Hereby, with moderate stress factors, the presence of an anxiolytic effect not changing the level of activity, was established [97].

In a double-blind, placebo-controlled, randomized study, the ability of *M. officinalis* total extract in a single dose of 600 mg to alleviate symptoms of laboratory-induced stress in healthy individuals, was found out. The most likely mechanism of *M. officinalis* drug action, is inhibition of the acetylcholinesterase in the central nervous system and a decrease in the activity of nicotinic and muscarinic receptors in the cerebral cortex [98].

Genus *Mentha* L.

The genus *Mentha L.* includes 25 species of perennial herbaceous plants [19]. The aerial part of the members of the genus is characterized by a high essential oil content, in the composition of which menthol, its esters and related compounds, as well as α-pinene, limonene, cineole, dipentene, pulegon, β-fellandren, etc. were found out [99, 100].

Flavonoids (naringenin, hesperidin), tannins, organic acids, carotenoids, and other biologically active substances were found out in the aerial parts and in *Mentha* L. aqueous extracts [101, 102]. The plants of this genus are widespread throughout the world, they are used in cooking and food industry as a spicy aromatic culture. The most studied and widely cultivated is *Mentha piperita* L. (peppermint) – a pharmacopeia species obtained by hybridization and consisting of many varieties.

M. piperita leaves serve a source of the essential oil and menthol. *M. piperita* infusion of leaves and collections containing leaves, has sedative, antispasmodic, choleretic, antiseptic and anti-inflammatory properties. The administration of *M. piperita* leaves extract to laboratory animals for 5 weeks, eliminates the effect of stress on plasma corticosterone and the metabolism of serotonin and dopamine in the brain, and the reduction in the animals' anxiety was also registered. The results are consistent with the anti-stress effect of *M. piperita* and suggest the role of cerebral serotonin and dopamine [104].

Mentha arvensis L., characterized by polymorphism, is of the greatest interest of the wild species of mint. It is very widespread in Russia and neighboring states in the temperate climate zone. *M. arvensis* infusion is used in traditional medicine as a sedative and appetite enhancer.

M. arvensis chemical composition is represented by essential oil (monoterpenoids and sesquiterpenoids: menthol, isomenthol, pinene, myrcene, linalool, geranial, camphene, sabinene, limonene), flavonoids (linarin), higher fatty acids (linolenic, linoleic, oleinic) [60].

It was notified that *M. arvensis* methanol extract from the leaves, potentiates pentabarbital-induced sleep [104].

Another member of this genus, investigated for the presence of neurotropic activity, is *M. aquatica* L., which grows in moist soil or along streams, and is widespread in Europe, Northwest Africa, central Russia and Asia.

Naringenin (5,7,4'-trihydroxyflavanone), isolated from *M. aquatica* aerial parts, has a pronounced anxiolytic effect. Intraperitoneally administered at the dose of 100 mg/kg, naringenin led to a significant decrease in basic and fine motor skills ($P<0.05$). The combination of naringenin (100 mg/kg) with midazolam (1.5 mg/kg) led to more significant anxiolysis compared to naringenin (100 mg/kg) with flumazenil at the dose of 3 mg/kg ($P<0.05$) [105].

Genus *Nepeta* L.

The genus of annual and perennial *Nepeta* L herbs, has about 250 species found in the temperate climatic zone of Europe, in Asia, North Africa, in the mountains of tropical Africa, etc. [72]. The main attention of researchers is drawn to *Nepeta cataria* L. (catnip), a perennial herbaceous plant found in the wild and also introduced into the culture. The aerial part of *N. cataria* contains up to 3.0% of essential oil containing more than 70% of nepetalactone, as well as terpineol, borneol, menthol, isomenolit, pinene, citral, linalool, geranial, camphene, sabinen, limonene. Tannins, flavonoids, phenylpropanoids, iridoids (nepetalactone, epinepeta-lactone, methylnepetonate), terpenoids, saponins were found in *N. cataria* aqueous-alcohol extract [60, 106]. Grown as a spicy aromatic culture, the infusion of *Nepeta* L herbs is used in folk medicine.

In addition to *N. cataria*, *N. grandiflora* Bieb and *N. persica* Boiss are of interest, too. Aqueous-alcohol extracts with an anxiolytic activity were obtained from their aerial parts [107]. *N. persica* also contains essential oil in which ne-petalactones and linalool were found out [108, 109]. Nepetalactones contained in essential oil from herb of representatives of the genus *Nepeta* L. possess anxiolytic, sedative and hypnotic activity [108, 109].

In Rabbani et al. (107), the effect of the aqueous–alcohol extract from *N. persica* aerial parts on the behavior of the laboratory animals in the «Elevated Plus Maze» test was investigated. When intraperitoneally administered to male NMRI mice, the studied extract at the dose of 50 mg/kg significantly increased the number of entries and the time spent in the open sleeve. This dose did not affect the locomotor activity of the animals and the duration of their sleep induced by ketamine. At the dose of 100 mg/kg, the extract increased the locomotor activity. Thus, it was established that the extract from *N. persica* aerial parts at the dose of 50 mg/kg, has an anxiolytic effect with less pronounced sedative and hypnotic effects than diazepam, and induces non-specific stimulation at the dose of 100 mg/kg [107].

N. cataria essential oil and nepetalic acid, significantly increased sleep induced by hexobarbital [110].

N. cataria aqueous–alcohol extracts showed two-phase effects on chick behavior: low and moderate dose levels (25–1800 mg/kg) led to an increase in the number of chicks falling asleep, while high dose levels caused a decrease in their number [111].

In Formisano et al. [112], the object of the research was *N. sibthorpii* Benth, a perennial herbaceous plant distributed in Greece, southern Albania and in south-eastern part of former Yugoslavia (now Northern Macedonia). In the experiments on rodents for the presence of a neuropharmacological activity, the preparations obtained from the herb were studied – the methanol extract (after the removal of the extractant), essential oil and the essential oil fraction containing epinepetalactone. All the drugs made changes in the general picture of behavior and potentiation of sleep induced by sodium pentobarbital. CNS depression is most likely associated with GABA-mediated effects of epinepetalactone. A sedative activity of ursolic acid isolated from *N. sibthorpii* herb, was also evaluated in mice. When administered orally at the dose of 2.3 mg/kg, ursolic acid had a significant depressant effect on the central nervous system. It was manifested in a decrease in the spontaneous motor activity [113].

N. sibthorpii ursolic acid isolated from the herb, has sedative and anticonvulsant effects [113]. The ursolic acid activity can be mediated through the GABA-energy system because it increases the waiting time for attacks induced by pentylenetetrazole (PTZ), a GABA-α receptor antagonist. In addition, ursolic acid exhibits a moderate affinity for the GABA-α receptor benzodiazepine site [112].

The paper presents data from a study of extracts from herb *N. glomerulosa* Boiss. – the total extract and its fractions – water, ethyl acetate and butanol. Studies were conducted in mice, it was found that all studied extracts at a dose of 50–200 mg/kg increased the duration of sleep induced by diazepam [113].

In Hosseini et al. [113], the data on the study of *N. glomerulosa* Boiss. (K. glomerular) extracts – the total extraction and its fractions (aqueous, ethyl acetate and butanol) are given. The studies were conducted on mice. It was found out that all the studied extracts at the dose of 50–200 mg/kg increased the duration of sleep induced by diazepam.

Clinical study. N. menthoides lyophilized aqueous extract was used in the treatment of depression. Twenty-two patients participated in a double-blind, randomized, controlled trial between April and September 2015. The patients were from two psychiatric clinics at Shiraz Medical University (Republic of Iran). Based on

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the structured clinical survey, as defined in the Diagnostic and Statistical Manual of Mental Disorders (5th ed.), the patients met the basic criteria for depression. They were randomly grouped to take *N. menthoides* extract or sertraline for 4 weeks. Compared to the control group, in the group receiving *N. menthoides* extract, the average values of the Beck questionnaire for detecting depression were significantly higher. In this group, which was examined within 2 weeks after the intervention, a lower relapse rate was detected. As a herbal formulation**,** the extract can be successfully used to normalize mood in patients with severe depression, since it was established that *N. menthoides* lyophilized aqueous extract has an antidepressant effect and prevents the relapse of depression [114].

Genus *Ocimum* L.

The members of the genus *Ocimum* L. are annual, less frequently short-living perennial herbaceous plants, sometimes sub-shrubs that grow wild in South America, Iran, China, the south of European Russia, the Caucasus, Central Asia and the Far East. *Ocimum* L. is cultivated in Western Europe, Asia, Africa, America. The genus has about 70 species. The greatest attention of researchers is drawn to *Ocimum basilicum* L., *O. sanctum* L. – syn. *O. tenuiflorum* L. (Tulsi) and *O. gratissimum* L. [115].

O. basilicum flowering aerial part (herb) contains 1.0–1.5% of essential oil, the main components of which are monoterpenes of a phenolic nature, anthocyanins, as well as phenolic glycosides, organic acids, vitamins, etc. [115]. The neurotropic properties of O. basilicum aerial part are associated with phenolic compounds and the essential oil containing methylchavicol (42.8%), geranial (13.0%), neral (12.2%) and β-caryophyllene (7.2%) [117]. O. basilicum is widely grown to obtain spices, its aqueous extract is used in folk medicine.

In the experiments on animals, anxiolytic, sedative, antidepressant, antistress effects of *Ocimum* L. essential oil and extracts from herbal raw materials of the species of the genus were established.

In experiments on the anxiolytic and sedative activities, the male Syrian mice were injected intraperitoneally with *O. basilicum* aqueous-alcohol extract at the doses of 100, 150 and 200 mg/kg and essential oil at the dose of 200 mg/kg 30 minutes before the test. It was found out that anxiolytic and sedative effects of the essential oil are higher than those of the aqueous-alcohol extract with the same doses. The extraction at the doses of 150 and 200 mg/kg and the essential oil at the dose of 200 mg/kg significantly increased the time spent by the mice in the open sleeves compared to the control group. None of the doses had a significant effect on the number of entries in the open sleeves. The aqueous-alcohol extracts, like the essential oil, reduced a locomotion of the mice compared to the control group [116].

An aqueous-alcohol extraction from *O. basilicum* leaves (in the author's designation "Sent-Ocim") prevents a depressive behavior in the rats sensitized with ovalbumin [117]. The animals were divided into three groups: the first was control, its animals were injected with saline, the second one was sensitized with ovalbumin, hereby, the extract was not used. The third group was divided into three subgroups, which were injected with hydroalcoholic extracts at the doses of 50, 100 and 200 mg/kg (Sent-Ocim 50, Sent-Ocim 100 and Sent-Ocim 200) against the background of sensitization with ovalbumin. In the «The open field» test, the number of intersections of the central zone was observed, the avoidance of which was directly associated with depression. The number of intersections of the central zone by the sensitized group animals was lower, and the number of intersections in the peripheral zone was higher than in the control group ($P<0.05$–$P<0.01$). The influence of Sent-Ocim 200 extraction significantly increased the number of intersections of the central zone ($P<0,05$), and in the subgroups with Sent-Ocim 50, Sent-Ocim 100 and Sent-Ocim 200, the number of intersections in the peripheral zone was lower than in the sensitized group ($P<0.01$–$P<0.001$). In the «The forced swim test», the immobility time in sensitized rats was higher, and the swimming and climbing times were lower than in the control group ($P<0.05$–$P<0.001$). In the animals of Sent-Ocim 200 group, the mobility was higher, just as the duration of swimming and climbing compared to the sensitized animals ($P<0.01$–$P<0.001$) [117].

O. gratissimum is an aromatic medicinal plant found in wild nature or cultivated throughout the tropics and subtropics. Hybrid *O. gratissimum* and *O. menthifolium* Hochst is known as *b. eugenolic*, which is a medicinal and spicy aromatic culture. It is used in official and traditional kinds of medicine. *B. eugenol* fresh leaves and inflorescences contains up to 0,6% of the essential oil. Its main component is eugenol and its esters [118].

A study on male albino mice showed that the methanol fraction of the extract obtained from *O. gratissimum* fresh leaves, has anxiolytic properties [118]. In order to detect sedative, anxiolytic, antidepressant and motility-coordinating effects of the influence of *O. gratissimum* essential oil on mice, the tests were performed using the "The open field», "Light-dark chamber", "The rotarod" and "The tail suspension" tests. The essential oil demonstrated calming, anxiolytic and antidepressant effects in mice and did not cause harmful effects on their motor coordination, which attributed by the authors to the synergistic effect of the components of *O. gratissimum* essential oil [119].

A spontaneous sedative effect was found in *O. gratissimum* essential oil of the thymol chemotype, which was rich in thymol and p-cymone and did not contain eugenol or 1,8-cineole. The authors also associate the observed effect with the synergistic interaction of the essential oil components [120].

O. sanctum is widely-spread in India as a spicy aromatic culture. In Ayurvedic medicine, drugs are obtained

from its aerial part. Flavonoids (circilineol, cirsimaritin, isotimusin, apigenin), phenylpropanoids (rosmarinic acid) were found out in the aqueous-alcohol extraction from the aerial part. Eugenol was found out in the composition of the essential oil [117].

O. sanctum extract from the leaves, induced a decrease in the duration of the state of immobility in rodents. This effect was enhanced under the influence of bromocriptine, an agonist of the dopamine D2 receptor, and was blocked by haloperidol and sulpiride, antagonists of the dopamine D2 receptor, which indicates that the antidepressant activity of the studied extract is associated with the involvement of the dopamine system and weakening of stress-induced changes associated with stress reduction serotonin in the brain in rodents [121].

O. sanctum extract obtained with 70% ethyl alcohol, showed the anti-stress activity in rats exposed to noise. In this study, Wistar albino rats were exposed to 100 dB broadband white noise 4 hours a day for 15 days. The analysis of norepinephrine, adrenaline, dopamine and serotonin contents in discrete regions of the rats' brain, performed by high-performance liquid chromatography (HPLC), indicates that a 15-day exposure to the noise stress can change the concentration of biogenic brain amines. The administration of *O. sanctum* extract had a normalizing effect on the discrete areas of the brain, controlled the change in neurotransmitter levels resulting from noise stress, thereby confirming the presence of the antistress activity in the extract [122].

The methanol extract from *O. sanctum* roots (after the removal of the extractant), was studied using «The forced swim» model. The intraperitoneal administration of the extract at the dose of 400 mg / kg increased the duration of swimming, which is associated with the antistress activity of the extract, while the effect was comparable to that of deschipramine, an antidepressant [123].

In the *in vitro* experiment it was established that the antistress activity of *O. sanctum* herbal raw material extracts is associated with inhibition of cortisol release, blocking of the CRHR1 receptor, and inhibition of the activity of type 11β-hydroxysteroid dehydrogenase and catechol-O-methyl transferase [124].

Genus *Origanum* L.

The genus Origanum L. has about 40 species and 18 hybrids, most of which are perennial herbaceous plants and shrubs, common in the eastern Mediterranean region [125].

Medicinal preparations *based on Origanum vulgare* L., are most widely used in folk and official medicine. In the aerial part, collected in the blossom period, the essential oil was found. It contained aromatic compounds (thymol, carvacrol, thymyl acetate, eugenol, trans-anethole thymol and carvacrol), monoterpenoids and sesquiterpenoids (terpineol, borneol, menthol, isomentol, pinene, citral, linalool, geranial, camphene, sabinene, limonene), triterpenoids (squalene, ursolic and oleanolic acids), steroids (sitosterol, daukasterin), carbohydrates (stachyose, raffinose), lignans (origalignanol), phenolpropanoids (rosmarinic ferulic, caffeic, protocatechuic), flavonoids (luteolin, apigenin, quercetin, naringenin, galangin, taxifolin), higher fatty acids [60]. The medicinal preparations obtained from *O. vulgare* herb, have a calming effect on the central nervous system, and are used for neurosis, insomnia, and hypertension.

The intraperitoneal administration *of O. vulgare* aqueous extract to the mice at the dose of 200 mg/kg had anxiolytic as well as sedative effects. In the «The open field test», it increased the number of exits to the open fields ($p<0.05$) and the duration of the time spent on them ($p<0.001$) compared with the animals that were administrated with saline. In addition, the extract reduced the locomotor activity of the mice ($p<0.05$), but, unlike diazepam, did not cause the muscle relaxant action [126].

Rezaie et al. provide an assessment of the anxiolytic effect of *O. majorana* extract on male rats, compared to diazepam. The extract was administered to the rats intraperitoneally 30 minutes before the experiment under the conditions of the «Elevated plus maze» test. The results showed a significant increase in the time spent by the animals in the open sleeves when the extract was administered at the doses of 200 mg/kg and 400 mg/kg. In addition, it was found out that the extract increases the duration of sleep induced by ketamine. It was found out that *O. majorana* extract at the dose of 200 mg/kg exhibits sedative and anxiolytic effects in the excess of those of diazepam at the dose of 1,2 mg/kg [127].

Genus *Perilla* L.

Perilla L. is a monotypic genus, the only member of which is *Perilla frutescens* (L.) Britton. It has two varieties: *P. frutescens* var. *crispa* (Thunb.) H. Deane and *Perilla frutescens* var. *hirtella* (Nakai) Makino [18].

Perilla is an annual herbaceous plant grown as an oilseed and food crop, initially in China and the countries of the Far East, then around the world, for a long time. *Perilla* L. is used in folk medicine. Rosmarinic and caffeic acids were found out in its aqueus-alcohol extract [128].

P. frutescens leaves are commonly found in traditional eastern collections, aqueous extracts which are mainly used to treat depression and anxiety disorders. Behavioral studies and chemical analyses showed that *P. frutescens* extracts, which exerted an antidepressant effect in «The forced swim test» contained rosmarinic acid. It was established that isolated rosmarinic and caffeic acids cause an antidepressant effect and exhibit an anxiolytic activity in a stress test. Neurochemical studies have shown that neither rosmarinic nor caffeic acids affect the absorption of monoamines or the activity of monoamine oxidase, which underlies the therapeutic value of existing clinically effective antidepressants.

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It was previously discovered that caffeic acid produces antidepressant and anxiolytic effects by modulating signals mediated by alpha-1-adrenergic receptors, and also weakens the downregulation of BDNF protein transcription (Brain-Derived Neurotrophyc Factor), which occurs as a result of forced swimming. These results indicate that rosmarinic and caffeic acids can have antidepressant and anxiolytic effects by a mechanism different from the mechanism of the action of the drugs currently used in clinical practice [128].

Genus *Rosmarinus* L.

The genus *Rosmarinus* L. has five species of evergreen shrubs. The most famous of them is *Rosmarinus officinalis* L. (medicinal (ordinary) rosemary) – a shrub or sub-shrub, with petiolate, evergreen leaves, the shape of the leaf blade is linear, the leaf is induplicate. *R. officinalis* place of origine is the western part of the Mediterranean. It is cultivated widely around the world: in Italy, France, Spain, Asia Minor, it is also grown on the southern coast of Crimea, the Black Sea coast of the Caucasus, in Azerbaijan and Central Asia. *R. officinalis* is one of the oldest medicinal plants, the leaves of which are used for food, as well as for obtaining drugs and performing rituals [129, 130].

In *R. officinalis* aerial part, there is up to 1.2% of the essential oil, in which α-pinene, 1,8-cineole, camphor, borneol, bornyl acetate are found, aqueous -alcohol extracts from the leaves contains diterpen carnosol, carnosolic and rosmarinic acids, salvigenin, rosmanol and cirsimaritin, flavonoids (apigenin), triterpenes and tannins [131].

For a long time, infusions from *R. officinalis* leaves, have been used in medicine as a means of improving digestion, choleretic, tonic, relieving stress, as well as in the post-stroke period, due to the ability to improve cerebral circulation.

R. officinalis aqueous-alcoholic leaves extract increases dose-dependently the number of entries and the time spent by the mice in the open sleeves. At the high doses its effect is similar to the effect of diazepam. In this case, the extract does not significantly affect the locomotor activity. The complex of flavonoids from *R. officinalis* leaves, especially apigenin, is able to penetrate the blood-brain barrier and, as a positive and allosteric regulator, enhance the effect of GABA on GABA receptors. Luteolin has sedative and anxiolytic effects, interacting directly with GABA receptors [132].

The substances isolated from *R. officinalis* leaves (diterpen rosmanol, flavonoids salvigenin and cirsimaritin), were examined in mice for acute toxicity, antinociceptive and antidepressant effects (in “The tail suspension” and “The forced swim” tests), effects on anxiety (“Elevated plus maze” and “Light-dark chamber” tests).

The studies revealed antinociceptive, antidepressant and anxiolytic properties of the compounds under study, realized by two-phase modulation of GABAA receptors. The anxiolytic activity of all three compounds did not increase under the influence of the antagonist of the benzodiazepine receptors of flumazenil, but was inhibited under the influence of the analeptic of petylenetetrazole (corazole), which indicates a mechanism of action through the GABAA receptors at the binding site, which is different from the site possessing affinity for benzodiazepine. It was also established that the isolated compounds do not cause signs of acute toxicity at the doses of 50 to 200 mg/kg [132].

Other studies have shown that total extract from *R. officinalis* leaves, favorably affects memory, eliminates anxiety, depression, and insomnia. The improvement in memory, is explained by inhibition of acetylcholinesterase in the brain, the remaining properties of the extract are associated with its effect on GABA receptors [130, 134].

Genus *Salvia* L.

One of the largest genera of the *Lamiaceae* family, including about 900 species, which are mainly represented by perennial herbaceous plants, shrubs and sub-shrubs [134]. All members of the genus are essential-oil-bearing. The most studied medicinal plant is *Salvia officinalis* L. Aqueous and aqueous-alcohol extracts from leaves, as well as essential oil, have been used in medicine for a long time. This is a perennial herb or subshrub, well-spread in wild nature in the countries of the Mediterranean and the Balkan Peninsula. This ubiquitous species is grown as a medicinal and spicy aromatic culture. *S. officinalis* leaves contain essential oil (up to 2.5%), as well as di- and triterpenes, phenylpropanoids, and derivatives of caffeic acid, including rosmarinic and lithospermic acids, flavonoids, tannins, etc. [135] The drugs based on *S. officinalis* leaves, have a disinfectant, anti-inflammatory, astringent, hemostatic, emollient and diuretic effect, and reduce perspiration. Leaves decoction has a stress-protective activity.

Carnosole and carnosolic acid isolated from *S. officinalis* leaves, inhibit binding of tert-butylbicyclofluoro [35S] thionate to the chloride channel of the GABA-benzodiazepine receptor complex in the brain tissue (at IC_{50} values of 57±4 μM and 33± 3μM, respectively), but have no effect on binding of [3H]-muscimol, [3H]-diazepam or [3H]-flunitrazepam. Therefore, the site of action of these compounds, apparently, is located directly on the chloride channel and differs from miltiron [136]. In another study, three flavones and two abitan diterpenes, functioning as active benzodiazepine receptors, were identified by fractionating a methanol extract from *S. officinalis* leaves. Some flavones, such as apigenin [137], luteolin [93], linarin [138] and hispidulin, exhibit anxiolytic effects through the GABAergic mechanism similar to benzodiazepines [139].

Apigenin, Hispidulin, and cirsimaritin competitively inhibit binding of 3H-flumazenil to the benzodiazepine receptor with IC_{50} values of 30, 1.3, and 350 mM, respec-

tively. The IC_{50} values of abietane diterpenes, 7-methoxysmanol and haldosol consist of 7.2 and 0.8 mM, respectively [140]. In addition to the official form, *S. aethiopis* L., *S. sclarea* L., *S. pleberia* R. Brown, *S. daghestanica* Sosn., *S. elegans* Vahl. and etc are also of scientific interest [60, 141].

In the aqueous-alcohol extract from *S. elegans* leaves, ursolic acid and flavonoid 5-O-(6-rhamnosylglucoside)-7-hydroxy-4'-methoxyflavonone were detected and isolated [142]. They showed antidepressant activity in mice [143]. Herrera-Ruiz et al. estimated the anxiolytic and antidepressant activities of the aqueous-alcohol extracts (the extractant was 60% ethyl alcohol) obtained from *S. elegans* flowers and leaves, in mice. The extract, administered orally, increased the time the mice spent on the light side in the "Light-dark chamber" test and the time of the animals' immobility subjected to forced swim. The administration of the extract also increased the animals' time, spent in the open sleeve, and the entrances to the open sleeves in the "Elevated plus maze" test. The same extract could not modify the spontaneous locomotor activity measured in "The open field test" [144].

The aqueous-alcoholic extract from *S. reuterana* leaves (100 mg/kg) had an anxiolytic effect in mice in the "Elevated plus maze" test [145].

According to Javdan et al [146], neuropharmacological effects of *S. hypoleuca* leaves extract administrated to Wistar line rats at the dose of 150 mg/kg/day for 10 days, potentiated pentobarbital-induced sleep, reduced the number of animals in the open sleeves outputs. *S. leriifolia* aqueous leaf extract increased the sleep induced by pentobarbital, at the doses of 1.15 and 1.57 g/kg, but the effects were weaker than that of diazepam [147].

S. sclarea essential oil significantly increased the hexobarbital anesthetic effects (drug "Evipan") at the doses less than 20% LD_{50} (520 mg/kg in male mice), but had no significant effect on the spontaneous locomotor activity and statokinetic reflexes [148].

The biologically active substances of *S. triloba* essential oil, prolong sleep induced by hexobarbital in rats. *S. triloba* ethanol extract showed moderate affinity for the benzodiazepine $GABA_A$ receptor site [149].

S. guaranitica is an officinal medicinal plant in Latin America. Its herbal raw materials are used to produce sedative drugs. It was proved that circyliol (5,3',4'-trihydroxy-6,7-dimethoxyflavone) and caffeic ethyl ester, which are part of the plant's ethanol extract, are ligands with a competitively low affinity for benzodiazepine receptors [150]. In another study, circiliol exhibited a dose-dependent hypnotic effect in a sleep induced by pentobarbital. Circiliol was found to be stronger in binding 3H-zolpidem (Ki = 20 μM) than in binding 3H-flunitrazepam (Ki = 200 μM) to rats' benzodiazepine receptors. Consequently, circiliol has sedative and hypnotic properties, probably acting on the so-called benzodiazepine receptor type I [151].

*S. haematodes (*syn.*S. pratensissubsp. haematodes* (L.) Arcang.) ethanol extract from the aerial part, has an antidepressant activity in mice. It significantly increased pentobarbital-induced sleep and decreased the rats' excitation induced by amphetamine. Sedation is also apparent from the results that indicated an increase in hypoxic survival time in mice [152, 153].

S. miltiorrhiza root is widely used in China to produce drugs for the treatment of neurasthenic insomnia [154]. From the extract obtained by diethyl ether from the roots of this plant, ten diterpenic quinones were isolated. In the carried out radioligant studies, they inhibit binding of [3H] -flunitrazepam to central benzodiazepine receptors with an IC_{50} of 0.3 to 36.2 μM. Among the isolated compounds, the highest activity (IC50 = 0.3 μM) was demonstrated by miltiron, which showed an increase in the affinity in the presence of 100 μM GABA. Miltiron induced muscle relaxation, sedation, dependence, and withdrawal symptoms in mice at the doses of 10–60 mg/kg, which were effective in a behavioral test. Consequently, miltironderivatives may represent a new class of plant-derived tranquilizers [155].

The aqueous-alcohol extract from *S. pleberia* aerial part, contains flavonoids (Hispidulin, homoplantoginin, nepetin, nepetrin, 6-hydroxyluteolin, apigenin, luteolin) and rosmarinic acid [155].

Johnston et al. [156] conducted animal experiments using compounds isolated from *S. pleberia*. Ethyl acetate fractions obtained from *S. pleberia* aerial part, are more active than methanol ones due to the content of rosmarinic acid, which is active at 10 mg/kg in the model of pentobarbital-induced sleep in mice.

Flavone Hispidulin (5,7,4'-trihydroxy-3'-methoxyflavone) isolated from *S. pleberia*, has agonistic GABA receptor activity.

Genus *Satureja* L.

The genus *Satureja* L. has up to 50 species represented by annual plants, shrubs and sub-shrubs, which are distributed mainly in the countries of Asia, the Middle East and the Mediterranean [157].

Medicinal preparations based on *Satureja hortensis* L., are widespread in folk and official medicine. *Satureja hortensis* L. is an annual herb that grows and is cultivated in southern Europe, Central Asia, Turkey, and the Caucasus. Aqueous extracts and essential oil from *Satureja hortensis* L. aerial part, have insecticidal, antibacterial and anthelmintic activity, and used for diseases of the gastrointestinal tract, headaches, dizziness, tachycardia, etc. The chemical composition of the essential oil is represented mainly by terpenes and aromatic compounds (carvacrol, thymol, p-tsimen, g-terpinene, α- and β-pinene, sabinene, limonene, carvone, caryophyllene oxide). Aqueous-alcohol extracts contain phenylpropanoids (rosmarinic, caffeic, isoferulic, chlorogenic acids), flavonoids (naringenin, quercetin, apigenin, kaempferol, luteolin and their glycosides) [157].

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S. hortensis aqueous-alcohol extract was examined for the antidepressant activity in the "Elevated plus maze", "Force swim" and "Forced immobilization" tests on Wistar rats. It was found out that the studied extract reduces depression in the test animals at the dose of 400 mg/kg [158].

Carvacrol, which is present in *S. hortensis* essential oil, was administered to the mice p.o. at the doses of 12.5, 25 and 50 mg/kg. It exhibited an anxiolytic effect in the «Elevated plus maze» test, which was leveled out under the influence of flumazenil, the antagonist of the benzodiazepine receptors. However, carvacrol did not show sedative or muscle relaxant properties and did not affect the motor activity [159].

Genus *Schizonepeta* (L.) Briq.

Genus Schizonepeta (L.) Briq. consists of 3 species of annual or perennial herbaceous plants, growing mainly in Siberia, Primorye and Northern China [160].

The most studied species is *Schizonepeta multifida* (L.) Briq., a perennial herbaceous plant growing in the herbage of meadow steppes, stepped and forested upland meadows in southern Siberia, Yakutia, the Far East, Central Asia and Mongolia. The aerial part of the plant contains up to 1.6 *S. multifida* dry extract in the dose range of 50–300 mg/kg, increases the number of water intakes in the conflict methodology according to Vogel. Thereby, the effectiveness of the extract (in the dose range of 100–300 mg/kg), in a number of parameters, exceeded that of the drugs of rhizomes with valerian roots [161].

S. multifida dry extract in the dose range of 50–200 mg/kg, has pronounced anxiolytic, antidepressant, nootropic and anticonvulsant activities, and at the dose of 300 mg/kg – moderate sedative properties. The anxiolytic effect is realized to a greater extent due to the essential oil and luteolin-7-O-glucoside included in its composition, to a lesser extent - due to ursolic acid. In an experimental therapeutic dose, *S. multifida* extract provides pronounced pharmacotherapeutic efficacy in chronic stressful situations, reduces the feeling of fear and anxiety, restores emotional status, helps maintain a memorable trace, limits the severity of stress changes in the internal organs of animals, as well as the formation of regressive forms of neurons in brain structures. The main pharmacological mechanisms that determine anxiolytic, antidepressant and neuroprotective effects of *S. multifida* dry extract are: restriction of hyperactivation of the sympatho-adrenal and hypothalamic-pituitary-adrenal systems, inhibition of free radical processes, activation of the antioxidant system of the body and GABA-energy metabolic normalization with its ability to provide antioxidant, membrane-stabilizing, stress-protective and antihypoxic drugs action [160].

Genus *Scutellaria* L.

Scutellaria L. is one of the largest genera, uniting about 350 species, widely-spread in temperate subtropical and tropical zones including Europe, North America and East Asia [162]. Most of them are perennial, rarely annual, herbaceous plants, less commonly shrubs and sub-shrubs. Unlike most members of the genus *Lamiaceae*, which are essential-oil-bearing and belong to the subfamily *Nepetoideae*. The members of the genus *Scutellaria* form the subfamily *Scutellarioideae* and are among the dyeing plants. The most famous member of the genus is *Scutellaria baicalensis* Georgi, a perennial herbaceous plant. Its areal comprises the Russian Baikal region, Amur region, PrimorskyTerritory, as well as Mongolia, China, and Korea.

The roots of *S. baicalensis* contain flavonoids (baikalin, scutellarin, baikalein, apigenin, luteolin, etc.), chalcones, isoflavones, biflavones, lignoflavonoids, phenylpropanoids, phytosterols, saponins, etc. This species is among the most popular medicinal plants in China, Mongolia and the Far East. Drugs from its roots have a pronounced sedative and antiepileptic effects [163].

Baikalin and vogonin are commonly considered the main active components of *S. baicalensis* flavonoids.

In the experiments on rats and mice (7.5–30 mg/kg), Baikalin (5,6,7-trihydroxyflavone 7-O-glucuronide) had an anxiolytic effect, but did not affect the motor activity of the mice [169]. Baikalin interacts preferably with subtypes of GABA-α receptors containing subunits of α-2 and α-3, in contrast to benzoadepins that do not have such a specificity [12].

Vogonin is the main *S. baicalensis* component, inducing anxiolysis in male mice at the dose of 7.5–30 mg/kg in the "Elevated plus maze" test [164]. Vogonin exhibits its neuroprotective and anxiolytic effects. Having a pronounced affinity for the active benzodiazepine centers of GABAergic ergic receptors, it inhibits the activation of microglia [165, 166].

In European medicine, the aerial part of *S. lateriflora* has been widely used for more than 200 years to obtain drugs – a mild relaxant and agent for treating anxiety, nervous tension and seizures [167]. Flavonoids baikalin and baikalein, are considered the main active compounds in *S. lateriflora* herb. Baikalein is defined as a ligand of the benzodiazepine receptor (with a weak affinity) and has shown sedative and anxiolytic effects that occurs through the GABA-α-nonbenzodiazepine sites [168].

S. lateriflora aqueous-alcoholic extract was studied in rats *in vivo* behavioral tests. The extract (after the removal of ethanol) a mixture with milk was introduced: in the test group, 100 mg of the amount of extractives in 1 ml of milk was administered; in the control group it was only 1 ml of milk. It was found out that the rats receiving the extract, showed a more risky and less anxious behavior than the rats in the control group [169].

Genus *Stachys* L.

The genus *Stachys* L. consists of more than 270 species distributed throughout the world. These are: peren-

nial, rarely annual herbaceous plants or shrubs [170]. Among them there are: *Stachys officinalis* (L.) Trevis. – syn.: *Betonica officinalis* L., *S. palustris* L., *S. lavandulifolia* Vahl., *S. tibetica* Vatke., *S. betoniciflora* Rupr.– syn. *Betonica foliosa* Rupr., *S. sylvatica* L. et al.

Flavonoids (luteolin, apigenin, scutellarine, stachyflaside, vitexin), quinones, iridoids (harpagide, harpagoside, acetylgarparide, ajugol, and ajugoside), phenolic acids, diterpenoids (stachysic acid, abietatriene, annuanone, stachylone) were detected in aqueous-alcohol extracts from various *Stachys* species. In the composition of essential oil, D-germacrene, β-fellandren, α- and β-pinenes, myrcene were detected [60, 170, 171]. Flavonoid glycoside apigenin-7-glucoside is present in *S. tibetica* aqueous-alcohol extracts, acipylene (66.4%), fenchyl alcohol (8.9%), α-pinene (8.2%) caryophyllene oxide (4.7%), menthol (1.7%) and geraniol (1.3%) are in the essential oil [172]. *S. betoniciflora* aerial parts contain flavonoids, apigenin derivatives, stachidrin nitrogen base, iridoids, essential oil [173].

For a long time, *Stachys* extracts from herbs of various types have been used in folk medicine, in particular, in cases of gynecological bleeding. *S. sylvatica* hydroalcoholic extract has pronounced hypotensive and sedative effects, the latter being superior to Leonurus L. tincture [173, 174].

The fractions obtained from *S. lavandulifolia* aerial part extraction with petroleum ether, ethyl acetate, butanol and water, were tested for a spontaneous locomotor activity and the behavior of mice in the "Elevated plus maze" model. The test samples (after the removal of organic solvents) were administered intraperitoneally to the male mice in various doses for 30 minutes before assessing their behavior. The aqueous-alcohol extract (50 mg/kg), the fractions obtained with petroleum ether (25 and 50 mg/kg), ethyl acetate (25 and 50 mg/kg) and water (50 mg/kg) significantly increased the time and number of entrances in the open sleeves. The butanol fraction up to 50 mg/kg did not significantly affect any of the measured parameters. A spontaneous locomotor activity was significantly reduced in the animals injected with each fraction, compared to saline. The ethyl acetate and water fractions showed the smallest and maximum decreases in activity, respectively. The anxiolytic effects of ethyl acetate, petroleum ether and water fractions may be associated with the content of flavonoids, phenylpropanoids or terpenoids [174].

S. lavandulifolia aqueous-alcoholic extract and its essential oil were administered intraperitoneally to the male mice in various doses 30 minutes before the behavior assessment. *S. lavandulifolia* extract at the dose of 100 mg/kg increased the period of time spent, spent by the animals in the open sleeves; and it decreased the number of entries in the open sleeves. Besides, it decreased the period of time, spent by the animals in the closed sleeves. The extract at the doses below 100 mg/kg, did not significantly affect any of the parameters measured on the «Elevated plus maze» model. This dose of the extract prolonged ketamine-induced sleep time and decreased a locomotor activity in mice. *S. lavandulifolia* extract has an anxiolytic effect with a relatively lower sedative activity than diazepam. *S. lavandulifolia* essential oil in the doses up to 100 mg/kg did not significantly affect the behavior of the mice [175].

Methanol extracts obtained from *S. tibetica*at roots and herbs at the doses of 200 and 400 mg/kg significantly increased the time and number of entries into the open sleeves ($P<0.01$), but reduced the time and number of entries into closed sleeves. At the same time, the extracts reduced the time spent by the animals in the center of the maze (latency) [176].

Kumar et al. isolated flavonoids from *S. tibetica* and evaluated their anxiolytic activity in Wistar rats. The number of entries and the percentage of entries in the open sleeves increased, while the number of entries and the duration of time spent in the closed sleeves, decreased in the group receiving apigenin-7-glucoside. Apigenin-7-glucoside significantly reduced the number of head dives in the «Elevated plus maze» test. Apigenin-7-glucoside showed anxiolytic potential comparable to the reference drugs apigenin and diazepam [177].

In the test on the social interaction, apigenin-7-glucoside at the doses of 25 and 50 mg/kg, decreased the aggressive behavior of albino rats, while the time of social interaction significantly increased in bright light, in familiar and unfamiliar conditions. In «The hole-board test», *S. tibetica* essential oil significantly increased the number of head dives in the holes, the number of entrances and the time spent in the open sleeves of the "Elevated plus maze" test, while in the "Light-dark chamber" test it showed an increase in the number of transitions and the time spent on the bright side. The results indicate that *S. tibetica* essential oil has an anxiolytic effect [177].

The effect of methanol extracts from four Balkan endemic taxa – *Stachys*: *S. anisochila* Vis. et Pancic, *S. beckeana* Dorfl. et Hayek, *S. plumosa* Griseb. and *S. alpina (L.) subsp. dinarica* - administered intraperitoneally in the range of 100–400 mg/kg, on the behavioral activity, was studied on the adult male Wistar rats in the "Elevated plus maze test", during the observation of the spontaneous locomotor activity, in the tests on strength and compression, mainly predicting anxiolytic, sedative and muscle relaxant actions. As a result, it was established that the studied *Stachys* extracts do not have any anxiolytic or muscle relaxant activity, and *S. beckeana* at 400 mg/kg has an anxiogen-like effect. The study using β-carboline-3-carboxylate-tert-butyl ether, a selective antagonist of benzodiazepine receptors, showed that the sedative effect of the methanol extract of *S. alpina subsp. dinarica* was partially mediated by GABA-α receptors containing the α-1 subunit. The behavioral effects of *S. anisochila* and *S. plumosa* extracts did not differ. Chlorogenic acid and verbascoside were identified in all the extracts. *S. anisochila*, *S. beckeana* and *S. alpina sub-*

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sp. dinarica flavonoid fraction consisted of isoscutellarin and hypoalectin glycosides, whereas in *S. plumosa* fraction had chrysoriol and apigenin glycosides. The results show a psychotropic potential of the flavonoids of four *Stachys* endemic taxa. *S. alpine subsp. dinarica* turned out to be the most promising for the preparation of a sedative drug [178].

Genus *Thymus* L.

The genus *Thymus* L. is one of the largest in the family *Lamiaceae*, it includes several hundred species, distributed mainly in Eurasia and North Africa. On the territory of Russia and neighboring countries, there are about 170 species [56]. The members of the genus are low-growing shrubs and sub-shrubs belonging to essential-oil-bearing plants.

In the composition of the essential oil from various plant raw material samples of species of the genus *Thymus*, the following substances were detected: thymol (12–61%), carvacrol (0.4–20.6%), 1,8-cineole (0.2–14.2%), p-cimen (9.1–22.0%), linalool (2.2–4.8%), borneol (0.6–7.5%), a-pinene (0.9–6.6%), camphor (up to 7.3%), etc. [180]. The most studied are *T. vulgaris* L. and *T. serpillum* L., the latter is more common in nature. The herb of both types is used to obtain drugs, which are widely used in medicine.

F. Komaki et al. showed an anxiolytic effect of the aqueous-alcoholic extract from *T. vulgaris* leaves when weekly administrated p.o. to the male rats of the Wistar strain in the “Elevated plus maze” model [180].

Thymol (monoterpene phenol – 2-isopropyl-5-methylphenol) is the dominant essential oil component of *T. vulgaris*, at the dose of 20 mg/kg, it significantly increases the time spent by Swiss albino mice in the open sleeves in the «Elevated plus maze» test [181].

Methanol extracts and *T. fallax* Fisch essential oil from the aerial parts of & C.A. Mey., *T. kotschyanus* Boiss. & Hohen., *T. pubescens* Boiss during “The forced swim” test significantly reduced the period of immobility of the animals, compared with the control, and showed a dose-dependent antidepressant activity. The test results showed that *T. fallax* extracts and essential oil have a greater antidepressant activity than those from *T. kotschyanus* and *T. pubescens* [182].

CONCLUSION

The review presents some of the results of the scientific research on the pharmacological activity of various fractions of biologically active substances, essential oils and individual compounds obtained mainly from the aerial parts of plants of *Lamiaceae* family members.

Most of the plant species considered play an important role in traditional medicine of different countries and have a therapeutic and prophylactic value in the stress-correction-therapy. Many of them, being pharmacopoeial, are sources of medicinal plant raw materials in modern pharmacy and medicine.

The members of the Lamiaceae family often become objects of the scientific invesigation, where a search for new sedative, anxiolytic and neuroprotective agents is carried on. A lot of attention is paid to both - relatively well-studied plant species (for example, from the genera Salvia, Stachys, Thymus) and insufficiently explored genera, including tropical and subtropical ones, which are not represented in the flora of Russia (Agastache, Clerodendrum, Clinopodium, Eremostachys, Leucas, ets.).

This review has comprised 71 species from 30 genera of the family. In spite of a fairly significant level of a previous study which can be notified in the analysis of this publication, a large number of potentially resource species remain unexplored. In the future, they may be of pharmacognostic interest and have a practical application, in particular, in the field of creating new drugs with neurotropic effects.

The carried out analytical review makes it possible to assess the current level of knowledge of the neurotropic activity of various substances obtained from the plant raw materials from the *Lamiaceae* Linddl. family and to establish promising areas of the scientific research for the creation of new drugs.

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All authors equally contributed to the research work.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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RESULTS OF A COMPARATIVE STUDY OF *NIGELLA SATIVA* L. SEEDS OILS COMPOSITION

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This article presents results of the chemical composition study of the seeds oils lipid complex of *Nigella Sativa* L. grown under various geographic conditions. The task of the comprehensive study of the chemical composition of the plant and its individual parts remains relevant due to the wide spectrum of its pharmacological activity.

The aim of this work is a comparative study of the fatty acid composition, a non-saponifiable fraction and the composition of essential oils of *Nigella Sativa* L. seeds grown in different regions of the world.

Materials and methods. The combination of chromatography-mass spectrometry and 1H-NMR spectroscopy methods made it possible to study the qualitative and quantitative composition of *Nigella Sativa* L. lipid complex seeds. All the experiments were carried out in accordance with the requirements of the State Pharmacopoeia, 14th Ed, given in the corresponding general pharmacopeial monographs.

Results. Profiles have been established and the content of fatty acids, sterines, triterpene alcohols, essential oils and thymoquinone found out in the lipid complex, has been estimated. The saponifiable portion of the complex is represented by triglycerides (81.7–95.3%), di- (3.9–15.2%) and monoglycerides (0.7–4.1%). They mainly contain linoleic (55.8–60.6%), oleic (21.8–24.6%), palmitic (10.0–12.8%), stearic (2.4–3.2 %) and cis-11.14-eicosadiene (2.3–2.6%) acids. In the lipid complex, the contents of sterines and triterpene alcohols were 0.4–0.7%; up to 70% of the fraction was represented by β-sitosterol (22.5–29.2%), cycloartenol (20.1–36.6%) and 24 methylenecycloartanol (9.5–19.9%). In the trace amounts (up to 1.0%), cholesterol has been detected in all the samples. In the lipid complexes, the content of thymoquinone ranged from 0.7 to 2.6%.

Conclusion. A comparative study of the seeds lipid complex of *Nigella Sativa* **L.** grown under various geographic conditions, has been carried out. The marker compounds as well as their content standards for determining the authenticity of raw materials (thymoquinone, para-cimen, cis-11.14-eicosadienic acid), have been identified.

Keywords: *Nigella sativa* L., fatty oil, essential oil, chromatography-mass spectrometry, NMR spectroscopy

РЕЗУЛЬТАТЫ СРАВНИТЕЛЬНОГО ИССЛЕДОВАНИЯ СОСТАВА МАСЕЛ СЕМЯН *NIGELLA SATIVA* L.

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RESEARCH ARTICLE

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В данной статье представлены результаты исследования химического состава липидного комплекса из семян черного тмина, выращенного в различных географических условиях. Актуальной остается задача всестороннего изучения химического состава растения и его отдельных частей, обусловленная широким спектром его фармакологической активности.

Цель данной работы – сравнительное исследование жирнокислотного состава, неомыляемой фракции и состава эфирных масел семян чёрного тмина, выращенного в различных регионах мира.

Материалы и методы. Совокупностью методов хромато-масс-спектрометрии и спектроскопии ЯМР ^{1}H изучен качественный и количественный состав липидного комплекса семян чёрного тмина. Все эксперименты проводили в соответствии с требованиями Государственной фармакопеи XIV издания, приведенными в соответствующих общих фармакопейных статьях.

Результаты. Установлены профили и оценено содержание жирных кислот, стеринов, тритерпеновых спиртов, эфирных масел и тимохинона, обнаруженных в липидном комплексе. Омыляемая часть комплекса представлена триглицеридами (81,7–95,3%), присутствуют ди- (3,9–15,2%) и моноглицериды (0,7–4,1%). Они содержат в составе преимущественно линолевую (55,8–60,6%), олеиновую (21,8–24,6%), пальмитиновую (10,0–12,8%), стеариновую (2,4–3,2%) и *цис*-11,14-эйкозадиеновую (2,3–2,6%) кислоты. Содержание стеринов и тритерпеновых спиртов в липидном комплексе составило 0,4–0,7%, до 70% фракции представлено β-ситостерином (22,5–29,2%), циклоартенолом (20,1–36,6%) и 24-метиленциклоартанолом (9,5–19,9%). В следовых количествах (до 1,0%) во всех образцах был обнаружен холестерин. Содержание тимохинона в липидных комплексах варьировалось в пределах 0,7–2,6%.

Заключение. Проведено сравнительное изучение липидного комплекса из семян черного тмина, выращенного в различных географических условиях, выявлены соединения-маркеры, а также нормы их содержания для определения подлинности сырья (тимохинон, пара-цимен, *цис*-11,14-эйкозадиеновая кислота).

Ключевые слова: чёрный тмин, *Nigella sativa* L., жирное масло, эфирное масло, хромато-масс-спектрометрия, спектроскопия ЯМР

INTRODUCTION

Since classical times, *Nigella sativa* L. has been cultivated in the Mediterranean, North Africa, Central Asia, India, and the Middle East. On the territory of Russia, it can grow and ripe in the North Caucasus, in Tatarstan [1–4].

Nigella sativa L. seeds are used as a spice (nigella) and oily raw materials. They can contain up to 70% oil [5].

Nigella sativa L. oil has a wide range of pharmacological activities and therefore is widely used in folk medicine of the East. Today, there is a great number of scientific papers devoted to the study of the pharmacological activity of this plant material [6–11].

Nigella sativa L. fatty seed oil is richer in palmitic acid and relatively rare fatty acids of C20 group than sunflower oil. Compared to palm oil, it is much richer in polyunsaturated fatty acids and also contains a lot of palmitic acid [5, 12, 13].

The unsaponifiable components of *Nigella sativa* L. seed oils are represented by a complex of sterines, monoterpenes, diterpenes and triterpenes. In its turn, *Nigella sativa* L. is a valuable resource of the essential oil, which consists of terpenes and products of their oxidation, condensation and cyclization – phenols, thymohydroquinone and thymoquinone. The total content of the essential oil in *Nigella sativa* L. seeds is from 0.5 to 3% of air dried raw materials [3, 14, 15].

Modern instrumental methods including gas chromatography and high-performance liquid chromatography with various types of detection, and a nuclear magnetic resonance method, were used to study the phytochemical composition of *Nigella sativa* L. seeds [14–21].

The composition of *Nigella sativa* L. essential oil contains a rather high content of thymoquinone. It is due to the fact that thymoquinone is the final oxidation product in this chain of terpenes, therefore, it is most accumulated in the oil.

Thymoquinone can undergo further transformations, for example, in the light it dimerizes, forming dithymoquinone, which indicates its photosensitivity. As a product of thymoquinone dimerization, dithoquinone is less studied and presumably, like thymoquinone, may have an antitumor effect [22–25].

Currently, information on the comparative chemical composition of *Nigella sativa* L. seeds according to the growth region, is not registered in the available literature. In this regard, it seems relevant to carry out this kind of research.

THE AIM of this work was a comparative study of the fatty acid composition, the unsaponifiable fraction and the composition of essential oils of *Nigella sativa* L. grown in different regions of the world.

MATERIALS AND METHODS

The samples of *Nigella sativa* L. seeds were obtained from 7 different eco-economic regions of the globe: Yemen, the Russian Federation (Republic of Tatarstan), India, Tajikistan, Ethiopia, Egypt, Israel in the period within 2017–2018. The authenticity of the raw materials was checked by a microscopic method in accordance with the requirements of the State Pharmacopoeia (14th Ed.), general pharmacopeial monograph 1.5.3.0003.15 “Technique of microscopic and microchemical studies of medicinal plant materials and herbal medicines” and general pharmacopeial monograph 1.2.1.0009.15 “Optical microscopy”. The studied oils had been obtained from *Nigella sativa* L. seeds in Soxhlet’s apparatus by method of circulating extraction. The seeds had been pre-crushed to a particle size passing through a 0.5 mm sieve. The test samples in the amount of 50.0 g were placed into a cartridge and loaded into Soxhlet’s apparatus. The extraction was carried out with n-hexane. After the extraction, the extractant was distilled off on a rotary evaporator IR-1MZ at the temperature of 40°C.

By this method of obtaining oils, both the lipid com-

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plex of *Nigella sativa* L. seeds and the essential component of the oil had been extracted [26]. Then the lipid complex was saponified and converted into a mixture of methyl esters.

Study of fatty acid oils composition by gas chromatography

The operating mode of Agilent6890N Chromatograph (Agilent Technologies, USA) was the following: capillary column VF-23 ms (Agilent Technologies, USA, 30 m length, 0.32 mm internal diameter, 0.25 µm phase thickness), the carrier gas was helium, the velocity of the carrier gas was 1.5 ml/min, the injector temperature was 280°C, the initial temperature of the chromatograph furnace was 50°C, then there was isotherm for 2 min; after that it was heated at the speed of 10°C/min up to 180°C, held up for 5 minutes, then heated up to 240°C at the rate of 5°C/min.

The total analysis time was 32 minutes. The sample was injected in a flow split mode (1:10). The fatty acids were identified by comparing the retention times of the peaks in the chromatograms of the test samples with the retention times of the peaks in the chromatogram of a standard sample – a mixture of 37 fatty acid methyl esters (Supelco® 37 component FAME mix, 10 mg/ml, methylene chloride, Cat. No CRM47885, Sigma-Aldrich, USA). Each sample was analyzed three times.

Sample preparation: fatty acid methyl esters were obtained by transesterification of glycerides. A sample weighed quantity of about 10.0 mg was placed in a 7.0 ml glass vial with a screw cap, then 1.0 ml of methanol and 100.0 µl of acetyl chloride were added.

The vial was closed and placed in a laboratory heater for 60 min at 80°C. After cooling the reaction mixture, 3.0 ml of double-distilled water was added to the vial, followed by 1.0 ml of n-hexane, and shaken. 1 µl of the upper layer of n-hexane was injected into a gas chromatograph.

The composition of unsaponifiable components of *Nigella sativa* L. seeds oils was studied by chromatography-mass spectrometry.

The operating mode of Agilent6890N Chromatograph (Agilent Technologies, USA) was the following: capillary column VF-23 ms (Agilent Technologies, USA, 30 m length, 0.25 mm internal diameter, 0.25 µm phase thickness), the carrier gas was helium, the velocity of the carrier gas was 1.5 ml/min, the injector temperature was 280°C, the initial temperature of the chromatograph furnace was 60°C, then there was isotherm for 3 min; after that it was heated at the speed of 10°C/min up to 290°C and held up for 20 minutes. The total analysis time was 46 minutes. The mass spectra recording mode was the following: magnetic sector mass detector JMSG C Mate II (JEOL, Japan), ionization energy of 70 eV, the source temperature of 270°C, scanning in the range of 40–400 Da at the speed of 2 scans/sec. The volume of the injected sample was 1 µl.

For the identification, standard samples of individual compounds and the NIST 14 mass spectral database were used; in case of the absence of mass spectra of the detected components in it, the structure was established on the basis of characteristic fragmentation processes and the data on the chromatographic properties of the studied compounds.

To calculate the retention indices, an analysis of the mixture of normal hydrocarbons (C6–C35) was performed under the selected chromatographic conditions. When determining the relative percentage of the components of essential oils in terms of their total content, the ionization coefficients were equalized. When determining the quantitative content of sterols and triterpene alcohols in terms of the internal standard, their ionization coefficients were equalized.

Sample preparation: using an automatic pipette dispenser, 10 µl of the essential oil was taken and placed in a 2.0 ml glass vial for chromatography, 1 ml of chloroform was added, and the vial was vigorously shaken. Then 1 µl of the solution was injected into GC-MS.

To isolate the unsaponifiable fraction, 100.0 mg of the sample was placed in a 5 ml glass vial, then 1 ml of potassium hydroxide solution, 2M, and 20 µl of an internal standard solution (cholestanol, 10.0 mg/ml) were added. Then the samples were kept for an hour at the temperature of 80°C and after cooling the reaction mass, 3 ml of bidistilled water was added. The unsaponifiable fraction was extracted in three portions of 1 ml of diethyl ester, the extracts were combined, passed through a cartridge with sodium sulfate, blown dry under the nitrogen current, and silicated before the analysis. To do this, 300 µl of BSTFA: acetonitrile (1:2) mixture was added to the dry residue and kept for 30 minutes at 80°C, then 1 µl of the solution was injected into the GC-MS device.

Study of oils composition by NMR spectroscopy

Quantitative NMR spectra of *Nigella sativa* L. seeds lipid complexes were recorded and processed using the Delta program (JEOL, Japan), which provides an instrument control, data collection and analysis.

The 1H-NMR spectra were recorded under quantitative conditions (32K points per spectrum, 16 accumulations, 90° pulse, 40 with a delay between pulses). For the quantitative determination, the integral signal intensity of chloroform was taken as 1. The content of thymoquinone in the sample was determined by the following formula:

$m(T) = n(CHCl_3) * I(T) * M(T)$,

where:

m (T) is the mass of thymoquinone in the sample,

n (CHCl3) is the content of the residual proton-containing deuterochloroform isotopomer in moles,

I (T) is the integrated signal intensity of thymoquinone at 6.51 or 6.57 ppm,

M (T) is the molecular weight of thymoquinone, equal to 166 Da.

RESULTS AND DISCUSSION

Physico-chemical properties of the obtained *Nigella sativa* L. seeds lipid complexes are presented in Table. 1.

The content of *Nigella sativa* L. seeds lipid complex-

es in terms of absolute dry raw materials amounted to about 30.4-37.8%. The highest yield was observed for the seeds from Russia (Tatarstan). The smallest yield was observed for the seeds from Ethiopia.

Figure 1 shows typical chromatograms of fatty acid methyl esters obtained from *Nigella sativa* L. seeds lipid complexes and methyl esters of Supelco® 37 component FAME mix by transesterification of triglycerides.

As Figure 1 shows, 5 main components have been revealed in the samples, which constitute a total of 98.7–98.9% of the fatty acids content; the remaining components have been detected in the trace amounts. The content of the predominant unsaturated acids (linoleic C18: 2 and oleic C18: 1) in the samples amounts to a total from 80.4% to 83.9%. The content of ichtrans isomers (elaidic – C18: 1n9t and linoleidic – C18: 2n6t acids) is very insignificant and does not exceed 0.05%. In its fatty acid composition, Nigella sativa L. seeds oil is close to sunflower oil, but contains much more palmitic acid. According to this indicator, it exceeds palm oil twice (Table 2). Cis-11.14-eicosadiene acid has also been detected in lipid complexes; in the samples, its content varied within 2.3–2.6%. This component can be considered one of the markers of the authenticity of *Nigella sativa* L. seeds.

Table 2 shows the results of the study of fatty acids compositions of seven *Nigella sativa* L. *seeds* samples from different regions of the world.

The results presented in Table 2, indicate the presence of comparable amounts of fatty acids in all the studied samples. A noticeable difference is observed only in the case of palmitic acid – up to 3.0%.

The results of the study of the unsaponifiable fraction of the *Nigella sativa* L. seeds lipid complexes by GC-MS, are presented in Table 3.

The above data shows that the content of sterols and triterpene alcohols depends on the country of origin of the seeds, however, a characteristic profile is preserved in all oil samples. The total content of sterols and triterpene alcohols was 400.3–719.7 μg/100.0 mg (0.4–0.7%). It has been found out that β-sitosterol is the main component of the fraction, its content was about 22.5–29.2%, campesterol and stigmasterol were detected in the amounts of 4.8–6.0% and 7.7–9.6%, respectively. Cycloartenol (20.1–36.0%) and 24-methylenecycloartanol (9.5–19.9%) are the dominant triterpene alcohols found in the lipid complexes of *Nigella sativa* L. seeds. In the trace amounts (up to 1.0%), cholesterol has also been detected in all the samples.

The low molecular weight fraction of unsaponifiable substances is represented by a set of mono-terpenes and their oxidation products: pinenes, limonene, r-cimol, carvacol, thymoquinone, trace amounts of free fatty acids – palmitic, stearic, linoleic, oleic and cis-11.14-eicosadiene. Free fatty acids indicate the residual activity of the lipase enzyme present in *Nigella sativa* L. seeds; this enzyme causes hydrolysis of acylglycerides.

A typical chromatogram of *Nigella sativa* L. essential oil is shown in Figure 3, and the composition of the essential oils of the seeds grown in various regions of the world, is shown in Table 4.

The composition of the essential oils includes more than 40 compounds, however up to 99.0% are represented by 16 compounds. The most common component of the essential oils of all the samples is para-cymol. Its content is 38.2–52.0%. A significant part (16.2–23.9%) is represented by monoterpenes - hydrocarbons formed by the combination of two isoprene fragments with the general molecular formula $C_{10}H_{16}$ C10H16 (136 Da). Thymol, which is a hydroxy derivative of para-cimol, has been detected in 2.5–4.9%. The proportion of thymoquinone in essential oils ranged from 10.1 to 22.9%.

A typical 1H NMR spectrum of *Nigella sativa* L. oil is shown in Figure 4. Unlike peaks in GC or HPLC chromatograms, where the signals correspond, as a rule, to individual components, the signals in the NMR spectra are associated with the presence of certain functional groups in the compounds. The 1H NMR spectra of vegetable oils contain, as a rule, 9 main signals corresponding to the functional groups of fatty acids that make them up: -CH_3 (0.82–0.94 ppm), -$(CH_2)_n$- (1.20–1.43 ppm), -OCO-CH_2-$\underline{CH_2}$ (1.55–1.69 ppm), -$\underline{CH_2}$-CH=CH- (1.93–2.13 ppm), -OCO-CH_2- (2.25–2.36 ppm), =CH-CH_2-CH= (2.73–2.87 ppm), -CH = CH- (5.29–5.43 ppm), as well as methylene and methine protons of the glycerol fragment -CH_2OCOR (4.10–4.35 ppm) and CHOCOR (5.23–5.29 ppm). If the experiment is performed correctly, a strict linear relationship between the signal areas and the content of the molecule fragments responsible for these signals in the sample under study is observed; it is based on the physical principle of NMR spectroscopy. By reference to the relationships between the signal areas, the content of various oil components can be calculated. The composition of *Nigella sativa* L. seeds lipid complexes determined by the 1H NMR method, is shown in Table. 5.

The results on the fatty acids composition obtained by the 1H NMR method, are quite close to the results obtained by the GC-FID method. There are also slight variations in the fatty acids compositions of oils from different regions of the world. The registration of 1H NMR spectrum of a thymoquinone standard sample, made it possible to identify characteristic signals in the spectrum that do not overlap with the main signals of fatty acids and glycerol functional groups. There are three different signals located at 6.57, 6.51 and 3.01 m. d., respectively, suitable to identify thymoquinone in the oils (Fig. 4). For the purpose of the quantitative assessment of the thymoquinone content, two signals located about 6.5 m. d., were selected. NMR spectroscopy is a direct method of the quantitative analysis that does not require any use of standard samples of the compounds being determined to assess their content in the objects of different Genesis. The results of 1H NMR show that the content of thymoquinone in the samples varies within the range of 0.7–2.6%.

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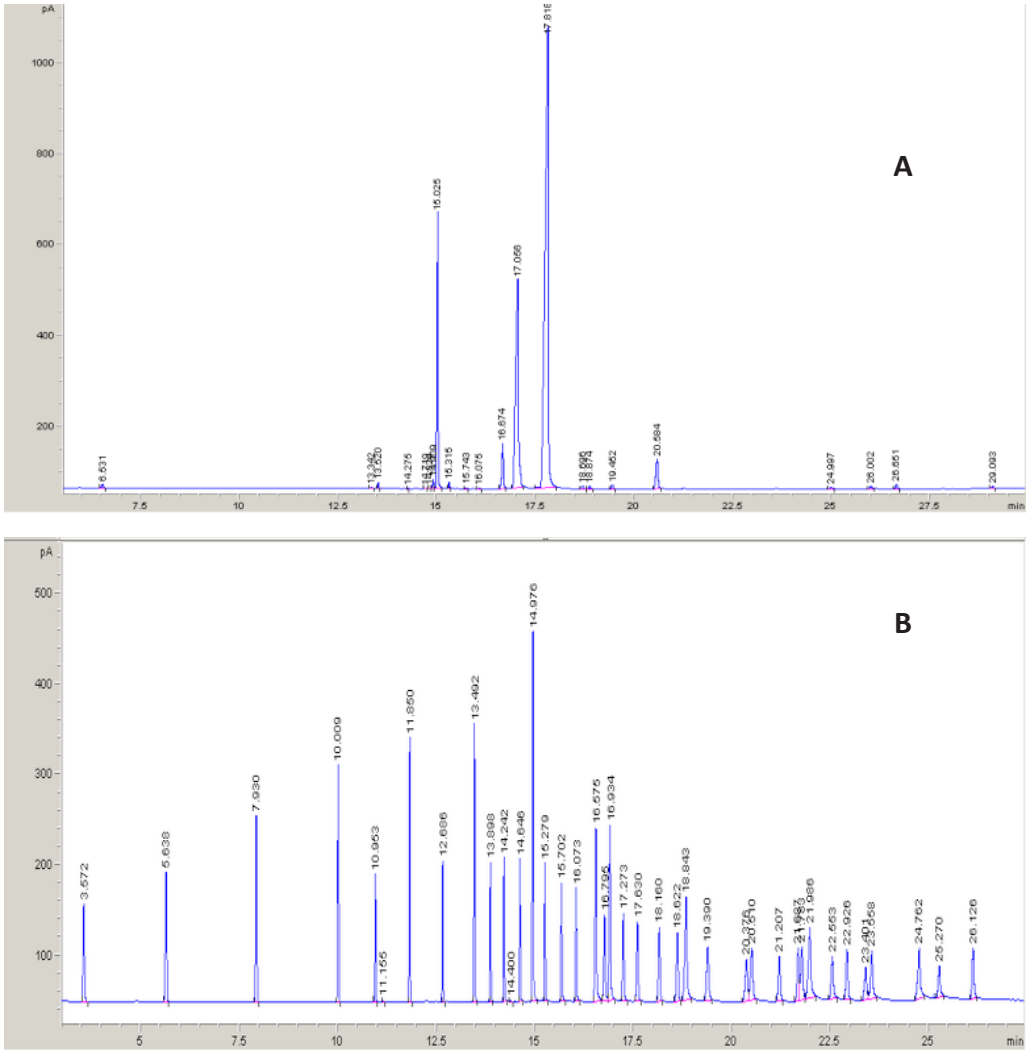

Figure 1 – Chromatogram of FA methyl esters from the lipid complex obtained from *Nigella sativa* L. seeds (A); chromatogram of methyl esters obtained from Supelco® 37 component FAME mix (B)

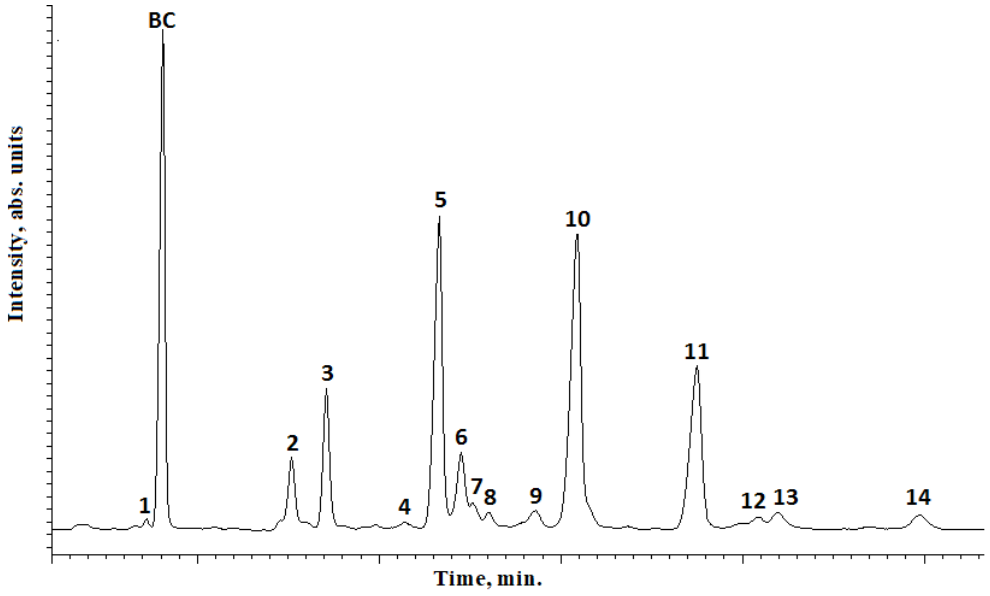

Figure 2 – A typical chromatogram for the total ion current of the *Nigella sativa* L. unsaponifiable fraction (the output region of sterols and triterpene alcohols)

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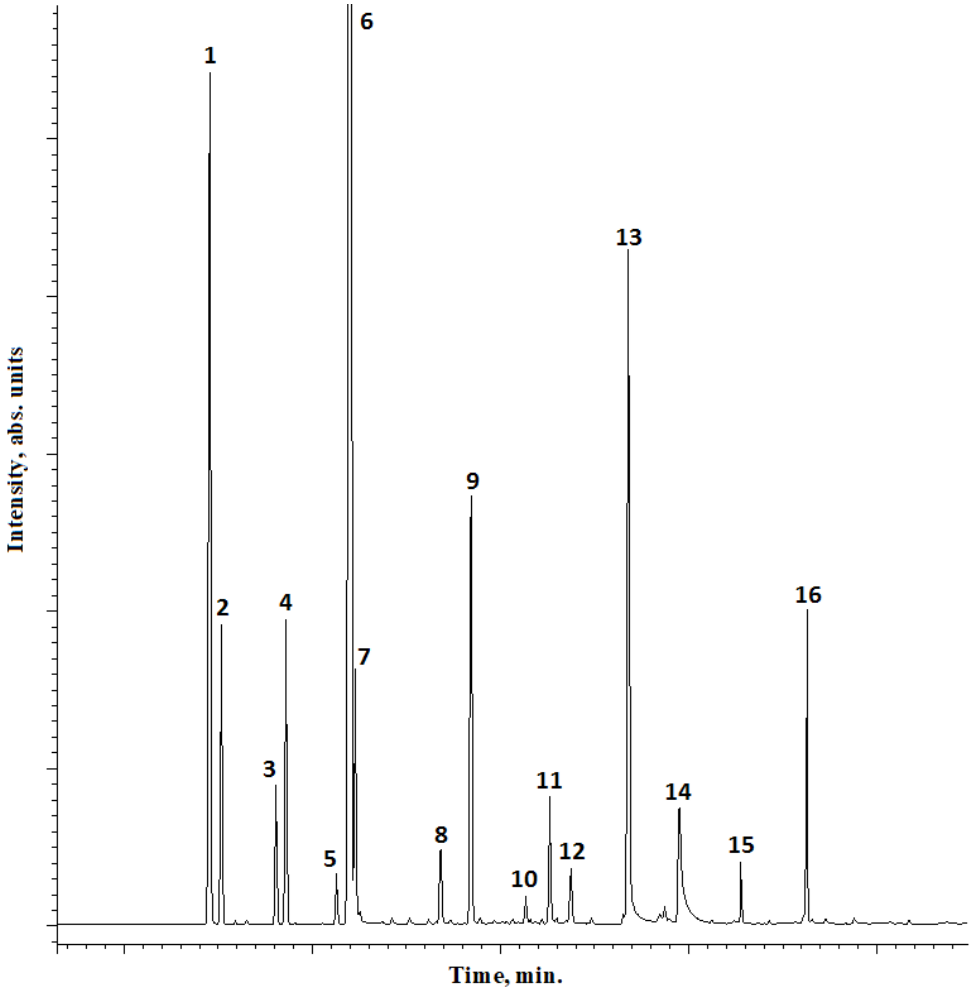

Figure 3 – Typical chromatogram of the total ion current of *Nigella sativa* L. essential oil

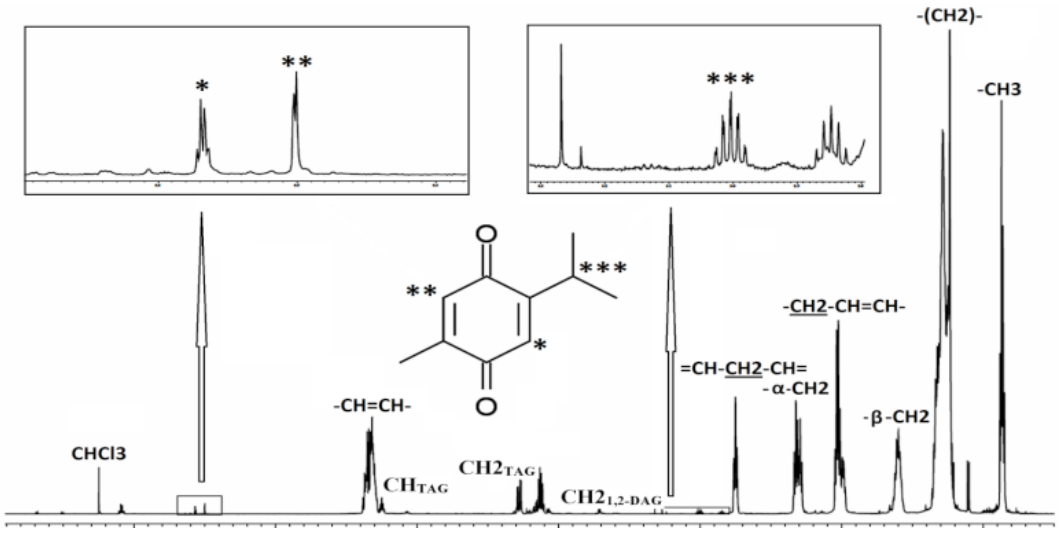

Figure 4 – Typical quantitative 1H NMR spectrum of *Nigella sativa* L. oil

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Table 1 – Physico-chemical properties of the obtained *Nigella sativa* L. seeds lipid complexes

No	Country of origin	Oil content, %	Oil characteristics			
			Viscosity, cPs	Refraction index	Density, мg/cm^3	Appearance
1	Yemen	35.8	39.3	1.4688	906.592	Yellow orange viscous opalescent liquid with an aromatic odor and a bitter spicy acrid taste
2	Tatarstan	37.8	40.8	1.4654	908.924	
3	India	34.8	41.1	1.4675	903.945	
4	Tajikistan	37.2	39.2	1.4644	892.137	
5	Ethiopia	30.4	37.0	1.4638	897.590	
6	Egypt	35.5	38.2	1.4677	908.304	
7	Israel	32.4	37.5	1.4687	907.865	

Table 2 – Fatty acids composition of *Nigella sativa* L. seeds lipid complexes according to the results of GC-FID

No	Acid name	Formula	Sanple, growth region, relative content of FA in the lipid complex, %						
			Yemen	Tatarstan	India	Tajikistan	Ethiopia	Egypt	Israel
1	Palmitic	C16:0	12.1	10.0	12.2	12.8	2.3	12.4	12.3
2	Stearin	C18:0	2.6	2.4	2.7	3.2	3.1	2.8	2.9
3	Oleic	C18:1	3.3	23,3	24.4	24.6	4.7	21.8	22.2
4	Linoleic	C18:2	8.3	60.6	57.1	55.8	6.3	59.4	59.0
5	Cis-11,14-eicosadiene	C20:2	2.6	2.5	2.3	2.3	2.3	2.4	2.5
6	Other fatty acids		1.1	1.2	1.3	1.3	1.3	1.2	1.1
7	Total		100.0	100.0	100.0	100.0	100.0	100.0	100.0

* The table shows average values of three parallel detections

Table 3 – Sterols and triterpene alcohols found in *Nigella sativa* L. seeds lipid complexes according to the results of GC-MS

No on chromatogram (Fig. 2)	Compound	Sample, growth region, content, mcg / 100 mg						
		Yemen	Russia (Tatarstan)	India	Tajikistan	Ethiopia	Egypt	Israel
BC	Cholestanol	200.0	200.0	200.0	200.0	200.0	200.0	200.0
1	Cholesterol	2.6	1.4	2.7	3.7	9.0	4.3	2.5
2	Campesterol	34.7	30.9	25.4	26.6	31.3	25.7	21.0
3	Stigmasterol	57.6	49.4	37.1	37.0	45.2	39.1	38.3
4	Cleosterol	3.6	5.8	4.1	2.4	1.3	3.3	2.6
5	Sitosterol + Lanosterol (traces)	165.6	143.7	108.4	117.3	119.9	129.2	116.9
6	Δ5-Avenasterol + sitostanol (traces)	44.1	41.9	28.6	37.5	35.1	39.5	20.3
7	β-amyrin	13.6	12.6	9.3	0.0	5.6	6.9	7.4
8	Otusifoliol	7.6	8.0	6.6	4.4	5.5	3.9	5.9
9	Gramisterin	13.3	16.4	11.2	4.5	5.1	6.6	7.6
10	Cycloartenol + Δ7-Avenasterin	210.7	190.8	120.9	164.6	188.8	89.9	95.1
11	24-methylenecycloartanol	126.1	106.3	69.5	54.6	49.9	89.1	73.0
12	Erythrodiol	10.6	8.8	6.4	2.5	2.0	3.7	3.9
13	Cytrostadienol	14.8	12.1	7.2	5.3	4.0	3.4	3.9
14	Uvaol	14.8	11.0	3.8	17.7	21.2	2.9	1.9
	Total	719.7	639.1	441.2	478.1	523.9	447.5	400.3

* The table shows the average values of three parallel detections

Table 4 – Compounds found in *Nigella sativa* L. essential oils seeds, according to the results of GC-MS

No. on chromatogram (Fig. 3)	Compound	Sample, growth region, relative content of component in essential oil, %						
		Yemen	Russia (Tatarstan)	India	Tajikistan	Ethiopia	Egypt	Israel
1	alpha-Thujene	8.8	7.9	12.9	12.1	7.6	9.3	8.6
2	alpha Pinene	0.6	1.9	3.3	3.2	1.7	1.8	1.8
3	Sabinene	1.3	0.8	0.9	1.7	0.7	0.9	1.2
4	beta-Pinene	3.2	2.2	3.3	3.4	2.9	2.5	3.7
5	beta-Myrcene	0.3	0.4	0.2	0.6	0.1	0.2	0.5
6	para-Cymene	44.6	38.2	47.9	46.6	49.2	52.0	49.9
7	Limonene	2.0	3.4	1.8	2.9	2.3	1.9	1.6
8	Cis-methoxythujene	0.8	1.0	1.0	1.2	1.3	0.9	1.3
9	Trans-methoxythujene	4.4	5.3	1.3	6.4	5.3	5.3	4.7
10	Unidentified compound	0.3	0.2	0.3	0.3	0.1	0.1	0.2
11	Terpenine -4-ol	1.2	0.8	0.8	1.5	1.5	0.9	1.4
12	Camphor	0.9	0.6	1.1	0.8	0.8	0.7	1.1
13	Thymoquinone	19.8	22.9	14.5	10.1	17.9	13.5	12.7
14	Thymolum	4.3	4.9	2.9	3.8	2.5	2.7	3.4
15	alpha-Longipinene	0.7	1.7	2.7	0.7	0.9	0.8	0.5
16	alpha-Longifolene	6.0	6.8	4.3	3.6	4.5	5.6	6.3
	Other compounds	0.8	1.1	0.8	0.9	0.7	0.9	1.1
	Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0

* The table shows the average values of three parallel detections

Table 5 – Composition of *Nigella sativa* L. seeds oil lipid complexes from different regions of the world according to the results of quantitative 1H NMR spectroscopy

Compound	Sample, growth region, content, %						
	Yemen	Russia (Tatarstan)	India	Tadjikistan	Ethiopia	Egypt	Israel
Palmitic +Stearic acids	15.2	16.3	16.0	15.9	14.3	15.6	14.7
Oleic	28.4	26.2	28.0	27.3	30.2	26.3	27.1
Linoleic	56.4	57.5	56.0	56.9	55.5	58.1	58.2
Linolenic	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Sum of acids	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Triacyglycerides	87.3	86.4	95.3	94.5	85.1	84.1	81.7
1,2-Diacylglycerides	4.7	5.7	1.7	1.9	5.5	5.6	7.2
1,3-Diacylglycerides	6.4	6.0	2.2	2.9	5.3	6.6	8.0
1-Monoacylglycerides	1.4	1.9	0.7	0.7	2.0	2.1	2.5
2-Monoacylglycerides	0.2	0.0	0.1	0.0	2.1	1.6	0.6
Sum of glycerides	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Thymoquinone	1.5	0.7	1.0	2.6	2.2	2.4	2.5

* The table shows the average values of three parallel detections

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The chemical composition data show that linoleic acid is the dominant fatty acid in the lipid complex, which may indicate the potential prospects of this plant material for creating drugs that affect lipid metabolism.

The results of the comparative analysis of *Nigella sativa* L. lipid complex have been obtained for the first time, the data of similar studies in the available literature have not been identified.

CONCLUSION

Thus, the profiles and the contents of fatty acids, sterols, triterpene alcohols, essential oils and thymoquinone found in *Nigella sativa* L. seeds lipid complexes, have been established and estimated. The component composition of the lipid complexes obtained from raw materials grown in different regions of the world, is very similar, their saponified portion is represented by triglycerides (81.7–95.3%), di- (3.9–15.2%) and monoglycerides (0.7–4.1%).

They mainly contain linoleic (55.8–60.6%), oleic (21.8–24.6%), palmitic (10.0–12.8%), stearic (2.4–3.2%) and cis-11.14-eicosadiene (2.3–2.6%) acids. The content of sterols and triterpene alcohols in the lipid complex was 0.4–0.7%; up to 70% of the fraction was represented by β-sitosterol (22.5–29.2%), cycloartenol (20.1–36.6%) and 24-methylenecycloartanol (9.5–19.9%). The content of thymoquinone in lipid complexes ranged from 0.7 to 2.6%.

The chemical composition of *Nigella sativa* L. seeds makes it possible to recommend this plant material as a source of essential fatty acids, thymoquinone and essential oils with a multivalent pharmacological target. This determines the need for further in-depth research of *Nigella sativa* L. from the standpoint of pharmacology and pharmaceutical development.

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AUTHORS` CONTRIBUTION

All authors equally contributed to the research work.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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RESEARCH ARTICLE

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HPLC METHODS OF FEXOFENADINE QUANTITATIVE ANALYSIS IN RABBITS' LIVER

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The investigation of pharmacokinetics of marker substrates of carrier protein P-glycoprotein (Pgp, ABCB1-protein) including fexofenadine, is one of the methods of its functional activity evaluation.

The aim of the study was to work out the HPLC methods of the quantitative determination of fexofenadine in rabbits' liver.

Materials and methods. The quantitative determination of fexofenadine was performed using Stayer chromatographic system (Akvilon, Russia) with UVV 104 ultraviolet detector. Reverse-phased chromatographic column Luna C18 100Å (250*4.6) was used with 5 µm granulation at 45°C. The concentration of fexofenadine was determined by methods of absolute peak area calibration.

Results. The work was conducted in the isocratic mode. The composition of the mobile phase consisted of deionized water, acetonitrile and glacial acetic acid at the ratio of 267.4:120:4.33 brought to pH=6.7 with triethylamine.

The sample processing was in the form of homogenization of 500 mg of ground liver in 500 µl of purified water with the subsequent centrifugation (1750 g) and selection of the supernatant. The proteins were precipitated by acetonitrile (2.5 ml) acidified with 375 µl of hydrochloric acid by shaking at 500 rev/min.

The supernatant was transported into a separate test tube, where methylene chloride, diethyl ether and ethyl acetate were added (2 ml each). Then the solution was again shaken for 10 minutes (500 rev/min). After that, the solution was centrifuged (1750 g) and the supernatant was evaporated on a rotor-vacuum evaporator at 50°C. 300 µl of the mobile phase was added to the dry residue, and 100 µl was injected into the chromatograph.

The method was validated in the linear range from 3 to 60 µg/g of fexofenadine with the acceptable intra- and intercycle accuracy, precision and stability. The method was tested on rabbits after the intravenous administration of fexofenadine at the dose of 11 mg/kg.

Conclusion. The HPLC methods of fexofenadine quantitative determination in the hepatic tissue of rabbits has been worked out. It can be used for the evaluation of the functional activity of Pgp in preclinical studies.

Keywords: P-glycoprotein, ABCB1-protein, fexofenadine, chromatography, pharmacokinetics, rabbits, liver

Abbreviations: Pgp – P-glycoprotein, HPLC – high performance liquid chromatography, rev/min – revolutions per minute

ВЭЖХ-МЕТОДИКА КОЛИЧЕСТВЕННОГО АНАЛИЗА ФЕКСОФЕНАДИНА В ПЕЧЕНИ КРОЛИКОВ

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Исследование фармакокинетики маркерных субстратов белка-транспортера гликопротеина-Р (Pgp, ABCB1-белка), к которым относится фексофенадин, является одним из способов оценки его функциональной активности.

Цель. Разработка ВЭЖХ-методики количественного определения фексофенадина в печени кроликов.

Материалы и методы. Количественное определение фексофенадина осуществляли с использованием хроматографической системы Stayer («Аквилон», Россия) с УФ детектором UVV 104. Применяли обращенно-фазную хроматографическую колонку Luna C18 100Å (250*4,6) с зернением 5 мкм при температуре 45°С. Определение концентрации фексофенадина проводили методом абсолютной калибровки по площади пиков.

Результаты. Исследование проводили в изократическом режиме. Состав подвижной фазы: вода деионизированная, ацетонитрил и ледяная уксусная кислота в соотношении 267,4:120:4,33, доведенные триэтиламином до pH=6,7. Пробоподготовка заключалась в гомогенизации 500 мг измельченной печени в 500 мкл воды очищенной с последующим центрифугированием (1750 g) и отбором надосадочной жидкости. Осаждение белка осуществлялось ацетонитрилом (2,5 мл), подкисленным 375 мкл кислоты хлористоводородной путем встряхивания на приборе Shaker (500 об./мин). Надосадочный слой переносили в отдельную пробирку, добавляли по 2 мл метилена хлористого, эфира диэтилового и этилацетата и повторно встряхивали 10 мин (при 500 об./мин). Затем центрифугировали (1750 g) и упаривали супернатант на роторно-вакуумном испарителе при 50°С. К сухому остатку добавляли 300 мкл подвижной фазы и 100 мкл инжектировали в хроматограф. Метод был валидирован в линейном диапазоне от 3 до 60 мкг/г фексофенадина с приемлемой внутри- и межцикловой точностью, прецизионностью и стабильностью. Методика была апробирована на кроликах после внутривенного введения им фексофенадина в дозе 11 мг/кг массы.

Заключение. Разработана ВЭЖХ-методика количественного определения фексофенадина в ткани печени кроликов, которая может использоваться для оценки функциональной активности Pgp в доклинических исследованиях.

Ключевые слова: гликопротеин-Р, ABCB1-белок, фексофенадин, хроматография, фармакокинетика, кролики, печень

Список сокращений: Pgp – гликопротеин-Р, ВЭЖХ – высокоэффективная жидкостная хроматография, об./мин – оборотов в минуту

INTRODUCTION

P-glycoprotein (Pgp, ABCB1-protein) is an efflux ATP-dependent membrane carrier protein that removes exogenous and endogenous substances of the lipophilic nature from cells into the intercellular space [1, 2]. Pgp possesses a wide substrate specificity and transports a range of medical substances: cardiac glycosides, hypotensive, antiarrhythmic, antiepileptic drugs, anticoagulants, cytostatics and other groups of drugs [3].

Being localized on the apical surface of enterocytes of small and large intestines, this transporter prevents absorption of the substrates in the endothelial cells of histohematic barriers.

It prevents their penetration into the barrier organs, on the biliary surface of hepatocytes and on the apical surface of epitheliocytes of renal tubules. Pgp promotes excretion of the substrates into bile and urine, respectively [1, 4]. Thus, Pgp plays an important role in pharmacokinetics of the drugs, which are its substrates: controls their absorption, distribution and excretion.

Functioning of Pgp can differ under the influence of many factors (hypoxic influences, hormonal level, drugs consumption), which may induce the development of undesired medical reactions in case of inhibition of the transporter, or reduction of effectiveness of the conducted pharmaceutical therapy if its activity increases [5, 6].

A study of Pgp functional activity will permit to optimize a medicinal treatment and to predict interactions between medical drugs [7]. One of the evaluation methods of Pgp functioning *in vivo,* is the examination of pharmacokinetics of its marker substrates in dynamics [6, 8]. A marker substrate is a substance, its pharmacokinetics (absorption, distribution and excretion) is determined by the tested carrier protein [6]. As a marker substrate of Pgp, H_1-histaminolytic drug of the 3rd generation – fexofenadine – is used.

An HPLC method with UV detection is a universal method of the quantitative analysis available at any laboratory, in contrast to the method of mass-spectrophotometric detection. There are known HPLC methods of fexofenadine concentration determination in blood plasma and in the homogenate of the brain that are used for testing a functional activity of Pgp at the level of the whole organism and in the blood-brain barrier [9–13]. However, in the studied literature, no HPLC methods of quantitative analysis of fexofenadine in the liver that could make it possible to evaluate the activity of the transporter of this localization and its role in the interactions between drugs at the stage of excretion, have been found.

THE AIM of the study was to develop the HPLC methods for the quantitative analysis of the Pgp marker substrate – fexofenadine – in the liver of rabbits.

MATERIALS AND METHODS

Animals

In the study, male rabbits of the Chinchilla breed weighing 3200–3500 g were used. The animals had been taken from the Kasimov-miacro breeding nursery (Kasimov, Ryazan region) and had the required veterinary certificates. After delivery from the nursery, the animals were examined by a veterinarian, kept in quarantine for 14 days, and after that they were placed to the convection vivarium of Ryazan State Medical University [14].

Each rabbit was kept in an individual cage on a litter for laboratory animals by lamplight with 12 daylight hours. The temperature in the room was maintained at 22±1°C, the relative air humidity was 45–65% [14]. The animals were fed in correspondence with State Standard P 50258-92. All the experiments with the animals were conducted in compliance with the international rules (Directive 86/609/EEC) and with the rules of Good Labo-

ratory Practice (Order of Health Ministry of RF No.199н dated 01.04.16).

The study protocol was considered and approved at the meeting of Commission for control of management and use of laboratory animals No.11 dated 28.01.2018.

The animals were euthanized by the overdose of zoletil (Zoletil 100, Virbac C.A., France) at the dose of 30 mg/kg. After that a sample of liver was taken. As a biological matrix, a homogenate of intact animals' liver was used, obtained from 6 different animals.

To test the used methods, the liver was extracted ~~in~~ 5, 10, 15, 30, 60 minutes after the administration of a fexofenadine solution (10 mg/ml) into the marginal vein of ear in the quantity of 1.1 ml/kg (n=3 for each time point). The preparation of the fexofenadine solution had been described in detail before [11]. The liver samples were kept in the frost chamber at –29°C.

Equipment

A quantitative determination of fexofenadine in the liver homogenate was carried out using Stayer chromatographic system (Akvilon, Russia) with ultraviolet spectrophotometric detector UVV 104 equipped with a 100 µl loop injector PEEK and an autosampler 7725i (Rheodyne, USA) with a 220 nm wavelength. A reversed-phase chromatographic column Luna C18 100Å (250*4.6) was used with 5 µm graining at 45°C. The samples were injected to the chromatograph loop with Microsyringes (Germany).

In the work, the following auxiliary equipment was used: Diax 9000 tissue grinder (Heidolph, Germany), Elmi CM 6M centrifuge (Elmi, Latvia), Vodolei D301 deionizer (Akvilon, Russia), VV-Micro rotor-vacuum evaporator (Heidolph, Germany), a device for shaking test tubes Shaker S 3.01 (Elmi, Latvia), Vortex laboratory medical shaker (Elmi, Latvia).

Materials

As a standard substance, fexofenadine hydrochloride was used (Sigma, Lot: R032H0, USA having the quality certificate ensuring 99.2% content of fexofenadine hydrochloride).

For the extraction of fexofenadine from the liver gomogenate and preparation of the mobile phase, the following reagents were used: acetonitrile "for HPLC" (Merck, Germany), CP hydrochloric acid (Ekos-1, Russia), CP glacial acetic acid (Ekos-1, Russia), triethylamine "for HPLC" (Lab-Skan, Poland), methylene chloride "for HPLC" (Lab-Skan, Poland), ethyl acetate (Lab-Skan, Poland), diethyl ether (Lab-Skan, Poland), deionized water obtained using Vodolei D301 deionizer (Akvilon, Russia).

Validation of Chromatographic Methods

Bioanalytical methods was validated according to the guidelines for the expertise of medicine remedies, the rules of medical drugs' bioequivalence study within the Eurasian Economic Union, EMA Guidelines on bioanalytical method validation, 2011 and FDA Guidance for Industry: Bioanalytical method of validation (draft guidance). U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evolution and Research (CDER). U.S. Government Printing Office: Washington, DC, 2013, against the following parameters: selectivity, a calibration curve (linearity), accuracy, precision, the lower limit of the quantitative determination, a sample transfer, stability of fexofenadine in the liver homogenate [15–17].

The content of fexofenadine in the homogenate of rabbits' liver was determined by a method of a peak area absolute calibration. Calibration solutions were prepared by adding the standard solution (100 µl) of the substance to the mixture of 400 µl of water and 500 µg (before homogenization) of the ground organ for obtaining the samples with concentrations of 15, 60, 120, 180, 240 и 300 µl and, further on, with final concentrations of 3, 12, 24, 36, 48 and 60 µg/g of the organ, respectively.

RESULTS AND DISCUSSION

Conditions of Chromatography

The study was conducted in the isocratic mode. The composition of the mobile phase was the following: a mixture of deionized water, acetonitrile and glacial acetic acid at the rate of 267.4:120:4.33, brought to pH=6.7 with triethylamine. In these conditions, the flow rate was 1.0 ml/min. and the retention time of fexofenadine was 17.14±0.79 min.

Sample Processing

In the process of working out the HPLC methods of quantitative analyses of the substances in the organs, it was rather problematic to achieve a high degree of their extraction from the cells, and the sample purity. It is very important since a high content of ballast compounds may lead to the contamination of the chromatographic column and considerably reduce the life of the precolumn filter.

In the work, the sample processing used for extraction of fexofenadine from the brain tissue of rats [11] based on precipitation of bioorganic molecules in the homogenate of the organ by addition of acetonitrile, has been conducted right from the beginning. Despite a high degree of the fexofenadine extraction from the liver cells, the visual evaluation of the obtained sample revealed a considerable turbidity of the solution, which evidenced the presence of compounds of a high molecular weight.

The methods of fexofenadine extraction from blood plasma [10], which consisted in adding a mixture of equal amounts of diethyl ether, methylene chloride and ethyl acetate to the acidified plasma, also led to obtaining a contaminated sample. Therefore, after the attempts to purify it by centrifuging, a stage of precipitation of proteins with acetonitrile had been added before the extraction.

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In the course of the work, the extent of the fexofenadine extraction from the liver homogenate, was checked in conditions of acidic, neutral and alkaline reactions of the medium. The most reproducible data were obtained when the extraction of fexofenadine was conducted from the acidified water-acetonitrile extract with the use of the mixture of diethyl ether, ethyl acetate and methylene chloride.

Further on, the extractability of fexofenadine from the water-acetonitrile extract was tested with different volumes of organic phases. The increase in the volume to 6 ml, led to an insignificant increase in the extent of fexofenadine extraction, therefore, a further increase in the amount of the extracting agents, was irrational. As a result, the following conditions of sample processing appeared the most optimal ones. Homogenization of 500 mg of the liver ground up with scissors, was conducted in 500 µl of purified water at 26 000 rev/min on Diax 9000 homogenizer within 1 min with subsequent centrifuging at 1750 g and separation of the supernatant. Precipitation of proteins was carried out with acetonitrile (2.5 ml) acidified with 375 µl of hydrochloric acid by shaking it on a Shaker device at 500 rev/min for 10 min.

The supernatant was transferred to a separate tube, where 2 ml of methylene chloride, diethyl ether and ethyl acetate were added, with re-shaking of the solution at 500 rev/min for 10 min. Then the solution was centrifuged at 1750 g for 10 min and the supernatant was evaporated on a rotary-vacuum evaporator at 50°C. The extraction coefficient of fexofenadine was 42.4%. 300 µl of the mobile phase was added to the dry residue, and 100 µl was injected into the chromatograph.

These conditions of sample processing permitted to achieve a reproducible coefficient of fexofenadine extraction, sufficient for the construction of calibration curves and calculation of the main validation characteristics of the methods.

Parameters of applicability of chromatographic system

The number of theoretical plates were more than 3100, the peak asymmetry coefficient was not more than 1.2.

Selectivity of chromatographic methods

The successive analysis of the sample with the concentration of fexofenadine equal to 60 µg/g and that of the pure liver homogenate sample after the sample processing, showed no peaks on the chromatogram of the intact homogenate corresponding to the peaks of the target substance by the retention time. The samples of the obtained chromatograms are presented in Figure 1. The coefficient of separation (resolution) of the fexofenadine peak and of the nearest peak of the coextractive substances, was calculated as the difference between the retention times of the mentioned peaks divided by the sum of their widths at half heights. The parameter was more than 2.

Calibration curve

To construct calibration curves, 6 standard fexofenadine solutions in the rabbits' liver homogenate of the following concentrations were prepared: 3, 12, 24, 36, 48 and 60 µg/g.

They were analyzed with the calibration graph construction showing the dependence of the concentration of a substance on the area of its chromatographic peak (Fig. 2).

This procedure was conducted 3 times – before each following stage of chromatography, which was necessary, according to the recommendations [15–17]. The following regression equations were obtained: $y = 0.0086 + 1.425$, $R^2 = 0.9981$; $y = 0.0095 + 1.3598$, $R^2 = 0.9989$; $y = 0.0092 + 1.1568$, $R^2 = 0.9995$. Calibration curves with their respective regression equations were used for calculation of accuracy and precision between the cycles in the given methods.

The correlation coefficients were more than 0.99. The deviations of the concentrations of calibration samples from nominal values calculated using three levels of linear dependence, are presented in Table 1.

Accuracy and Precision

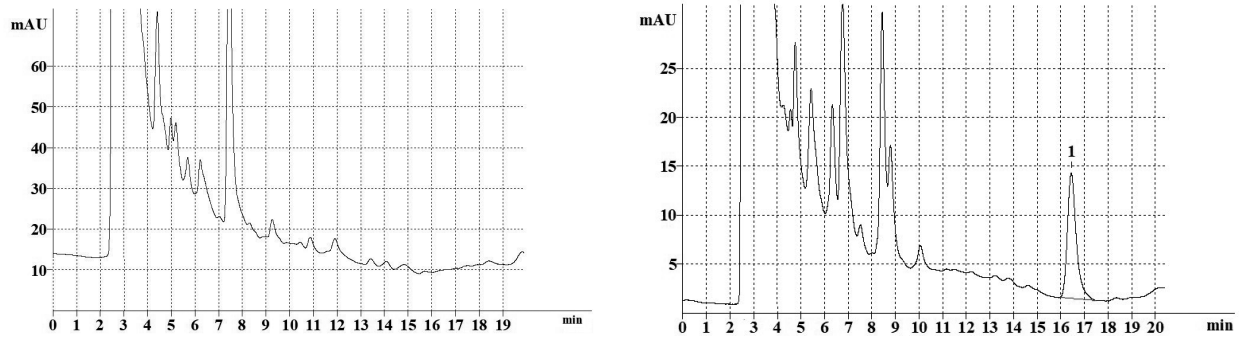
Accuracy and precision were evaluated by the analysis of the samples of intact rabbits' liver homogenate with adding a standard fexofenadine solution to obtain the concentrations of 3, 12, 24 and 48 µg/g.

The precision values (relative to the standard deviation) and the accuracy values (a relative error) corresponded to the accepted norms (not more than 20% for the lower limit of the quantitative determination and not more than 15% for other points) [15–17] (Table 2).

Lower Limit of Quantitative Determination

The lower limit of the quantitative determination of the methods was evaluated on the basis of linearity, accuracy and precision. The lower limit of the quantitative determination was assumed the minimal fexofenadine concentration in the liver homogenate within the range of linear dependence in which the substance can be determined with accuracy and precision not more than 20%. The lower limit of the quantitative determination of fexofenadine by this methods was 3 µg/g.

The signal/noise ratio based on the fexofenadine peaks at the level of the lower limit, was not less than 10. The limit of fexofenadine detection by this method was about 1.6 µg/g. The signal/noise ratio was about 3 [18].



A **B**

Figure 1 – Chromatogram of liver homogenate sample

Note: A – intact homogenate of liver; B - with addition of fexofenadine standard up to the concentration of 3 μg/g

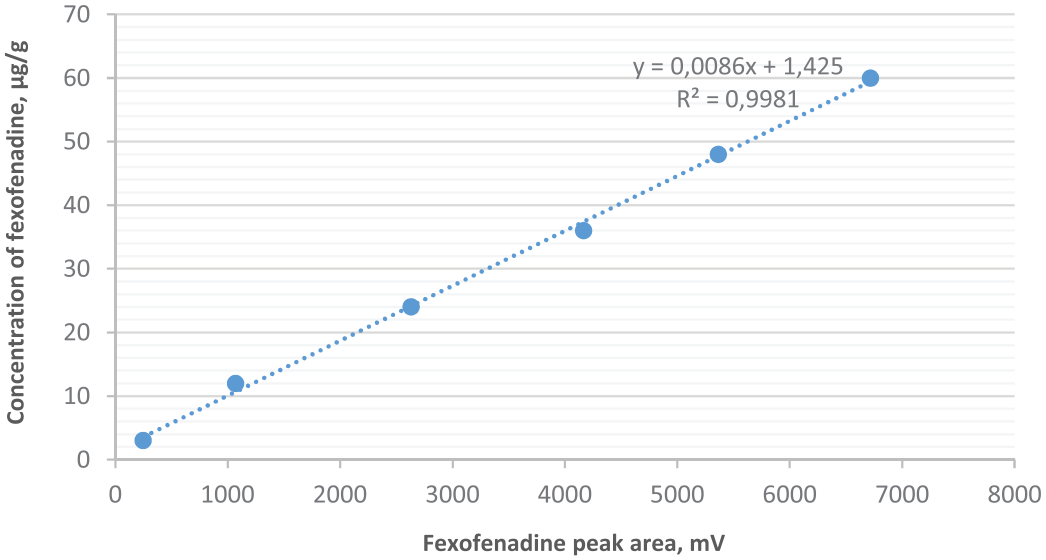


Figure 2 – Calibration curve of interdependence in fexofenadine concentration in the liver homogenate and its chromatographic peak area

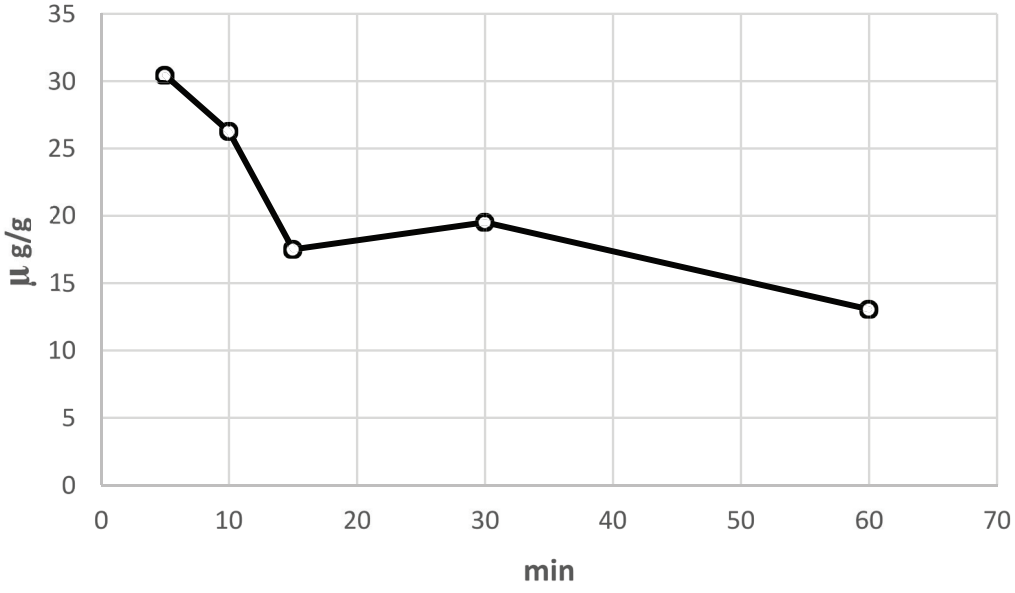


Figure 3 – Concentration of fexofenadine in the liver after its intravenous administration at the dose of 11 mg/kg (n=3 for each time point, M±SD)

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Table 1 – Deviations of concentrations of fexofenadine calibration samples from nominal values calculated by equations of linear dependences

Nominal concentration, μg/g	Graph 1		Graph 2		Graph 3	
	Calculated concentration, μg/g	Accuracy,%	Calculated concentration, μg/g	Accuracy, %	Calculated concentration, μg/g	Accuracy,%
3	3.56	18.57	3.53	17.53	3.41	13.65
12	10.63	11.46	11.50	4.13	11.28	6.03
24	24.06	0.26	23.23	3.20	24.53	2.20
36	37.25	3.48	36.51	1.42	36.02	0.07
48	47.57	0.90	48.86	1.79	48.68	1.41
60	59.21	1.31	59.27	1.22	60.02	0.03

Table 2 – Accuracy and precision of methods of fexofenadine quantitative determination in rabbits' liver homogenate within and between cycles

Nominal Concentration, μg/g	Mean Concentration, μg/g	Mean Accuracy, %	SD	Precision, %
Within cycles				
3	3.52	17.20	0.053	1.52
12	10.85	9.60	0.23	2.16
24	23.80	5.35	1.45	6.09
48	44.92	6.42	0.94	2.10
Between cycles				
3	3.37	12.35	0.14	4,02
12	10.82	9.81	0.44	4.07
24	25.29	5.38	1.16	4.57
48	48.99	4.17	2.35	4.79

Stability

The stability of standard fexofenadine solutions was evaluated by their three-fold chromatography after three frost/defrost cycles and dilution to the concentration of 10 μg/ml. Each frost cycle lasted 24 hours at –29°C in the frost chamber followed by defrost at the room temperature for 2 hours. No reliable differences were found out between the fexofenadine concentrations before and after the described manipulations.

To evaluate the stability of fexofenadine in rabbits' liver homogenate kept in the frozen state, the samples were prepared in the concentration of 48 μg/g. Half of the samples were analyzed immediately after the preparation, and the rest of them were prepared after storage in the frozen condition within 60 days. 3 independent samples from each party were examined. The mean concentration was 46.44 μg/g, the mean accuracy was 3.26%.

Sample Transfer

In the successive analysis of the sample with the fexofenadine concentration of 48 μg/g and the sample of the blank liver homogenate, no peaks were present in the chromatogram of the pure (intact) liver homogenate corresponding to the fexofenadine peak by the retention time, which evidenced the absence of the sample transfer.

Approbation of Methods

A study of pharmacokinetics of Pgp marker substrates, is a leading method in the investigation of functioning of the protein carrier *in vivo* [14]. The concentration determination of the marker substrate in blood after its single peroral administration, characterizes the functional activity of Pgp at the level of the whole organism [6]. However, a more complete and tissue-specific evaluation of functioning of Pgp, requires the use of methods permitting to study the functional activity of the transporter locally in the organs responsible for absorption, distribution and excretion of substances. This is done by a quantitative determination of the concentration of marker substrates in different organs and tissues [11, 19].

As a marker substrate, fexofenadine possesses a number of advantages: it does not undergo biotransformation, does not cumulate in an organism, it possesses a wide range of therapeutic effects, rarely causes side effects, it is available in price and can be bought from pharmacies without prescription [6, 9, 10]. This drug is primarily excreted with bile (80%) [20], therefore a method of its quantitative distribution in the liver permits evaluation of the functional activity of the transporter in this organ. An increase in the concentration of fexofenadine in the liver, indicates inhibition of the functional activity of Pgp, the reduction of its level evidences the induction of the activity of this transporter.

Rabbits are recommended as a test system for the investigation of functioning Pgp *in vivo* since these animals have the highest homology between amino acid sequences with the transporter of a human being [21]. Pgp of rabbits and humans is coded by one *mdr1* gene, and not by two (*mdr1* and *mdr2)* like in other rodents (mice and rats) [22–24]. Besides, the mechanisms of regulation of the transporter are similar in a human being and a rabbit [21, 25].

To test the developed methods, the concentration of fexofenadine in the rabbits' liver was analyzed 5, 10, 15, 30, 60 minutes after its intravenous administration at the dose of 11 mg/kg.

Concentrations of fexofenadine in the liver homogenate were 30.4±0.88, 26.3±3.17, 17.5±2.90, 19.5±1.49 μg/g, respectively (Fig. 3). It confirms the possibility of using the developed methods for the determination of concentrations of the substance under study in the rabbits' liver and for the subsequent analysis of functioning of the carrier protein Pgp.

CONCLUSION

Thus, HPLC methods was for quantitative determination of fexofenadine in the hepatic tissue of rabbits has been worked out. It is characterized by efficiency, sensitivity, specificity, high resolution, reproducibility and linearity in the range of the concentrations that can be used for the evaluation of the functional activity of Pgp in preclinical trials.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

All the authors have equally contributed to the research work.

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RESEARCH ARTICLE

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BETAMETHASONE ADMINISTRATION AS A TREATMENT OF CHOICE IN LOCAL POST-TATTOO COMPLICATIONS

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The aim of the study is to determine the effectiveness of betamethasone in the treatment of local post-tattoo complications, depending on the mode of administration.

Materials and methods. The work was carried out on 90 male rats which had been tattooed (n = 30 – a negative control group; n = 30 – a comparison group: a subcutaneous administration of 1 ml of a betamethasone solution; n = 30 – an experimental group: an administration of 1 ml of a betamethasone solution using a tattoo machine), 15 – intact rats. Withdrawal from the experiment took place on the 3rd, 10th and 21st days. The skin samples were fixed in 10% formalin, followed by histological posting and manufacturing of micropreparations, then staining with hematoxylin and eosin, according to Van Gieson. A morphometric study included determination of the volume fraction (VF) of the epidermis; dermal fibers; pigment; inflammatory cells; macrophages (%), as well as the pigment depth (μm) and the severity of edema.

Results. The study found out that in the process of the betamethasone administration using a tattoo machine, the drug was uniformly administrated over the entire area of the tattoo; hereby, the phenomena of edema and inflammatory infiltration were insignificant. The dermal fibers were located in each layer with no signs of edema and with single cells of inflammation, respectively. The data of the histological processing were completely consistent with the results of morphometry: it was found out that in the experimental group, edema significantly decreased, the volume fraction of the pigment and macrophages decreased, and the volume fraction of the dermal fibers increased. The estimation of the inflammatory reaction was carried out according to the morphometric parameters of the volume fraction of inflammatory cells and had significant differences in all the experimental groups, decreasing in the following series: the negative control group> the comparison group> the experimental group> the group of intact animals ($p < 0.05$).

Conclusion. Based on the data obtained, the effectiveness of betamethasone in the treatment of local post-tattoo complications has been proved. In this case, the treatment of choice is the administration of this drug not traditionally subcutaneously, but using a tattoo machine that enables the targeted delivery of the substance to the area of the pathological process.

Keywords: betamethasone, tattoo, morphometry, tattoo pigment, macrophages

Abbreviations: NC – negative control, VF – volume fraction

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ПРИМЕНЕНИЕ БЕТАМЕТАЗОНА В ЛЕЧЕНИИ МЕСТНЫХ ПОСТТАТУАЖНЫХ ОСЛОЖНЕНИЙ

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Цель. Определить эффективность применения бетаметазона в лечении местных посттатуажных осложнений в зависимости от способа введения.
Материалы и методы. Работа выполнена на 90 крысах-самцах, которым наносили татуировки (n=30 – негативный контроль; n=30 – группа сравнения: подкожное введение 1 мл раствора бетаметазона; n=30 – опытная группа: введение 1 мл раствора бетаметазона с помощью тату-машинки), 15 – интактные крысы. Выведение из эксперимента на 3, 10, 21 сут. Образцы кожи фиксировали в 10% формалине с последующей гистологической проводкой и изготовлением микропрепаратов, окраской гематоксилином и эозином, по ван Гизону. Морфометрическое исследование включало определение объемной доли (ОД) эпидермиса; волокон дермы; пигмента; клеток воспалительного ряда; макрофагов (%), а также глубину залегания пигмента (мкм) и выраженность отека.
Результаты. В ходе исследования установлено, что введение бетаметазона с помощью тату-машинки препарат равномерно вводился на всю площадь татуировки, в связи с этим, явления отека и воспалительной инфильтрации были незначительными. Волокна дермы располагались соответственно каждому слою без признаков отека и с единичными клетками воспаления. Данные гистологического исследования полностью согласовывались с результатами морфометрии, в результате которой было установлено, что в опытной группе достоверно уменьшался отек, снижалась объемная доля пигмента и макрофагов, увеличивалась объемная доля волокон дермы. Оценка воспалительной реакции проводилась по морфометрическим параметрам ОД клеток воспалительного ряда и имел достоверные различия во всех экспериментальных группах, уменьшаясь в ряду: группа негативного контроля > группа сравнения > опытная группа >группа интактных животных ($p<0,05$).
Заключение. На основании полученных данных доказана эффективность применения бетаметазона при лечении местных посттатуажных осложнений. При этом наиболее предпочтительным является введение данного препарата не традиционно подкожно, а с использованием тату-машинки, дающей возможность адресной доставки вещества в зону патологического процесса.
Ключевые слова: бетаметазон, татуаж, морфометрия, татуировочный пигмент, макрофаги
Сокращения: НК – негативный контроль, ОД – объемная доля

INTRODUCTION

The search for the development and implementation of treatment regimens for patients with post-tattoo complications is an urgent problem of modern medicine in general, and dermatology in particular. In modern society, having tattoos is no longer associated with a certain social status of its owners, however, due to the appearance of numerous tattoo parlors, the number of complications resulting from tattooing is steadily growing [1].

On the basis of a multicenter clinical trial, a classification of complications arising from the tattoo procedure has been presented [2]. One of the most common post-tattoo local complications is contact dermatitis and the formation of keloid scars [3, 4].

The attempts to treat inflammation in the tattoo area using local non-steroidal and steroidal anti-inflammatory drugs, are single [5], and the data obtained are contradictory. These factors determine the relevance of this study.

THE AIM of the study is to determine the effectiveness of betamethasone in the treatment of local post-tattoo complications, depending on the mode of administration.

MATERIALS AND METHODS

Experimental animals

The experiment was performed on 105 nonlinear sexually mature male rats (stock), weighing 280–300 g. The rats were kept under standard vivarium conditions,

with a natural change in the daily cycle, a free access to extruded food and water. The contents and manipulations were carried out in accordance with order No. 755 of the USSR Ministry of Health dated 08/12/1977, and the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, March 18, 1986) [6, 7].

Study design

The design of the experiment is shown in Fig. 1.

At the first stage of the experiment, after anesthesia (injected intraperitoneally with chloral hydrate 350 mg/kg) and preoperative showering обработки операционного поля (the dorsal area область спины), 90 rats were intradermally tattooed with **a** black pigment (Corona Colors Inc., USA), 2 cm^2 in area, using a Long Time Liner tattoo machine of a rotary type. A characteristic feature of this device is its operation in a gentle mode, providing a penetration depth of 0.5 mm into the tissue. The control group was represented by 15 intact animals.

At the second stage, the rats were divided into 3 groups: a negative control group (without pharmacological correction, n = 30), a comparison group (with a traditional subcutaneous administration of 1 ml of a betamethasone solution, n = 30), an experimental group (an administration of 1 ml of a betamethasone solution using a tattoo apparatus, n = 30). Betamethasone is a glucocorticosteroid, its trade name is Diprospan® suspension for injection 7 mg/ml (2 mg + 5 mg/ml); 1 ml ampoule, blister pack 1, cardboard pack 1; EAN Code: 4602210000038; No. P N013528 / 01, 2008-07-04 from MSD Pharmaceuticals LLC (Russia); manufacturer: Schering-Plough Labo N.V. (Belgium) ATX H02AB01 Betamethasone. Betamethasone (Diprospan) is a drug of choice in dermatovenerology in the local treatment of dermatitis, eczema, psoriasis and other skin diseases.

The animals were withdrawn from the experiment on the 3rd, 10th and 21st days.

Histological processing

180 fragments of the skin with tattoos were taken; 30 fragments of the skin of intact animals were taken as a control. After fixing the material in a 10% neutral formalin solution (the exposition time was 24 h), a standard histological posting was performed by keeping the fixed material in ethyl alcohol in the ascending concentration (from 70° to 100°) and chloroform. After pouring into paraffin and manufacturing blocks, serial histological sections were obtained, followed by staining with hematoxylin and eosin, according to Van Gieson (for determining the connective tissue).

The microphotographs were taken using a "Leica DM 100" microscope with a digital camera, magnification ×100, ×200.

Morphometric research

The morphometric research was carried out in accordance with the established principles of quantitative morphological studies, according to which measurements are made on microphotograms obtained by photographic documentation of serial sections (and determining the number of objects on at least 10 glasses in 10 fields of view) [8].

Using the program "Video test Morpho", the following data were determined: the volume fraction of epidermis (%); the volume fraction of dermal fibers (%); the volume fraction of the pigment (%); the volume fraction of inflammatory cells (%); the volume fraction of macrophages (%), as well as the depth of the pigment (μm) and the severity of edema.

Statistical processing of results

The gained results were processed using the STSTISTICA 7.0 application software package (StatSoft, USA). The following data were determined: M ± SEM, nonparametric Wilcoxon's test, Student's t-test and confidence indices (p). The results were considered reliable at $p < 0.05$.

RESULTS

Effectiveness of betamethasone during the tattooing process depending on the mode of administration according to the results of histological processing

When performing the histological block of the study of the rats' skin samples, it was found out that on the 3rd day, in the rats of the intact group, the skin was represented by two layers: epidermis and derma. The epidermis included horny, prickle-cell and basal layers adjacent to the basement membrane; the derma consisted of papillary and reticular layers (Fig. 2).

In all the skin samples in the groups with tattooing, the signs of the traumatic damage were reported in *tattooing with a black pigment* and an inflammatory reaction in response to this damage. When assessing the skin layers in the negative control group, the epidermis was characterized by preserving all its layers, where the epithelium was represented by a basal layer that was fairly tightly adjacent to the basement membrane without signs of damage.

The remaining layers of the epidermis (prickle-cell, granular, horny) were preserved and clearly visualized when stained with hematoxylin and eosin. In the papillary and reticular layers of the derma, an accumulation of the black pigment was reported. It was located both diffusely and perivascularly, which was accompanied by a reaction of the vascular bed and the development of a pronounced inflammatory reaction. So, in the early period, there was a slight perivascular edema and the presence of neutrophils mainly in the reticular layer of the derma. The boundary between the reticular and papillary dermal layers was not clearly detected due to the edema and inflammatory infiltration. The dermal fibers were more loose compared to the skin samples of the intact animals (Fig. 3).

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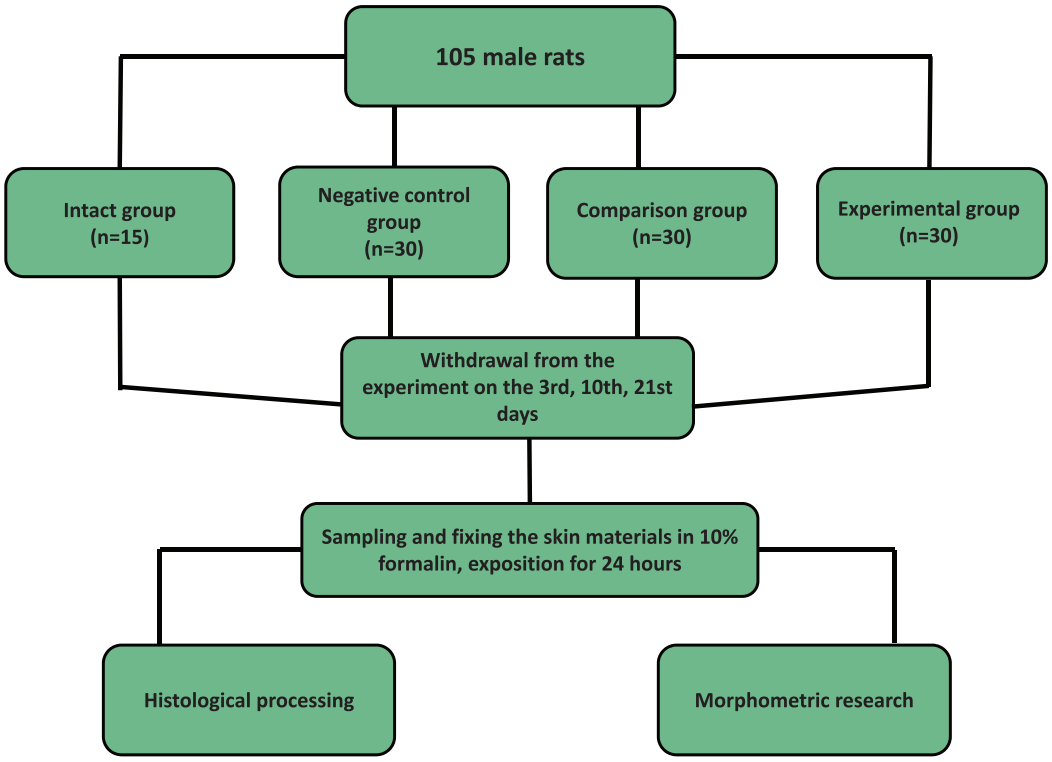

Figure 1 – Design of the experiment

Note: NC – a negative control group (without pharmacological correction); a comparison group – subcutaneous administration of betamethasone; an experimental group – a betamethasone administration using a tattoo machine

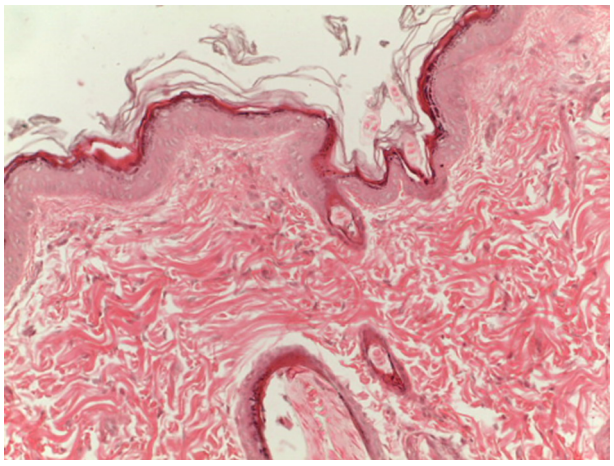

Figure 2 – Histological structure of the skin of the positive control group rats (intact animals) on the 3rd day of the experiment. Stained with hematokilin and eosin. A 100 × magnification

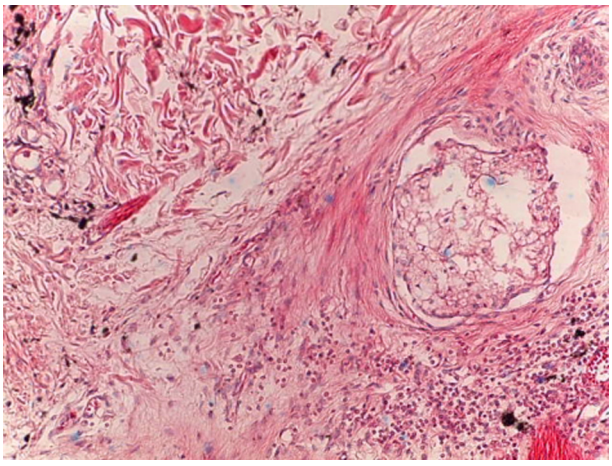

Figure – 3. Histological structure of the skin of the negative control group rats (the skin of the tattoo area without treatment) on the 3rd day of the experiment. Stained with hematokilin and eosin. A 100 × magnification

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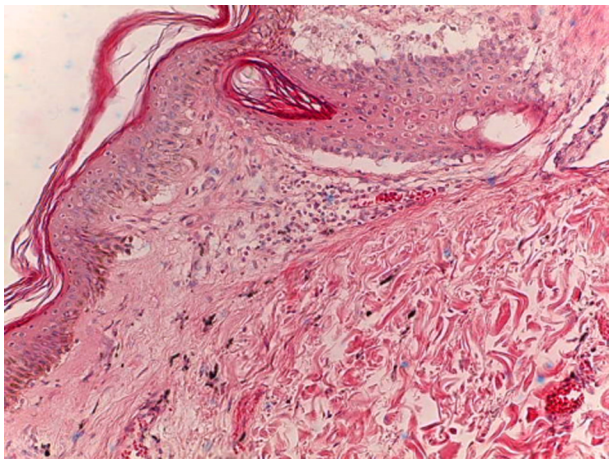

Figure 4 – Histological structure of the skin of the comparison group animals (intradermal betamethasone administration) on the 3rd day of the experiment. Stained with hematokilin and eosin. A 100 × magnification

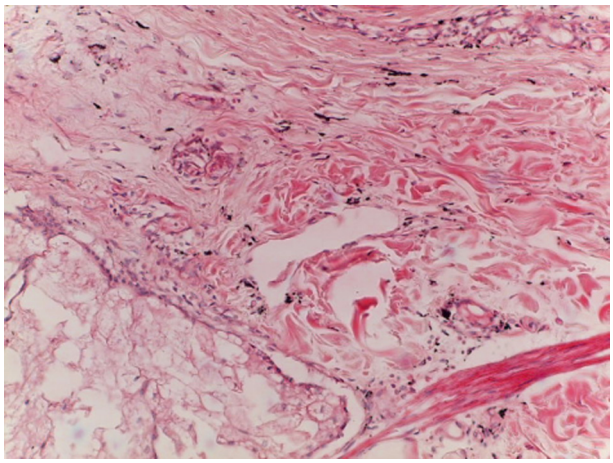

Figure 5 – Histological structure of the skin of the experimental group rats (betamethasone administration using a tattoo machine) on the 3rd day of the experiment. Stained with hematokilin and eosin. A 100 × magnification

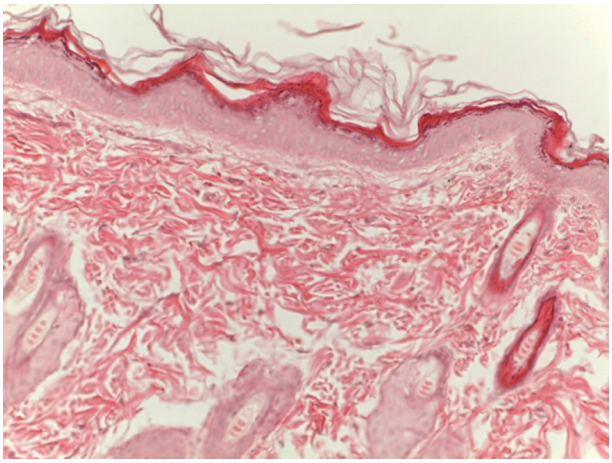

Figure 6 – Histological structure of the skin of the positive control group rats (intact animals) on the 21st day of the experiment. Stained with hematokilin and eosin. A 100 × magnification

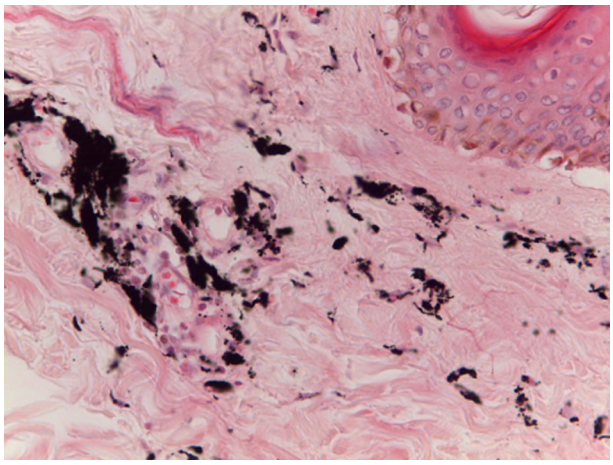

Figure 7 – Histological structure of the skin of the negative control group rats (skin of the tattoo area without treatment) on the 21st day of the experiment. Stained with hematokilin and eosin. A 100 × magnification

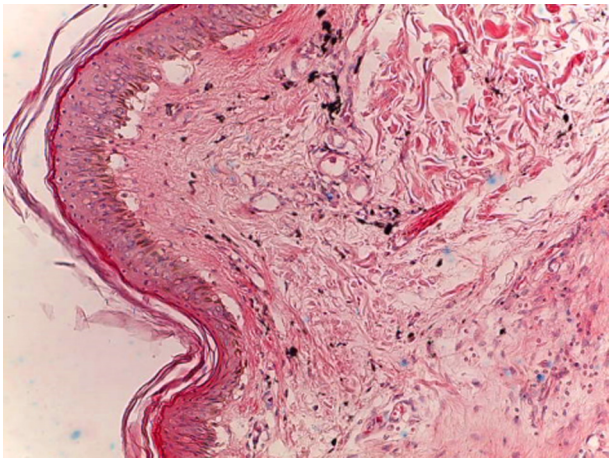

Figure 8 – Histological structure of the skin of the comparison group animals (intradermal betamethasone administration) on the 21st day of the experiment. Stained with hematokilin and eosin. A 100 × magnification

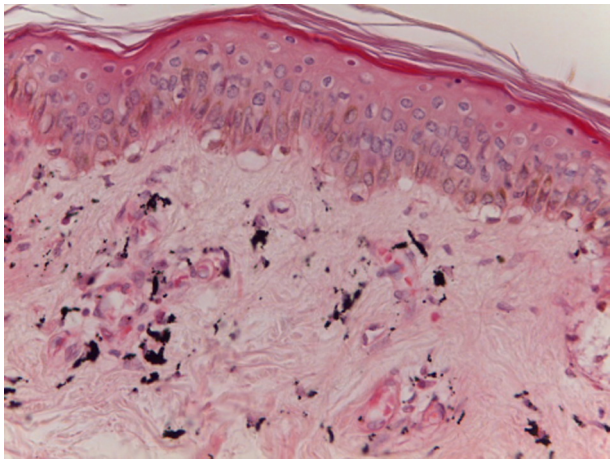

Figure 9 – Histological structure of the skin of the experimental group rats (betamethasone administration using a tattoo machine) on the 21st day of the experiment. Stained with hematokilin and eosin. A 100 × magnification

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In the comparison group (with intradermal betamethasone administration into the tattoo area), the edema of the derma was less pronounced, the fibers were located more densely to each other, and the papillary and reticular layers of the derma were visualized. The sites of inflammatory infiltration represented by neutrophils and lymphocytes were reported, while the cellular reaction was less pronounced and manifested unevenly, persisting mainly in the deep layers of the derma. Inflammatory infiltrates located perivascularly, as well as around the appendages of the skin, were detected. The structure of the epidermis had no significant differences from the structure of the skin of the intact group animals: the basal, prickle-cell, granular and horny layers were visualized (Fig. 4).

In the skin samples of the experimental group (with intradermal betamethasone administration using a tattoo machine), the drug was uniformly injected over the entire tattoo area, in this regard, the phenomena of edema and inflammatory infiltration were insignificant. So, the papillary layer was represented by a loose, unformed connective tissue, the fibers were tightly attached to the basement membrane of the epidermis. A clear boundary between the papillary and reticular layers, where the latter was represented by a dense unformed tissue, with the inclusion of skin appendages, was visualized. Single lymphocytes and neutrophils around the blood vessels and appendages of the skin, were determined. The dermal fibers were located in each layer without signs of edema, respectively (Fig. 5).

On the 10th day of the experiment, the skin samples of the intact animals had a typical histological structure that did not differ from that on the 3rd day of the experiment. In the negative control group, on the 10th day of the experiment, the epidermis had a typical structure that did not differ from that in the group of intact animals, the cells were tightly attached to the basement membrane. Morphological changes concerned the papillary and reticular layers of derma. In this case, there were phenomena of edema, visually manifested in a connective tissue shedding. An inflammatory infiltrate was determined, and in this model, its severity slightly decreased compared to the 3rd day of the experiment. According to the cellular composition, single neutrophils, lymphocytes, and plasmocytes were detected. It should be noted that at this time of the experiment, macrophages absorbing the black pigment, were identified; they were located predominantly perivascularly and were characterized by incomplete phagocytosis. An insignificant reaction of connective tissue cells was also reported. There fibroblasts and fibrocytes with randomly located fibers which can lead to the formation of scar tissue or keloid scar, were determined.

In the comparison group, on the 10th day, there was a decrease in edema and inflammatory response. Histologically, all the skin layers represented by epidermis, derma and hypoderma, were determined. The basal cells were located on the membrane, in a tight contact with the prickle-cell layer adjacent to the granular one and the subsequent horny layer. The papillary dermal layer tightly adhered to the basement membrane of the epidermis, the fibers of the loose tissue were located somewhat randomly. Single lymphocytes, neutrophils and plasma cells were detected. A black pigment with a diffuse arrangement of granules was revealed in both reticular and papillary layers, and in single macrophages. In the reticular layer there were appendages of the skin, represented by intact hair follicles, sweat and sebaceous glands.

A histological processing of the animal skin samples of the experimental group on the 10th day, did not show significant differences from the skin fragments in the comparison group. All the layers of the epidermis, the structure of the basement membrane were preserved, a clear boundary was determined between the papillary and reticular layers of the derma. The black pigment was located both in the papillary and reticular layers, in the form of small clusters located mainly perivascularly. A small number of macrophages with black pigment inclusions was determined. Single lymphocytes and neutrophils were observed only in the deep layers of the derma near the hair follicles and sebaceous glands.

On the 21st day of the experiment, the structure of the skin samples of the intact rats corresponded to the histological norm (Fig. 6).

On the 21st day, in the negative control group, the skin samples from the tattoo area showed morphological changes from the derma, while the epidermis was fully consistent with the intact rats' skin structure. It should be notified that in the derma, there were cells of the lymphocytic series, represented by single groups of lymphocytes and plasmocytes, which are the basis for the development of granulomatous complications with the formation of nonspecific intradermal granulomas. Such pathological manifestations of the skin reaction to an alien pigment, lead to visual changes in the skin topography, and are the most common reason for patients to contact a dermatologist with complaints of a violation of the aesthetic appearance of the tattoo.

On the 10th day of the experiment, the pigment volume decreased slightly due to the presence of both resident macrophages and free phagocytes. The number of cells of the fibroblastic series increased significantly, and single fibrocytes were reported. These factors were associated with the presence of a severe inflammation at the early stages, and secondary tissue damage. In some cases, a thickening of the connective tissue fibers was observed. It was due to hyalinosis resulting in the formation of scars. According to the morphological structure, these scars were similar to keloid ones (Fig. 7).

When studying the skin samples of the comparison group on the 21st day of the experiment, a complete absence of cellular elements was revealed. It indicates an inflammatory process due to the peculiarities of the

pathological process at the earlier stage of the experiment. In this regard, secondary tissue damage, as well as chemo-induced fibroblast migration, was reduced, which did not cause a pronounced synthesis of the connective tissue fibers resulting in the formation of scars. So, the papillary layer of the derma which was closely adjacent to the basement membrane of the epidermis, as well as the reticular layer in which the appendages of the skin were located, were determined. The pigment distribution was shaped relatively nonuniformly as small clusters in both reticular and papillary layers (Fig. 8).

The greatest effect in reducing the inflammatory response and prevention of complications was achieved in the experimental group. In the study carried out on the 21st day, it was found out that the skin structure corresponded to the histological norm with visualization of the main layers: epidermis, derma, hypoderma (Fig. 9).

The presence of the black pigment in the reticular derma, was reported in the form of small clusters caused by the presence of histiocytes in the connective tissue. The dermal fibers were arranged according to its layers and had a characteristic structure.

Morphometric evaluation of betamethasone effectiveness in tattoo dynamics depending on the mode of administration

The results of the morphometric study of the skin samples in the tattoo area in the rats of the studied groups in the dynamics of the experiment, are presented in Table 1.

During the experiment, the volume fraction (VF) of the epidermis had no significant differences from the VF of the epidermis in the group of intact animals and averaged 16.49±1.19% (p> 0.05) in all the experimental groups.

When determining the VF of the dermal fibers, it was found out that in the tattooed animals, there was a significant decrease in this indicator compared to the intact rats. The most pronounced decrease in this morphometric indicator, was determined in the negative control group on the 3rd day of the experiment (60.02±4.02%), p <0.05. As the duration of the experiment increased, the VF of the dermal fibers increased, too, and on the 10th day, in the animals of the comparison and experimental groups, there were no significant differences from the VF in the intact group.

The maximum VF values of the tattoo pigment were determined in all the experimental groups on the 3rd day and significantly decreased as the duration of the experiment increased. At the same time, there was an increase in the VF of macrophages; however, this indicator significantly decreased in the dynamics of the experiment. Thus, the macrophage VF was minimal in the negative control group, varying from 1.74 ± 0.11% (on the 3rd day) to 0.6 ± 0.05% (on the 21st day), p <0.05.

The assessment of the inflammatory response was carried out according to the morphometric parameters of the VF of the inflammatory series cells (neutrophils, lymphocytes, monocytes). This indicator had significant differences in all the experimental groups, decreasing in the series in the following way: the negative control group> the comparison group> the experimental group> the group of intact animals (p <0.05).

The greatest betamethasone effectiveness in morphometry was determined when evaluating an interstitial edema, which had significantly reduced by the 10th day of the experiment as a result of intradermal administration of the drug, and completely leveled when introduced using a tattoo machine (p <0.05).

When studying the depth of the tattoo pigment, it was found out that in the dynamics of the experiment, in the negative control rats an increase of this indicator by 1.97 times was recorded in all the animals; in the comparison group – by 1.75 times and in the experiment – by 1.82 times (p <0.05).

DISCUSSION

The intact animals' skin had a typical structure, which corresponded to the histological norm. In all the experimental groups, the main pathomorphological changes were registered in the derma. The accumulation of the black pigment was registered in the papillary and reticular layers; it was located both diffusely and perivascularly. The pigment accumulation was accompanied by a reaction of the vascular bed and the development of a pronounced inflammatory reaction.

In the negative control group in the early period, the border between the reticular and papillary layers of derma was not clearly defined due to edema and inflammatory infiltration. The dermal fibers were looser compared to the skin samples of the intact animals. In the reticular layer of the derma and hypoderma, skin appendages were determined; there was neutrophilic infiltration and an admixture of lymphocytes around the sebaceous glands and hair follicles, which can become a source of purulent complications. The results obtained are consistent with the data of foreign authors, who point out a risk of post-tattoo complications, mainly when using a black tattoo pigment [9, 10].

By the end of the experiment, the border between the papillary and reticular layers had become fuzzy, due to the development of a fibroblastic reaction and an increased production of connective tissue fibers; it resembled the picture of a "young" scar in the process of formation. The data obtained do not contradict the literature data on the possibility of the formation of keloid scars in the tattoo area, regarded as a local procedure complication [11].

In the comparison group (with intradermal betamethasone administration into the tattoo area), the edema of the derma was less pronounced, the fibers were located more densely to each other, and the papillary and reticular сетчатый layers of the derma were visualized.

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Table 1 – Morphometric parameters of the skin in the tattoo area of the rats in the studied groups in the experiment dynamics (M ± m)

Groups of Animals	Experiment duration		
	The 3rd day	The 10th day	The 21st day
VF of epidermis,%			
Intact	16.21±0.81	17.01±0.85	15.99±0.79
Negative control	16.82±1.51	16.77±1.84	16.01±1.12
Comparison group	16.74±1.17	16.59±1.49	16.33±1.31
Experimental group	16.43±1.31	16.68±1.01	16.29±1.14
VF of dermal fibers,%			
Intact	83.79±4.18	82.7±4.13	84.01±4.20
Negative control	60.02±4.02*	69.82±4.19*	78.65±5.51*#
Comparison group	66.0±4.62*	75.23±6.77	79.43±5.56
Experimental group	67.39±4.87*	74.8±4.49	79.51±6.36
Pigment depth, μm			
Intact	0	0	0
Negative control	42.0±2.13*	67.9±4.75*#	82.77±6.62*#
Comparison group	40.53±2.43*	70.6±4.23*#	71.0±4.97*#
Experimental group	40.21±2.81*	61.33±3.68*#	73.22±5.85*#
VF of pigment, %			
Intact	0	0	0
Negative control	8.77±0.61*	5.11±0.46*#	4.43±0.49*#
Comparison group	9.58±0.86*	4.98±0.34*#	3.97±0.19*
Experimental group	9.33±0.65*	5.52±0.39*#	3.99±0.23*#
VF of inflammatory series cells, %			
Intact	0	0	0
Negative control	12.65±1.01*	7.5±0.82*#	0.31±0.02
Comparison group	6.35±0.45*	3.1±0.21*#	0.17±0.01
Experimental group	5.7±0.39*	2.9±0.23*#	0.11±0.01
VF of macrophages, %			
Intact	0	0	0
Negative control	1.74±0.11*	0.8±0.07*#	0.6±0.05*#
Comparison group	1.33±0.11*	0.1±0.008#	0.1±0.007
Experimental group	1.15±0.10*	0.1±0.011#	0.1±0.005
Severity of edema (+++)			
Intact	–	–	–
Negative control	+++*	++*#	–#
Comparison group	++*	+*#	–#
Experimental group	**++***	**–***#	–

Note: * – significant differences with the group of intact animals (p <0.05); # – significant differences compared with the previous period (p <0.05)

The inflammatory reaction was less pronounced and nonuniformly manifested, persisting mainly in the deep layers of the derma and dynamically decreasing as the duration of the experiment increased. On the 21st day of the experiment, secondary tissue damage was reported, herewith the chemically induced migration of fibroblasts was reduced, which did not cause a pronounced synthesis of the connective tissue fibers resulting in the formation of scars.

The greatest effect in relation to the inflammatory reaction and the prevention of complications, was achieved in the experimental group. In the skin samples of the experimental group (with intradermal betamethasone administration using a tattoo machine), the drug was uniformly injected over the entire area of the tattoo; in this regard, the phenomena of edema and inflammatory infiltration were insignificant. The dermal fibers were located in each layer, respectively, with no signs of edema and with single cells of inflammation. The carried out histological research was fully consistent with the results of morphometry. It was found out that in the betamethasone administration using a tattoo machine, edema significantly decreased, the VF of the pigment, macrophages and inflammatory cells decreased, too, and the number of dermal fibers increased (p <0.05).

The data obtained are extremely relevant, which is confirmed by foreign authors' publications. They indicate an increase in post-tattoo complications and the development of skin diseases, such as atopic dermatitis, allergic reactions provoked by the tattoo procedure [12–15]. Granulomatous diseases, up to skin sarcoidosis, are recorded somewhat less frequently [16]; the prerequisites for its occurrence were identified during the histological research. Thus, the inflammatory nature of the skin diseases, against the background of tattooing and the location of the tattoo pigment in the deep layers of the derma, determine the consistency of using betamethasone as the drug of choice and explain the effectiveness of its administration using a tattoo machine.

CONCLUSION

As a result of the carried out experimental study, the effectiveness of betamethasone in the treatment of local post-tattoo complications has been proved. In this case, the treatment of choice is the administration of this drug not traditionally subcutaneously, but using a tattoo machine. That enables the targeted delivery of the substance to the area of the pathological process, and can be recommended for clinical trials.

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AUTHORS' CONTRIBUTION

All the authors have equally contributed to the research work.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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RETROSPECTIVE ANALYSIS OF ADVERSE DRUG REACTION REPORTING FORMS ASSOCIATED WITH PENICILLIN FAMILY ANTIBIOTICS (PCNE-DRP 9.0) BASED ON DRUG-RELATED APPROACH

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A widespread use of β-lactam antibiotics such as penicillins in practical medicine, and its authorized use in special categories of patients (e.g. children, pregnant and lactating women, the elderly) requires a critical investigation of their safety as well as the obligatory risk assessment before conducting antibacterial pharmacotherapy.
The aim of the work was the conduction of a retrospective study of adverse reactions cases, the identification and analysis of drug-related problems (DRP) associated with the use of penicillin family antibiotics.
Materials and methods. The objects of the study were adverse drug reactions (ADR) associated with the use of penicillin family antibiotics in inpatient and outpatient facilities, as well as the cases of self-treatment, which were recorded in the official ADR reports and then inputted in the regional (Republic of Crimea) database of spontaneous reports called ARCADe (Adverse Reactions in Crimea, Autonomic Database). The covered period is 2009–2018. The analysis of DRP was carried out using the 9.0 version of the qualification system DRP PCNE (Pharmaceutical Care Network Europe Foundation).
Results. The data analysis of ADR *reporting forms* has revealed that Amoxicillin clavulanate and Amoxicillin were the most frequent cause of ADR. A high incidence of penicillins ADR in pediatric patients (from 0 to 18 years) – 142 cases – has been found. The clinical manifestations of reactions included drug hypersensitivity reactions (309 cases), dyspeptic disorders (28 cases) and disorders of the central nervous system (5 cases). The incidence of serious ADR was 113 cases (33% of the total number of ADR in the study), which indicates a rather high risk of developing severe ADR for penicillins, resulted in a significant decrease in the quality of patients' lives.
Conclusion. The detection of DRP using the PCNE V9.0 approach is a useful and promising tool important to improve the quality of pharmacotherapy and their adherence to treatment. The highest DRP values which were observed for Amoxicillin clavulanate and Amoxicillin, may indicate a high frequency of irrational use of these drugs.
Keywords: penicillins, adverse reactions, drug-related problems, DRP, Amoxicillin clavulanate, Amoxicillin
List of abbreviations: DRP – drug related problems; ADR – adverse drug reactions; INN – international non-patented name

РЕТРОСПЕКТИВНЫЙ АНАЛИЗ КАРТ-ИЗВЕЩЕНИЙ О НЕЖЕЛАТЕЛЬНЫХ РЕАКЦИЯХ АНТИБИОТИКОВ ПЕНИЦИЛЛИНОВОГО РЯДА С ПРИМЕНЕНИЕМ МЕТОДА СИСТЕМЫ ПРОБЛЕМ, СВЯЗАННЫХ С ЛЕКАРСТВЕННЫМИ ПРЕПАРАТАМИ

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RESEARCH ARTICLE

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PHARMACY & PHARMACOLOGY

Широкое использование β-лактамных антибиотиков группы пенициллинов в практической медицине, официально допустимое их назначение особым категориям пациентов (дети, беременные и лактирующие женщины, лица пожилого возраста) требует серьезного отношения к изучению безопасности и оценке рисков при проведении антибактериальной фармакотерапии.

Целью работы было ретроспективное изучение случаев нежелательных реакций (НР), а также выявление и анализ проблем, связанных с применением лекарственных препаратов (*Drug-related problems*, DRP) группы пенициллинов.

Материалы и методы. Объектами исследования послужили случаи развития НР при применении группы пенициллинов в стационарных, амбулаторных учреждениях, а также при использовании препаратов в виде самолечения, т.е. карты-извещения о НР, зарегистрированные в региональной (Республика Крым) базе спонтанных сообщений ARCADe (*Adverse Reactions in Crimea, Autonomic Database*) за период 2009–2018 гг. Изучение и анализ DRP, проводились с использованием обновленной версии квалификационной системы DRP PCNE (*Pharmaceutical Care Network Europe Foundation*) V9.0.

Результаты анализа карт-извещений о НР позволили выявить, что препаратами-«лидерами» по частоте развития НР являются амоксициллина клавуланат и амоксициллин. Стоит отметить высокую частоту развития НР на фоне применения пенициллинов у пациентов детского возраста (от 0 до 18 лет) – 142 случая. Клиническими проявлениями НР на антибиотики представленной группы были реакции лекарственной гиперчувствительности (309 случаев), диспепсические расстройства (28 случаев) и нарушения со стороны центральной нервной системы (5 случаев). Частота серьезных НР составила 113 случаев (33% от общего количества НР), что свидетельствует о достаточно высоком риске развития тяжелых НР при применении пенициллинов, сопровождающихся значительным снижением качества жизни пациентов.

Заключение. Выявление DRP при помощи метода PCNE V9.0 является важным и перспективным инструментом, необходимым для повышения качества фармакотерапии пациентов и улучшения их приверженности к лечению. Наиболее высокие показатели значений DRP наблюдались при применении амоксициллина клавуланата и амоксициллина, что свидетельствует о высокой частоте нерационального назначения данных препаратов.

Ключевые слова: пенициллины, нежелательные реакции, проблемы, связанные с лекарственными препаратами, DRP, амоксициллина клавуланат, амоксициллин

Список сокращений: DRP – проблемы, связанные с лекарственными препаратами; ЛС – лекарственные средства; МНН – международное непатентованное название; НР – нежелательные реакции; ПСС – причинно-следственная связь

INTRODUCTION

The history of the penicillin clinical use began in 1940 after the first experimental study of penicillin in mice, which confirmed a high antibacterial activity of this drug against staphylococci [1]. Currently, penicillin medicines are the basis of modern antibacterial chemotherapy and have an important place in the treatment of various infectious diseases [2].

A widespread use of β-lactam antibiotics in practical medicine, their officially authorized prescription for special categories of patients (children, pregnant and lactating women, the elderly) requires a serious attention to safety studies and risk assessment [3, 4]. A study of adverse drug reactions (ADR) of antibacterial drugs by Jung I.Y. et al. in South Korea, confirms the high frequency of ADR associated with the use of penicillins (16% of the total number of cases for chemotherapeutic agents). The main manifestations were allergic reactions and gastrointestinal disorders [5]. Numerous studies on the safety of the penicillin group also have been conducted on the territory of the Russian Federation [6, 7]. They made it possible to identify a high incidence of penicillin ADR, most often associated with ignoring patients' allergic anamneses, overdosage, and the non-compliance with the recommended frequency of administration.

THE AIM of the work was a retrospective study of ADR cases, as well as the identification and analyses of the drugs (penicillins) related problems (DRP) using the DRP PCNE V9.0 qualification system.

MATERIALS AND METHODS

The objects of the study were ADR developed after the use of penicillin medicines in inpatient or outpatient facilities, as well as the ones related to the use of the drugs for self-treatment. The ADR reporting forms recorded in the regional (Republic of Crimea) database of spontaneous reports called ARCADe (Adverse Reactions in Crimea, Autonomic Database) received in 2009–2018 period, were analyzed.

The detection of the cases of interest was carried out by the codes of the Anatomical and Therapeutic Chemical (ATC) classification of drugs proposed by World Health Organization [8].

During the analyses, the instructions for medical use of the State Drug Registers of the Russian Federation and Ukraine (for the cases registered before 2014 when the Republic of Crimea became the part of Russian Federation) were checked. In accordance with the ATC classification, penicillins are assigned the J01C – beta-lactam antibiotics, penicillins.

The seriousness of ADR was established in accordance with the definition given in paragraph 51 of Article 4 of Federal Law No. 61-FZ dated April 12, 2010 "On the Circulation of Medicines" [9].

The assessment of the causal relationship was carried out in accordance with the recommendations of the WHO Uppsala monitoring center [10]. According to this classification, 6 degrees of a causal relationship are distinguished, and only the first 3 degrees (certain, probable, possible) refer to a high degree of causality and allow to interpret adverse events as "adverse drug reactions".

Drug-related problems (DRP) are defined as "any circumstance or event related to drug therapy that actually or potentially prevents the patient from receiving the intended benefits of the pharmacotherapy" [11–19]. The study and analysis of DRP were carried out using the updated (9th) version of the DRP PCNE (Pharmaceutical Care Network Europe Foundation) qualification system, adopted on June 1, 2019 [20]. The appearance of a new

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classification category "Intervention Acceptance", as well as the update of the standard categories (P – problems, C – causes, I – interventions and O – the status of the problem or outcomes), is one of the characteristic features of this version of the PCNE system. Thus, Category "P" is divided into three groups: P1-effectiveness, P2 – safety, P3 – other. The causes of DRP, standardized by code "C", are classified as follows:

- C1 – Drug selection
- C2 – Drug form
- C3 – Dose selection
- C4 – Treatment duration
- C5 – Dispensing
- C6 – Drug use process
- C7 – Patient related
- C8 – Patient transfer related
- C9 –Other.

In Section I (planned interventions) interventions are divided into 4 classes: I1 – interventions at prescriber level; I2 – interventions at patient level; I3 – interventions at drug level; I4 – other interventions and activities. Options for the outcome of the intervention (code "A" – Acceptance) are as follows: the intervention is accepted (A1), the intervention is not accepted (A2) and there is lack of information about the acceptance of certain interventions (A3). Among the outcomes of DRP (code "O"), there are 3 main alternatives: the DRP problem is solved, partially solved or not solved.

The evaluation of the DRP analysis results was carried out by Matveev AV, Krasheninnikov AE, Egorova EA. Each case of ADR was evaluated by two researchers independently of one another, and in case of disagreement, the third opinion was taken into account (Koniaeva EI). Such an analysis makes it possible to identify the most likely causes of the development of ADR in each case [21]. The minimum amount of DRP characterizes a high degree of safety of pharmacotherapy, and high DRP values, on the contrary, indicate a significant risk of potential complications when prescribing the drug.

Clopper-Pearson method was used to calculate limits of confidence intervals.

RESULTS AND DISCUSSION

In order to study the ADR of the penicillin group drugs (J01C), 342 reports (2009–2018) were selected in the regional database ARCADe. It amounted to 5.01% (95% CI: 4.5–5.6%) of the total number of adverse reactions recorded during the covered period (6825 reports). Among all cases of ADR development due to the use of antimicrobial agents for systemic use (1771 cases), the frequency of ADR associated with the use of penicillins was 19.3% (17.5–21.3%), which indicates a high risk of adverse effects.

The analysis of 342 ADR reports of the pharmacological group "J01C" by the frequency in the context of its representatives, is of practical interest is (Table 1).

A significant predominance of amoxicillin ADR is most likely due to the high frequency of prescriptions of these drugs by doctors in outpatient and inpatient settings [22]. It is worth noting that European (2017) and WHO guidelines (2017) recommend the use of Amoxicillin and Amoxicillin-clavulanate as first-line drugs for infections of the lower and upper respiratory tract (mild and moderate severity), infections of the skin and soft tissues, as well as for infection of urinary system [23].

The analysis the patients' age categories with recorded ADR caused by penicillin family antibiotics, is also of scientific interest. In 142 cases (41.52% with 95%CI 36.2–46.9), ADR were observed in pediatric patients (from 0 to 18 years). The distribution analysis of ADR in children was carried out in accordance with Geppe NA' classification with the following results: 0–28 days old – 14 cases (4.1%; 2.3–6.8%); 29 days – 12 months old – 40 cases (11.7%; 8.5–15.6%); 1–3 years old – 34 cases (10; 7–13.6%); 4–7 years old – 19 cases (5.6%; 3.4–5.6%); 8–10 years old – 7 cases (2%; 0.8–4.2%) and 11–18 years old – 28 cases (8.2%; 5.5–11.6%). 200 records contained information about the development of adverse effects after penicillins administration in patients over 18 years old. The frequency of cases in this age group with subgroups is presented in Figure 1. A study of gender characteristics made it possible to determine that the majority of ADR were observed in female patients (196 cases, 57.3% with 95% CI 51.9–62.6%).

Table 1 – Frequency of ADR reporting forms on penicillins

INN	TC-code	Amount of reporting forms, abs. value	Percentage of reporting forms in the total amount of ADR cases (95% CI)
Monocomponent drugs			
Amoxicillin	J01CA04	111	32.5 (27.5–37.7)
Ampicillin	J01CA01	14	4.1 (2.3–6.8)
Benzylpenicillin	J01CE01	11	3.2 (1.6–5.7)
Benzathine benzylpenicillin	J01CE08	2	0.6 (0.1–2.1)
Combinations			
Amoxicilline clavulanate	J01CR02	186	54.4 (48.9–59.8)
Amoxicilline sulbactam	J01CR01	18	5.3 (3.1–8.2)

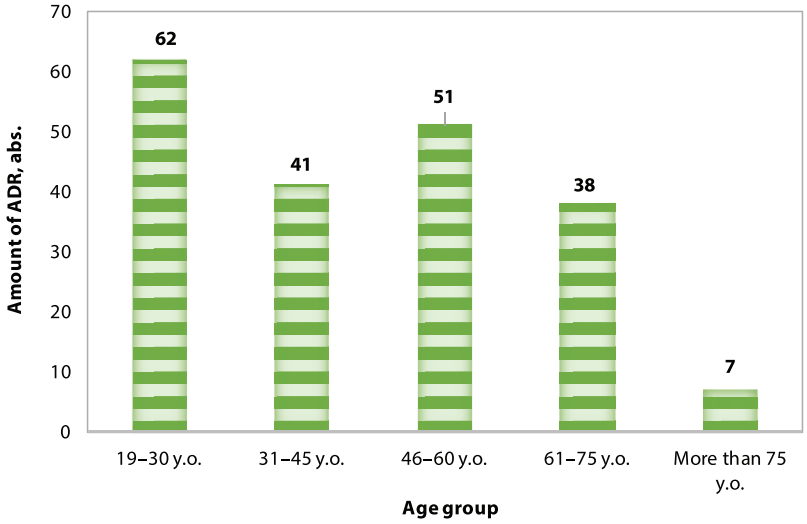
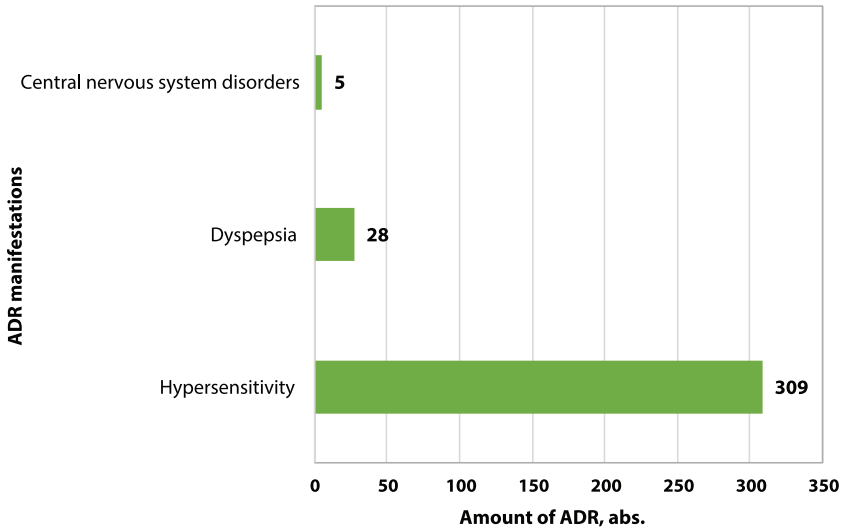


Figure 1 – The frequency of penicillins ADR in age categories of adult patients



Note: CNS – central nervous system

Figure 2 – The distribution of penicillin family antibiotics ADR according to their clinical manifestations

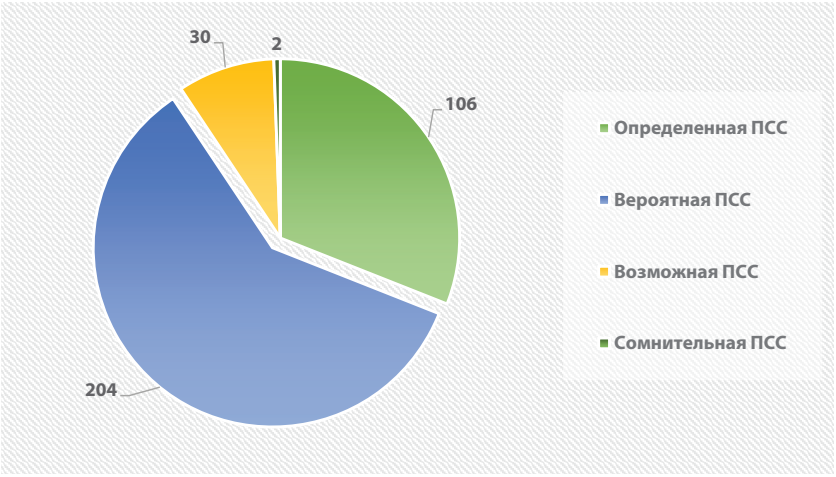


Figure 3 – Distribution of ADR cases by the type of casual relationship according to the WHO-UMC approach

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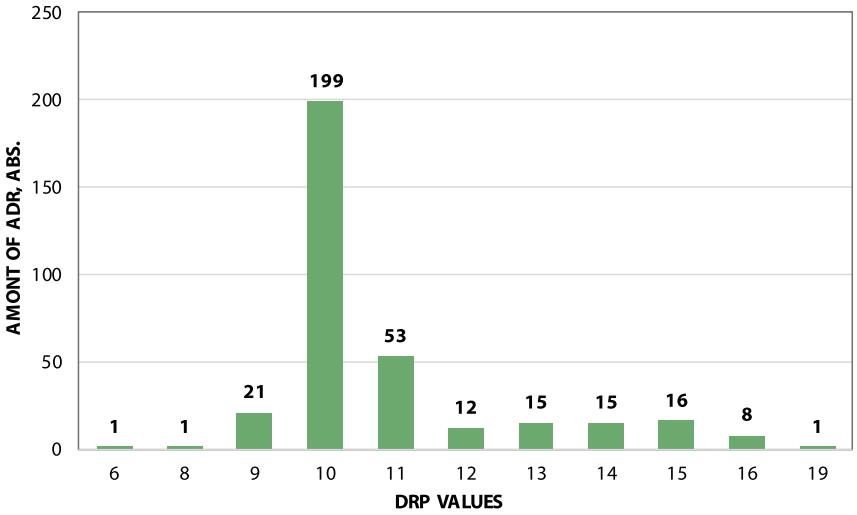

Figure 4 – Distribution of ADR cases by the quantity of total DRP value

Table 2 – Median (max:min) indices, DRP values in cases of ADR in the administration of penicillin family antibiotics according to standard qualification grades

INN / DRP subcategory	Amoxicillin	Ampicillin	Benzyl-penicillin	Benzathine benzyl-penicillin	Amoxicilline-clavulanate	Amoxicilline-sulbactam
Category P – Problems						
P1. Effectiveness	0 (0:0)	0 (0:0)	0 (0:0)	0 (0:0)	0 (0:0)	0 (0:0)
P2. Safety	1 (1:1)	1 (1:1)	1 (1:1)	1 (1:1)	1 (0:1)	1 (1:1)
P3. Other	0 (0:2)	0 (0:2)	0 (0:0)	0 (0:0)	0 (0:2)	0 (0:1)
Category C – Causes						
C1. Drug selection	0 (0:3)	0 (0:1)	0 (0:2)	0 (0:0)	0 (0:3)	0 (0:1)
C2. Drug form	0 (0:0)	0 (0:0)	0 (0:0)	0 (0:0)	0 (0:1)	0 (0:0)
C3. Dose selection	0 (0:2)	0 (0:2)	0 (0:0)	0,5 (0:1)	0 (0:3)	0 (0:2)
C4. Treatment duration	0 (0:0)	0 (0:0)	0 (0:0)	0 (0:0)	0 (0:0)	0 (0:0)
C5. Dispensing	0 (0:2)	0 (0:0)	0 (0:0)	0,5 (0:1)	0 (0:1)	0 (0:0)
C6. Drug use process	0 (0:1)	0 (0:1)	0 (0:0)	0 (0:0)	0 (0:2)	0 (0:1)
C7. Patient related problems	0 (0:1)	0 (0:1)	0 (0:0)	0 (0:0)	0 (0:1)	0 (0:1)
C8. Patient transfer related problems	0 (0:1)	0 (0:1)	0 (0:1)	0 (0:1)	0 (0:1)	0 (0:1)
C9. Other	1 (0:1)	1 (0:1)	1 (1:1)	1 (1:1)	1 (0:1)	1 (1:1)
Category I – Interventions						
I1. Interventions at prescriber level	2 (0:2)	2 (1:2)	2 (1:2)	2 (2:2)	2 (0:2)	2 (2:2)
I2. Interventions at patient level	1 (0:2)	1 (1:2)	1 (0:2)	1,5 (1:2)	1 (0:2)	1 (1:1)
I3. Interventions at drug level	1 (0:1)	1 (1:1)	1 (1:1)	1 (1:1)	1 (0:1)	1 (1:1)
I4. Other interventions and activities	1 (0:1)	1 (1:1)	1 (1:1)	1 (1:1)	1 (0:1)	1 (1:1)
Category A – Acceptance						
A1. Intervention is accepted	1 (0:2)	1 (1:1)	1 (1:1)	1 (1:1)	1 (0:1)	1 (1:1)
A2. Intervention is not accepted	0 (0:0)	0 (0:0)	0 (0:0)	0 (0:0)	0 (0:1)	0 (0:0)
A3. Other	0 (0:1)	0 (0:0)	0 (0:0)	0 (0:0)	0 (0:1)	0 (0:0)
Category O – Outcomes						
O. Status of problem	0 (0:1)	0 (0:1)	0 (0:1)	0,5 (0:1)	0 (0:1)	0 (0:1)
O1. Solved	1 (0:1)	1 (0:1)	1 (0:1)	0,5 (0:1)	1 (0:1)	1 (0:1)
O2. Partially solved	0 (0:0)	0 (0:0)	0 (0:0)	0 (0:0)	0 (0:0)	0 (0:0)
O3. Not solved	0 (0:1)	0 (0:1)	0 (0:1)	0,5 (0:1)	0 (0:2)	0 (0:1)

Table 3 – Total Median (Max:min) values in cases of ADR caused by individual members of penicillin family antibiotics according to standard qualification grades

INN	Category «P»	Category «C»	Category «I»	Category «A»	Category «O»	Sum of DRP values
Amoxicillin	1 (1:3)	2 (1:6)	5 (2:6)	1 (1:2)	1 (0:2)	10 (8:16)
Ampicillin	1 (1:3)	2 (2:5)	5 (4:6)	1 (1:1)	1 (1:2)	10 (10:15)
Benzylpenicillin	1 (1:1)	2 (2:4)	5 (3:6)	1 (1:1)	1 (1:2)	10 (10:11)
Benzathine benzylpenicillin	1 (1:1)	3 (2:4)	5,5 (5:6)	1 (1:1)	1,5 (1:2)	11 (10:14)
Amoxicilline-clavulanate	1 (1:3)	2 (1:9)	5 (2:6)	1 (0:2)	1 (0:2)	10 (6:19)
Amoxicilline-sulbactam	1 (1:2)	2 (2:5)	5 (5:5)	1 (1:1)	1 (1:2)	10 (10:14)

The development of penicillin ADR most often occurred after the oral administration (265 cases, 77.5% with 95%CI 72.7–81.8%) and less often after the parenteral administration (intravenously – 52 cases, 15.2% (11.6-19.5%); intramuscularly – 25 cases, 7.3% (4.8–10.6%)).

A study of the clinical manifestations of adverse reactions that occur in patients against the background of the administration of penicillin family antibiotics, revealed an absolute predominance of drug hypersensitivity reactions of varying severity (urticaria, pruritus, skin hyperemia – 298 cases (87.1%; 83.1–90.5%), angioedema – 9 cases (2.6%; 1.2–4.9%), anaphylactic shock – 2 cases (0.6%; 0.1–2.1%)). The distribution of the remaining cases of ADR by their clinical manifestations is presented in Figure 2. In 28 cases (8.2%; 5.5–11.6%) against the background of the administration of penicillin family antibiotics, the patients had various dyspeptic disorders (nausea, bloating, diarrhea, spastic pains). Disorders of the central nervous system (5 cases; 1.5% 95%CI 0.5–3.4%) were manifested in the forms of dizziness, darkening in the eyes, weakness and tinnitus.

An important step in the drug safety analysis, is the identification and assessment of the cases of serious ADR that require the doctor to timely withdraw the drug, hospitalize the patient and / or conduct emergency pharmacotherapy. In the case of the studied group of drugs, the frequency of serious reactions was 113 cases (33%; 28.1–38.3%), which indicates a rather high risk of developing severe ADR, accompanied by a significant decrease in the quality of patients' lives The distribution of such cases in accordance with the criteria of their severity, was presented by the following results: death – 1 case (0.3%; 0-1.6%), threat to patient's life – 8 cases(2.3%; 1–4.6%), temporary disability – 50 cases (14.6%; 11–18.8%), hospitalization or extension of its term – 54 cases (15.8%; 12.1–20%). The patient's death (1 y.o.) occurred as a result of the development of an anaphylactic shock (face cyanosis, respiratory and cardiac arrest) against the background of the administration of Amoxicillin-clavulanate suspension for an acute respiratory disease. It is worth notifying that, simultaneously with the suspected drug, the child was prescribed a syrup containing Phenylephrine, Salbutamol and Bromhexine, as well as Hephenadine tablets (5 mg). In most cases, the development of angioneurotic oedema posed a threat to the lives of patients and required emergency pharmacotherapy with glucocorticoid and anti-allergic drugs.

The analysis of drug correction cases to stop ADR clinical manifestations is of further interest. Despite a high incidence of non-serious events, the administration of drugs to relief ADR was necessary in the absolute majority of cases – 293 (85.7%; 81.5–89.2%). In remaining 49 cases (14.3%; 10.8–18.5%), ADR did not require additional pharmacotherapy. Among the pharmacological groups prescribed for the correction of ADR, antiallergic agents for oral and external use, glucocorticoids (Dexamethasone, Prednisolone) and sorbents prevailed.

An important step in safety assessing when using drugs, is to determine the causality between the ADR manifestations and the clinical and pharmacological characteristics of the drug [10]. One of the algorithms for determining the causal relationship is the WHO-UMC algorithm proposed by specialists of the WHO Center for Drug Safety Monitoring (Uppsala, Sweden).

The results obtained for causality, make it possible for doctors to correctly assess the current clinical situation and timely take the necessary treatment and preventive measures. The results of the causality analysis are presented in Figure 3. It is worth paying attention to the predominance of a certain and probable type, which indicates a high risk of developing adverse reactions due to the use of penicillins.

The second stage of the reporting forms analysis, was aimed at studying the problems related to the use of drugs (DRP). The calculation of the total DRP values for the cases of ADR, yielded the following results: DRP values between 5–8 were found in 2 cases (6 DRP – 1 case of HP, 8 DRP – 1 case), in 21 reporting cards the

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total number of DRP amounted to 9, and in 199 cases – 10 DRP. In the remaining 120 cases of ADR, the DRP values were higher than 10 (the minimum was 11 DRP, the maximum – 19 DRP), which may indicate the likelihood of an irrational choice of the drug, interaction of penicillins with other drugs, errors in the selection of individual doses or the frequency of administration. The frequency distribution of individual DRP values for penicillin family antibiotics is shown in Fig. 4. The calculation of the total number of DRP for all cases of ADR (3712 DRP) made it possible to determine the average number of DRP, which amounted to 10.85 per 1 patient.

A quantitative analysis of the problems associated with the use of various members of penicillin family antibiotics according to the main classification categories, is also of interest. The indicators of the minimum, maximum values and the DRP median for each of the drugs, are presented in Table 2.

The study of individual categories of the DRP system revealed the fact that for all the drugs under study, the maximum number of the drugs associated problems, was recorded in section "I" (Intervention). High DRP values, in this case, are due to the interventions performed by the doctor in the form of withdrawn the suspected drug and prescribing drugs for the ADR correction.

A detailed study of the results of DRP calculating according to the qualification category "C" (ADR causes) revealed the fact that the main reasons for the development of drug related problems, are various violations of the dosage regimen (low drug dose / low frequency, high drug dose / high frequency, unclear or incorrect recommendations on the dose regimen and frequency of administration). In accordance with the classification of DRP PCNE V9.0, the information on drug dosing violations is presented in section C3 – dose selection. The results of this section analysis of the classification, can be presented as follows: the dose of the suspected drugs was exceeded in 9 cases (2.6% of the total number of cases of penicillins ADR; 1.2–4.9%), the use of low doses of the drugs (below the minimum therapeutic doses) – in 6 cases (1.75%; 0.6–3.8%), the absence of indications of a dosage regimen or unclear instructions for use (for example, "1 tablet" without indicating the strength of the action) – in 19 cases (5.6% of cases; 3.4–8.5%). The main reasons for the development of penicillin related problems were individual hypersensitivity reactions, the manifestations of which were allergic reactions of varying severity.

The analysis of the final DRP values for individual members of penicillin family antibiotics showed that the maximum DRP value was observed in the administration of Amoxicillin-clavulanate (19 problems) and Amoxicillin monopreparations (16 problems) (Table 3). A study of these cases confirmed the irrational use of antibacterial drugs in acute respiratory viral infections with a violation of the dose regimen, which led to such high rates of DRP. The minimum DRP values (6 and 8 problems) were observed with the use of the same drugs. The corresponding cases of ADR have been associated with the development of allergic reactions against the background of their rational use.

Calculation of the median DRP showed the highest values for Benzathine benzylpenicillin, for the other drugs the median values were identical and amounted to 10 DRP / case, while the largest diapason between the minimum and maximum values of DRP was found for Amoxicillin-clavulanate (max: min – 6:19), and the smallest – for Benzylpenicillin (max: min – 10:11).

CONCLUSION

The results of the analysis of the ADR reporting forms revealed the fact that Amoxicillin-clavulanate and Amoxicillin are the "leaders" in the frequency of ADR development in the penicillin family antibiotics.

It is worth notifying that a high frequency of ADR against the background of penicillins administration in pediatric patients (from 0 to 18 years) is represented by 142 cases. The clinical manifestations of the antibiotics ADR were drug hypersensitivity reactions (309 cases), dyspeptic disorders (28 cases) and disorders of the central nervous system (5 cases). The frequency of serious adverse reactions was 113 cases (33% of the total number of ADR), which indicates a rather high risk of developing severe penicillins complications accompanied by a significant decrease in the quality of patients' lives.

The highest DRP values were observed against the background of Amoxicillin-clavulanate and Amoxicillin administration, the minimum DRP values were observed against the background of Benzylpenicillin preparations.

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AUTHOR'S CONTRIBUTION

All authors equally contributed to the research work.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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ОРИГИНАЛЬНАЯ СТАТЬЯ

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PROBLEMS OF PHARMACEUTICAL PROVISION OF POPULATION WITH HYPOLIPIDEMIC DRUGS: THE CASE OF THE VOLGOGRAD REGION (THE RUSSIAN FEDERATION)

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The aim of the study is to study the regional hypolipidemic drugs market, external and internal factors affecting their level of consumption, including the information awareness of the final customers about this pharmacotherapeutic group and the adherence to treatment with these drugs.
Materials and methods. The study was carried out using the methods of SWOT and STEP-analyses to assess the factors affecting the consumption of the studied group of drugs, as well as the questionnaire method of final customers and assessing their compliance using the Morisky-Green questionnaire.
Results. The influence of environmental and internal factors on the level and structure of the consumption of hypolipidemic drugs has been studied, hereby, the problems of the group and ways to solve them have been outlined, and an increase or decrease in the need for hypolipidemic drugs at the regional level, have been predicted. The assessment of the information awareness and preferences of the final customers of hypolipidemic drugs has been carried out, and insufficient awareness of patients about the drugs under study, has been revealed. The compliance of the final customers has been studied. A low level of the compliance of the patients to the prescribed hypolipidemic therapy has been established.
Conclusion. Modern advances in the treatment of cardiovascular diseases, based on fundamental achievements of science and practice, have created a high evidence base for the choice of strategies for pharmacotherapy with hypolipidemic drugs. The main ways to increase information awareness and compliance of the final customers are: development and intensification of educational programs to increase the level of knowledge and information awareness of doctors and pharmaceutical professionals, establishing the *Doctor-Patient* partnering relationships, increasing the trust level to the doctor and, as a result, the level of the patient compliance уровень; the development of materials for increasing the information awareness among the final customers about hypolipidemic drugs and hypolipidemic therapy in general.
Keywords: hypolipidemic drugs, statins, pharmaceutical provision, SWOT-analysis, information awareness, compliance

ПРОБЛЕМЫ ЛЕКАРСТВЕННОГО ОБЕСПЕЧЕНИЯ НАСЕЛЕНИЯ ГИПОЛИПИДЕМИЧЕСКИМИ ЛЕКАРСТВЕННЫМИ ПРЕПАРАТАМИ НА ПРИМЕРЕ ВОЛГОГРАДСКОЙ ОБЛАСТИ

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RESEARCH ARTICLE

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Цель. Изучение регионального рынка гиполипидемических препаратов (ГЛП), внешних и внутренних факторов, влияющих на уровень их потребления, в том числе, информированности конечных потребителей о данной фармакотерапевтической группе и приверженности лечению препаратами данной группы.
Материалы и методы. Исследование проведено с использованием методов SWOT и STEP-анализа для оценки факторов, влияющих на потребление исследуемой группы препаратов, а также методом анкетирования конечных потребителей и оценки их комплаентности с использованием опросника Мориски-Грина.
Результаты. Изучено влияние факторов внешней и внутренней среды на уровень и структуру потребления группы гиполипидемических препаратов, что позволило наметить проблемы группы и пути их решения, прогнозировать увеличение или уменьшение потребности в гиполипидемических препаратах на региональном уровне. Проведена оценка информированности и предпочтений конечных потребителей гиполипидемических препаратов, выявлена недостаточная информированность больных о препаратах исследуемой группы. Изучена комплаентность конечных потребителей. Установлен низкий уровень комплаентности больных, которым назначена гиполипидемическая терапия.
Заключение. Современные достижения в лечении сердечно-сосудистых заболеваний, базирующиеся на фундаментальных достижениях науки и практики, создали высокую доказательную базу для выбора стратегий фармакотерапии гиполипидемическими препаратами. Основными путями повышения уровня осведомленности и комплаентности конечных потребителей являются: разработка и усиление образовательных программ с целью повышения уровня знаний и информированности врачей и фармацевтических работников; установление партнерских отношений между врачом и пациентом, повышающих уровень доверия лечащему врачу, и, как следствие, уровень комплаентности больного; разработка материалов, повышающих уровень осведомленности конечных потребителей о ГЛП и гиполипидемической терапии в целом.
Ключевые слова: гиполипидемические препараты, статины, лекарственное обеспечение, SWOT-анализ, информированность, комплаентность

INTRODUCTION

A high level of cardiovascular morbidity in Russia, makes the problem of optimization the drug supply of patients with this pathology paramount. Atherosclerosis is one of the main pathogenetic factors underlying the development of coronary heart diseases, cerebrovascular diseases, hypertension, macro- and microvascular complications of diabetes, which remain the main causes of untimely deaths and early disability of the population [1, 2]. According to the latest recommendations, hypolipidemic drugs and, first of all, statins, are important parts of the basic therapy of cardiovascular diseases. The demand for drugs of this group is constantly growing, which is dictated by the results of recent studies, significantly expanding proposed clinical uses in both – diseases of the circulatory system, and in non-cardiac pathologies [3]. The importance and relevance of hypolipidemic therapy in the complex treatment of patients with cardiovascular diseases, diabetes mellitus, and obesity, has been established [4].

In recent years, dozens of clinical studies of statins have been carried out. They showed a significant decrease in the risk of developing myocardial infarctions and sudden coronary deaths while taking them [5-7]. In addition, statins, due to their pleiotropic effects, are widely used in rheumatology, gastroenterology, pulmonology (Table 1).

The success of a clinical use of hypolipidemic drugs (HLD) is determined by many factors: the individual selection of the drug, based on its special class-specific properties, its availability, information awareness of intermediate and final customers about special warnings and precautions for use of the drug prescribed by the doctor, patient adherence to treatment, and many others [25].

The analysis of the structure of hypolipidemic drug consumption shows that it can differ from the average Russian ones in different regions and even within the same city. This is of fundamental importance for assessing the rational justification and population consumption of specialty drugs [26].

The formation of a complete idea about of the processes taking place in regional markets is impossible without assessing the influence of external factors on the consumption level of a particular group of drugs.

Russian medical practice is characterized by a low degree of adherence to hypolipidemic therapy:

– according to various data sources, up to 70% of patients completely stop taking statins within 6 months after the start of treatment. It blocks solving the medical problem and causes the development of complications [27];

– short term treatment, which is common in Russia, is a fundamentally incorrect approach, since hypolipidemic therapy is effective only if the appropriate drugs are taken constantly;

– in Russia, a serious problem is a prescription of low, often ineffective, doses of hypolipidemic drugs, which is associated with their lower costs, as well as with unreasonable fears of side effects and unawareness of the need for treatment with higher doses. It has been established that if the patients have to partially pay for statin treatment, their adherence decreases by 5%; herewith, the adherence decreases even more in low-income patients [28].

In this regard, for the patients who are not able to purchase the original hypolipidemic drugs, it is important to recommend generics from well-known manufacturers that have proven bioequivalence and an optimal quality-to-price ratio.

Thus, the main reasons why Russian patients interrupt the prescribed therapy, can be the following ones: the lack of a quick and noticeable improvement in health during the treatment (the lack of confidence in the need for therapy due to the lack of pronounced symptoms of hypolipidemic and, accordingly, the lack of a pronounced improvement in well-being); fears about side effects, although they arise only in 1–5% of cases and, most often, decrease or completely disappear within a few weeks from the start of treatment; high costs of original hypolipidemic drugs and some generics; the lack of adequate explanatory work on the part of doctors and pharmaceutical professionals and the lack of patient awareness of the benefits and advisability of hypolipidemic therapy.

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Table 1 – Spectrum of clinical statin uses

Proposed clinical uses due to hypolipidemic statin effects	Proposed clinical uses due to pleiotropic statin effects
Primary hypercholesterolemia (types IIa, IIb)*	Ventricular arrhythmia [14]
Homozygous familial hypercholesterolemia *	Hyperuricemia [15]
Hypertriglyceridemia (Fredriksson, type IV)*	Chronic obstructive lung disease [16]
Complex hypercholesteremia and hypertriglyceridemia *	Rheumatoid arthritis and vasculitides [17]
Primary dysbetalipoproteinemia (hyperlipidemia, Type III)	Choledocholithiasis, pancreatitis [18]
Primary prevention of basic cardiovasculare complicating diseases (apoplectic attack, infarction, arterial revascularization)**	Osteoporosis [19]
Ischemic heart disease (secondary prevention) [8]***	Gastric and duodenal ulcers [20]
Acute coronary syndrome [9, 10]	Chronic kidney disease [21]
Chronic heart failure [11]	Glomerulonephritis [22]
Diabetes mellitus type II [12]	Non-alcoholic fatty liver disease [23]
Cardiometabolic syndrome, obesity [13]	Diffuse scleroderma [24]

Note:* – as a supplement to the diet and when other non-drug treatment methods are ineffective; ** – in adult patients without clinical signs of ischemic heart disease but with an increased risk of its development; *** – in order to reduce overall mortality, prevent myocardial infarction, reduce the risk of apoplectic attacks and transient ischaemic attacks, slow down atherosclerotic vascular disease progression

Table 2 – Results of SWOT analysis of hypolipidemic drug group on the Volgograd region pharmaceutical market

S (Strengths)	O (Opportunities)
– Presence of unique pharmacotherapeutic properties in hypolipidemic drug group; – Availability of a wide range of hypolipidemic drugs on the pharmaceutical market of the region; – A wide range of hypolipidemic drug applications in dyslipidemia, various diseases of atherosclerotic origin, as well as non – cardiac pathologies; – Availability of a large number of generic drugs with proven effectiveness, which makes the group accessible to diverse communities; – Availability of hypolipidemic drugs of imported and domestic origin, satisfying the needs of different groups of patients (older people often prefer domestic drugs); – Continuous expansion of the evidence base for hypolipidemic drugs, confirming their high effectiveness.	– Health situation in the region, preventive orientation of health services; – High level of CVD in the country and in the region; – Demographic structure in the region (percentage growth of elderly population in need for hypolipidemic therapy); – Scientific and technological progress in the pharmaceutical industry; – Environmental deterioration of the region; – Development of market relations in economics; – Increase in sales on pharmaceutical market, a wide range of medicines and related products; – Availability of customers' target segments (with real and potential customers); – Availability of various companies – distributors with a wide range of hypolipidemic drugs; – Appearance of new hypolipidemic drugs.
W(Weaknesses)	**T (Threats)**
– Need to consult a doctor for hypolipidemic drugs prescription; – High costs of a number of drugs; – Possibility of expressed side effects and a wide range of complications; – Insufficient information awareness of doctors and pharmaceutical professionals concerning this drug group.	– Low solvency of the population; – Low pension costs; – Unstable financial position of customers; – Fluxions of inflation rate, exchange rates, prices for hypolipidemic drugs; – High customs duties taxes for imported hypolipidemic drugs.

THE AIM of the study is to study the regional hypolipidemic drugs market, external and internal factors affecting their level of consumption, including the information awareness of the final customers about this pharmacotherapeutic group and the adherence to treatment with these drugs.

MATERIALS AND METHODS

The study included three stages.

At the first stage, SWOT and STEP analyses of the factors affecting the consumption of hypolipidemic drugs were performed. The STEP analysis was used to study the influence of environmental factors on the market of hypolipidemic drugs in the Russian Federation and the Volgograd region. The STEP analysis made it possible to assess the impact of social, technological, economic and political factors affecting the market and the consumption of hypolipidemic drugs from the perspective of the possibility to reflect them in the "Opportunities" and "Threats" sections of the SWOT analysis.

At the same time, the analysis of internal factors affecting the consumption of hypolipidemic drugs in the region was carried out. Subsequently, the data were reflected in the "Strengths" and "Weaknesses" sections of the SWOT analysis.

At the second stage, an assessment of the information awareness and preferences of final customers who had applied to the pharmacy with hypolipidemic drug prescriptions, was carried out. To determine the information awareness and preferences of the patients applying with prescriptions for hypolipidemic drugs, a survey was conducted. The authors worked out questionnaires, including a block of sociological questions, as well as questions regarding the *Doctor-Patient* interaction when prescribing hypolipidemic drugs. The factors affecting buying and regular intake of prescribed hypolipidemic drugs, the sources of information on these drugs, knowledge of their trade names, were taken into consideration. The questionnaire did not include questions regarding the final customers' compliance.

In order to assess the information awareness and preferences of the final customers, 390 visitors were surveyed at 20 pharmacies in the Volgograd Region, which made it possible to study this market segment and obtain the required information.

At the third stage of the study, the compliance of final customers with hypolipidemic therapy was studied. To assess the level of adherence to hypolipidemic therapy in the patients of the Volgograd region, the Moriski-Green questionnaire (the Moriski-Green Medication *Adherence Scale*) proposed by Morisky D.E. and Green L.W. in 1985, was used. The questionnaire contained 4 questions regarding the administration of drugs, the *rates* ranged from *0 point* in the positive answer ("Yes") and to 1 *point* in the negative answer ("No"):

1. Have you ever forgotten to take your drugs? ("Yes" – 0, "No" – 1);
2. Can you sometimes be inattentive to the hours of medication? ("Yes" – 0, "No" – 1);
3. Do you skip taking medications if you feel good? ("Yes" – 0, "No" – 1);
4. If you feel unwell after taking your medicine, do you skip the next dose? ("Yes" – 0, "No" – 1).

According to the questionnaire, the patients with 4 gained points were considered *compliant.* The patients whose score was 2 points or less, were considered *non-compliant*. The patients whose score was 3 points, were considered *not quite compliant* and referred to the risk group for developing noncompliance. The study was conducted on the basis of the same 20 pharmacies in the Volgograd region, but other visitors not included in the previous sample, also participated in it. This group of respondents were not offered the questionnaires used at the second stage of the study.

The pharmaceutical professionals turned to the individuals purchasing a hypolipidemic drug with a request to fill in only the Moriski-Green questionnaire. Thus, 125 visitors were interviewed. Further on, they were distributed by age and gender. In total, 68 (54.4%) men and 57 women (45.6%) took part in the study.

RESULTS AND DISCUSSION

At the first stage of the study, the influence of environmental factors referring to the hypolipidemic drugs from the perspective of their traceability in the "Opportunities" and "Threats" sections of the SWOT analysis, has been studied.

The influence of social factors. At the regional level, the range of social services can vary significantly. The necessity for patients to purchase modern and more effective hypolipidemic drugs for money, the absence of these drugs in preferential programs, are social factors that directly affect the level of consumption of these drugs and, as a result, the effectiveness of hypolipidemic therapy.

In recent years, the dynamics of the ageing coefficient has indicated a significant shift in the population structure to older ages [29]. On the one hand, an increase in the number of elderly people leads to an increase in the number of the people who need hypolipidemic therapy, which increases the need for hypolipidemic drugs in the region (the "Opportunities" section). On the other hand, retirement-age people belong to the category of disadvantaged population and, due to their low solvency, demonstrate a lower level of adherence to hypolipidemic therapy than the working population [30]. This, of course, will be traced in the "Threats" section of the SWOT analysis. The increase in cardiovascular morbidity and the presence of a large number of risk

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factors for their development, lead to the following. A lot of economically active population also require hypolipidemic therapy in spite of their higher solvency than pensioners, and are well-aware of the need for hypolipidemic therapy as a preventive measure. In the SWOT analysis, it will be traced in the "Features" section.

The influence of technological factors. Scientific and technological progress is reflected in new technologies for the prevention and treatment of cardiovascular diseases, in new treatment standards, the use of innovative, highly effective drugs. These factors are reflected in the pharmaceutical industry (the "Opportunities" section). In addition, scientific and technological progress promote the introduction of new on- and off-line tools (programs) in pharmacies that can optimize the system for providing the population and medical institutions with medicines, minimizing the risk of both overstocking and defects (the "Opportunities" section). At the same time, scientific and technological progress leads to the deterioration of the ecological state of the environment. It negatively affects the health status of the population, with the risk of developing cardiovascular diseases. On the whole, these factors lead to an increase in the need for appropriate medications and can also be attributed to the "Opportunities" section.

The influence of economic factors. Unstable exchange rates, unfavorable economic conditions on the world market, high customs duties taxes on imported hypolipidemic drugs and inflation, lead to an increase in the costs of hypolipidemic drugs. In the SWOT analysis, all of the above-listed factors can be reflected as "Threats". A high level of unemployment, low solvency, and, as a result, the instability of the financial situation of the region's population, lead to spending the money primarily on necessities of life (food, communal public services), which will be reflected in the "Threats" section in the SWOT analysis. It is obvious that with an increase in the purchasing power of the residents of the region, patients get the opportunity to purchase more expensive and often more effective modern hypolipidemic drugs with their own money.

With a decrease in this indicator, the emphasis shifts towards cheap non-branded generics, which undoubtedly affects the effectiveness of hypolipidemic therapy and, as a consequence, the course of the primary disease in connection with which по поводу которого the drugs are used.

The influence of political factors. The state support in the form of creating a scientifically-based social policy to reduce mortality from diseases of the circulatory system depends to a great extent on the provision of the population with hypolipidemic drugs, and their rational use through the system of pharmaceutical benefits. In recent years, the policy of the state as a whole and the region, in particular, has been aimed at the financial support of the low-income population, and at purchasing hypolipidemic drugs for them. It has also been proved by world practice that in addition to preventive measures, periodic health examination is of great importance. The above can be reflected in the SWOT analysis in the "Opportunities" section. The regional administrations' policy regarding price determination and pharmaceutical benefits, significantly affects the region's pharmaceutical market.

The analysis of the internal environment showed that the "Strengths" section, i.e., the opportunities increasing the intake of hypolipidemic drugs, can be attributed to their unique pharmacotherapeutic properties. A high demand of the population for these drugs in conditions of a high level of cardiovascular morbidity in the Volgograd region can also be attributed there [31]. The factors increasing the intake of hypolipidemic drugs include the expansion of the range of proposed clinical uses of this drug group, as well as the presence of a wide range of the drugs of domestic and foreign production (the "Strengths" section in the SWOT analysis). The "Weaknesses" section of this drug group includes the need for the dispensation of the prescription drugs, insufficient information awareness of doctors about the characteristics of this group, low information awareness of pharmaceutical specialists and final customers [32] (Table 2).

The study resulted in establishing the fact that using the SWOT analysis made it possible to predict trends in relation to an increase or decrease in the demand for hypolipidemic drugs under the influence of a number of external and internal factors. This type of analysis optimizes outlining the problems of this drug group and the ways to solve them, predicting an increase or decrease in the need for certain drug groups, and also developing ways to provide the population of the region with hypolipidemic drugs.

At the second stage of the study, an assessment of the information awareness and preferences of the final customers who applied to the pharmacy with hypolipidemic drug prescriptions, were detected. The carried out survey made it possible to obtain the results presented below.

The set of sociological issues presented in the questionnaires, made it possible to distribute respondents (390 people) by gender and age, place of residence, occupation, education, by the level of monthly income per family member. *By gender:* 251 (64.3%) people were men and 139 (35.7%) – women. According to the WHO classification, the respondents were distributed according to their age as follows: the persons aged 25–44 years old – 5 people (1.3%); 45–59 years old – 160 people (40.9%); 60 – 74 years old – 185 people (47.5%); 75 years old and older – 40 people (10.3%). *By place of*

residence: 264 respondents (67.8%) lived in the city of Volgograd; 126 respondents (32.2%) were residents of the Volgograd region. *By occupation:* 142 people (36.3%) were employees, workers; 109 (28.0%) were different level principals; 123 (31.6%) – old age pensioners (unemployed); 16 (4.1%) – housewives, unemployed. *By the level of monthly income per family member:* up to 10,000 rubles – there were 73 respondents (18.6%); from 11,000 to 20,000 rubles – 147 (37.8%); from 21,000 to 30,000 rubles – 119 (30.5%); from 31,000 to 40,000 rubles – 36 (9.3%); over 40,000 rubles – 15 (3.8%).

Since hypolipidemic medicines belong to the group of prescription drugs, the vast majority of respondents (all 390 of them) pointed out that they receive information about the prescribed drugs from the attending physician or another specialist. Thus, it is such intermediate consumers as doctors that are the main segment affecting the demand and structure of the hypolipidemic drugs intake. When initially contacting a pharmacy, a patient has a doctor's prescription for hypolipidemic drugs. However, hypolipidemic drugs are often sold over the counter to the patients who have already been undergoing hypolipidemic therapy for some time and who have repeatedly applied for the drug. The all – Russian tendency to self – medication or treatment on the recommendation of people without specialized knowledge in relation to the study group is less evident, except for the customers' requests for various biologically active additives with hypolipidemic actioss, the effectiveness of which is very doubtful.

The hypolipidemic drugs prescribed by the doctor are often / always purchased by 322 surveyed patients (82.6%), which indicates a high initial motivation of final customers to obtain prescribed hypolipidemic drugs. When choosing a hypolipidemic drug (if the doctor provides such an opportunity) for 247 (63.4%) final customers, its cost is of decisive importance not only for unemployed pensioners, for whom the price of the drug plays a leading role, but also for the people with the income from 31,000 to 40,000 rubles and more). And high efficiency is of decisive importance only for 143 (36.6%) of the respondents. The safety and the absence of side effects in hypolipidemic drugs, are important for 176 (45.1%) of the respondents. It should be notified that the second and third possible answers were indicated by the employees with an average monthly income per family member of 20,000 rubles and more. For unemployed pensioners, the price of the drug remains a decisive factor.

When asked about the preferences for the drugs, the majority of the respondents (328 people, i.e. 84.1%) answered that the manufacturer of the drug does not matter to them, since the medicine prescribed by the doctor must be effective regardless of the country of production. Further, the respondents were asked to indicate the names of the hypolipidemic drugs they were familiar with. The most recognizable were Atorvastatin (37.6%), Simvastatin (34.2%), Nicotinic acid (28.3%), Atoris (21.6%), Torvakard (18.7%) and Krestor (14.8%). Some of the proposed drugs (for example, rosuvastatin generics) were familiar to none of the respondents.

The last point of the questionnaire made it possible to clear up what information about the drug prescribed by the doctor is transmitted to the patient and in what form.

The questionnaire showed that only 51.3% of the final customers learn about the multiplicity and dosage regimen of taking hypolipidemic drugs from the signature in the prescription. Only 19.4% of these receive written information about the time of taking medicines and the duration of the treatment course. Thus, about 80% of patients are not aware that hypolipidemic therapy should be long – term (from several years to a lifelong period).

The remaining 48.7% of respondents either receive the required information (about the dosage regimen, side effects and undesirable drug combinations) from the doctor orally (76%), or simply get free – floating prescriptions without any comments from the specialist (26%). In the latter case the patients either have to turn to a pharmacy for help, or independently find the required information in electronic and printed directories. Thus, almost half of the final customers do not have enough information about hypolipidemic drugs, which reduces the adherence to hypolipidemic therapy and adversely affects the treatment of the underlying disease, worsening the prognosis.

At the third stage of the research, the compliance of the final customers was studied (Table 3).

The results of the Morisk – Green test in all age groups, demonstrate a low level of patients' adherence to the prescribed hypolipidemic therapy.

Table 1 shows that the leading indicator of a low adherence in all the age groups, is skipping the drug due to the patients' forgetfulness. Among the respondents, only 1 person scored 4 points, i.e., he was completely adherent to the carried out therapy. The highest average score is observed in the age group of 45–59 years old (2.5 in males, 2.3 in females). In the age groups of 60–74 years old and 75 years old and older, the average score is a little lower (2.3 in men and 2.2 in women). These data correlate with the results of the studies indicating a lower level of compliance in the patients of the retirement age. In all age groups, the women showed a little lower levels of adherence to the treatment.

CONCLUSION

Modern advances in the treatment of cardiovascular diseases, based on the fundamental achievements of science and practice, have created a high evidence base for the choice of pharmacotherapy strategies with hypolipidemic drugs.

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Table 3 – Results of patients' taking hypolipidemic drugs depending on their age and gender (according to the Morisk – Green test)

Question naire	Age group 45–59 years old (middle age)**				Age group 60–74 years old (elderly / old age)				Age group 75 years old and older (senile age)			
	Men n=27		Women n=23		Men n=34		Women n=28		Men n=7		Women n=6	
	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No
Have you ever forgotten to take your drugs?	26* 96%	1 4%	23 100%	–	34 100%	–	28 100%	–	7 100%	–	6 100%	–
Can you sometimes be inattentive to the hours of medication?	10 37%	17 63%	8 35%	15 65%	16 47%	18 53%	14 50%	14 50%	3 43%	4 57%	3 50%	3 50%
Do you skip taking medications if you feel good?	3 11%	24 89%	4 16%	19 74%	7 21%	27 79%	5 18%	23 82%	2 29%	5 71%	1 17%	5 83%
If you feel unwell after taking the medicine, do you skip the next dose?	1 4%	26 96%	4 17%	19 83%	2 6%	32 94%	3 11%	25 89%	–	7 100%	1 17%	5 83%
Average score	2.5±0.24		2.3±0.23		2.3±0.21		2.2±0.21		2.3±0.17		2.2±0.18	

*Note: * – absolute number; ** – distribution by age categories, carried out in accordance with the WHO classification*

A wide range of proposed clinical use, due, inter alia, to the presence of pleiotropic effects, determines a high demand of the population for hypolipidemic drugs. According to Russian and international recommendations, statins are the first choice drugs in the treatment of dyslipidemia. The factors contributing to an increase in the consumption of drugs of the studied group include: a continuously expanding evidence base confirming their effectiveness, unique pharmacotherapeutic properties of modern hypolipidemic drugs, their wide range, a great choice of generics with an optimal quality – to – price ratio. The factors that prevent the optimal consumption include: insufficient information awareness of intermediate and final customers about the assortment and pharmacotherapeutic properties of the drugs under study, the need for medical prescriptions, and high costs of original drugs. The lack of information awareness of final customers that occurs in the Volgograd region, leads to the fact that, despite a high level of trust in the attending physician, the patients demonstrate a low level of adherence to the treatment and an insufficient level of knowledge about hypolipidemic drugs, peculiarities of their administration, the expected safety and effectiveness. All the above mentioned factors lead to a decrease in the effectiveness of hypolipidemic therapy.

The development of measures to optimize the consumption of hypolipidemic drugs involves, first of all, providing patients with effective and safe drugs with a high tolerability profile. The main ways to increase the level of information awareness and compliance of the final customers of the Volgograd region are:

– development and intensification of educational programs in order to increase the level of proficiency and information awareness of doctors and pharmaceutical professionals regarding modern hypolipidemic therapy strategies, new drugs, their nomenclature, characteristic features of pharmacotherapeutic effects, side effects and drug interactions;

– establishing the *Doctor – Patient* partnering relationships, increasing the level of trust in the attending physician and, as a result, the level of patients' compliance;

– development of the materials that increase the level of information awareness of final customers about hypolipidemic drugs and hypolipidemic therapy in general, and ensuring their availability in pharmacies.

Thus, improving the quality of the drug supply that meets the needs of all market participants, and the rational use of drugs at the regional level, is a priority for Russian healthcare.

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AUTHORS' CONTRIBUTION

I.N. Tyurenkov – idea, development of research design, consultation on the problems of carrying out all the stages of the study; **Yu.S. Knyazeva** – literature analysis, article writing, research planning; carrying out all the stages of the study, formalization of the list of references; **L.M. Ganicheva** – consultation on the problems of planning, methodology and implementation of the study; **N.Sh. Kaisheva** – consultation on the problems of conducting individual stages of the study.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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