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USE OF SORPTION PROCESSES IN THE TECHNOLOGY OF DRUG DELIVERY SYSTEMS

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The aim *of this research is the review of scientific and technical literature regarding possibility of using sorption processes in the technology of drug delivery systems.* ***Materials and methods.*** *The materials are the following electronic resources: eLIBRARY, CyberLeninka, PubMed. The methods of review are analysis and synthesis. The study covers the scientific literature from 1996 up to the present time.* ***Results.*** *Sorbents are used as carriers for various medicinal peroral substances, they are also dispensers of various compounds in the form of polymeric eye films and stents in the human body. The delivery of medicinal substances occurs with the help of sorption processes of mass transfer. Currently, the following medical substances are used as carriers for medicinal substances: activated carbon, mineral sorbents (medical clays, synthetic sorbents), polymers and their biosimilars. 6 groups of pharmaceutical substances are registered for the production of enterosorbents in Russia and they can be used as sorbent carriers in the sorption drug system. They are: activated carbon, colloidal silicon dioxide, polyvinylpyrrolidone, dioctahedral smectite, polymethylsiloxane polyhydrate. As a result of the study, the model of the sorption drug system has been developed. It consists of sorbent carrier, active pharmaceutical ingredient and excipients that provide the desorption. Desorption of the active pharmaceutical ingredient may contribute to its modified release. The technology for obtaining sorption medicinal systems requires further study and development of modeling methods, searching for experimental pharmacological models and technological methods, which make it possible to obtain sorption dosage form with modified release.* ***Conclusion.*** *The review of the sorption processes used in the technology of drug delivery systems has been carried out. The model of the sorption drug system has been developed.*

Keywords: *sorption drug system, release modification, drug delivery*

ИСПОЛЬЗОВАНИЕ СОРБЦИОННЫХ ПРОЦЕССОВ В ТЕХНОЛОГИИ СИСТЕМ ДОСТАВКИ ЛЕКАРСТВЕННЫХ ВЕЩЕСТВ

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Цель *– обзор научной и технической литературы, касающейся возможности использования сорбционных процессов в технологии систем доставки лекарственных веществ.* ***Материалы и методы****. В качестве материалов исследования использовали электронные ресурсы eLIBRARY, CyberLeninka, PubMed. Методы исследования – анализ и обобщение научной литературы за период с 1996 года по настоящее время.* ***Результаты.*** *Сорбенты выступают в роли носителей для различных лекарственных веществ при приеме per os, а также в роли дозаторов различных соединений в организме человека при использовании полимерных систем доставки в виде глазных пленок и стентов. Доставка*

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*лекарственных веществ происходит при помощи сорбционных процессов массопереноса. В роли носителей для различных веществ используются следующие сорбенты: активированный уголь, минеральные сорбенты – медицинские глины, синтетические сорбенты – полимеры и их биоаналоги. В России зарегистрировано 6 групп фармацевтических субстанций, предназначенных для производства лекарственных препаратов энтеросорбентов, которые возможно использовать в качестве сорбента-носителя в сорбционной лекарственной системе: активированный уголь, кремния диоксид коллоидный, поливинилпирролидон, смектит диоктаэдрический, полиметилсилоксана полигидрат. Разработана модель сорбционной лекарственной системы, состоящая из сорбента-носителя, активного фармацевтического ингредиента и вспомогательных веществ, обеспечивающих десорбцию. Десорбция активного фармацевтического ингредиента может способствовать его модифицированному высвобождению. Технология получения сорбционных лекарственных систем требует дальнейшего изучения и разработки методов моделирования, поиска экспериментальных фармакологических моделей и технологических методик, позволяющих получить сорбционную лекарственную форму с модифицированным высвобождением. **Заключение.** Проведен обзор использования сорбционных процессов в технологии систем доставки лекарственных веществ, разработана модель сорбционной лекарственной системы.*

Ключевые слова: *сорбционная лекарственная система, модификация высвобождения, доставка лекарственных веществ*

INTRODUCTION

According to the information of Ministry of Health, in 2016 there were 237 million cases of registration of various diseases per 146.8 million people in Russia [1]. The statistical indicators show a high overall morbidity of the population. National security of the country, among other things, implies pharmaceutical security, i.e. the availability of drugs produced in Russia, which is impossible without the introduction of high-tech domestic developments in this area. Currently, specialists are faced with the task of developing and introducing new active pharmaceutical substances, developing new formulations of dosage forms of various directions of action, improving the production technology of dosage forms. These areas are the main ones in the state program "Strategy for the development of the pharmaceutical industry of the Russian Federation for the period up to 2020". The existing range of active pharmaceutical substances on the Russian pharmaceutical market is represented mainly by foreign manufacturers. Domestic development to improve the technology of obtaining new dosage forms are lagging behind foreign ones [2].

The development of a new dosage forms in pharmaceutical technology are going on in two directions:

1. development of new dosage forms with improved characteristics (release modification, low percentage of side effects) based on clinically approved substances. The actual direction is the study and use of mass transfer processes of drugs to modify their release;
2. development of new pharmaceutical ingredients and traditional dosage forms based on them [3, 4].

The promising direction in the development of drug delivery systems is the use of sorption technologies. Sorption methods can be used to inject medicinal substance (MS) into the sorbent under the conditions of reversibility of the process and desorption of the medicinal substance into the organism. The sorbent is pre-saturated with the necessary medicinal substance and the system is used in the desorption mode [5]. A medical substance has a large active area on the sorbent carrier due to the sorption of the monomolecular layer. This effect can reduce the dosage of the medicinal substance while maintaining therapeutic activity. The resulting sorption drug system can perform the function of the transport for delivering medicinal substance to the body.

THE AIM of this research is review of scientific and technical literature on the possibility of using sorption processes in the technology of drug delivery systems. The set up a problem are as follows: to review the use of sorption processes in the technology of drug delivery systems; to develop the sorption drug system model.

MATERIALS AND METHODS

The materials are the following electronic resources: eLIBRARY, CyberLeninka, PubMed. The methods of review are analysis and synthesis. The study covers the scientific literature from 1996 up to the present time.

RESULTS AND DISCUSSION

Modern pharmaceutical technology has the problems, the solution of which will be the new ways of developing effective drugs. The subject of the study is the dosage form with optimal bioavailability and a target action.

Currently, dosage forms are classified into three generations by therapeutic effect (Ischenko V.I., 2016):

1. traditional dosage forms are characterized by systematicity and periodicity of the action, in

which the major part of the injected substance is metabolized and does not reach the target;
2. dosage forms with systemic action and modified drug release (transdermal therapeutic systems, delivery systems based on sorption processes of mass transfer);
3. dosage forms characterized by target action in organs, tissues, cells and even in cell structures and controlled release (liposomes).

Traditional dosage forms are characterized by the immediate and uncontrolled release of drugs. Promising dosage forms are those with modified release and characterized by changes in the mechanism and nature of the release of medicinal substance [6]. Modification of medicinal substance release can be achieved in the following ways:

1. technological and nanotechnological ways mean the production of micro-size dosage forms, production of nanoscale dosage forms with a directed effect on a biological target;
2. physical-chemical ways mean the use of excipients that alter the solubility, absorption, distribution or elimination, as well as the formation of complexes or changes in the structure of the drug molecule [4, 7, 8].

Technological methods of modification consider sorption drug systems in which the drug is physically or chemically bound to a solid carrier in order to modify its release during subsequent desorption. Sorption drug system will reduce the dosage and frequency of drug administration. In this aspect, biopharmaceutical research on the creation of a new-generation of drugs is of particular relevance.

There are several ways to obtain sorption medicinal systems:

1. joint dispersion of drugs with a solid carrier in mills of various types. Grinding a polymer when it is used as a solid carrier can be done in the low temperature range, since it increases its ability for abrasion;
2. mixing drugs with a solid carrier in solvent medium, followed by removal of the solvent by evaporation [9].

Polymeric sorption systems with modified release of active pharmaceutical ingredients is the structure in which the polymeric carrier and the drug are in the complex with physiological activity and regulated pharmacokinetics [10-11]. In gastroenterology, dried cultures of living bacteria – probiotics – are widely used. The biomass of live bacteria adsorbed on the stone activated carbon is improved by dosage forms with respect to traditional lyophilized probiotics. The nomenclature of sorption probiotics is represented in Russia by the following medicinal preparation: “Bifidumbakterin Forte”, “Probifor” and “Florin Forte”. The technology of producing probiotic preparations is based on sorption processes aimed at sorption of microorganisms with activated carbon. Sorption ensures the survival of microorganisms in the acidic environment of the stomach, interaction with the parietal layer of the intestinal mucosa and an increase in the therapeutic effect [12, 13].

The carbon-mineral sorbent “SUMS-1” with bifidobacteria immobilized on its surface, showed a high adsorption activity and the probiotic therapeutic effect [14]. When immobilized on the “SUMS-1”, the sorbent polysaccharide from fucoidan algae, the elution of the latter into an aqueous solution was 50±10%. Combined dosage forms for enteral use have shown high efficacy in the treatment of burn wounds. [15, 16]. “SUMS-1” with an immobilized fibrinolysis inhibitor can be recommended in complex therapy for the treatment of inflammatory periodontal diseases [17].

Experimental studies of the treatment of burns and purulent wound surfaces have shown the effectiveness of using vulneororption by sorption medicinal systems with antibiotics [18–20] and herbal remedies [21–23].

Clinical observations of the last decades indicate side effects of antibiotics, and therefore interest in silver preparations is renewed. Silver-containing sorbents (SIAL-S), which showed good detoxifying and antioxidant properties have been developed [24]. Experimental studies indicate that «SIAL-S» sorbent may be an implant to fill in bone cavities, while maintaining osteoreparative properties in an infected wound [25].

The adsorptive immobilization of inulinase on ultra-crosslinked sorbents makes it possible to reuse the heterogeneous biocatalyst obtained in the enzymatic production of fructose. Protein adsorption was carried out on the polymeric sorbent [26], mesoporous silica [27].

In Babanina et al [28], methods for obtaining medical clays intercalated with Analgin and Amoxicillin are presented, as well as the influence of the synthesis methods (coprecipitation and hydration) on the degree of intercalation and the kinetics of release of active anions are considered. The advantages and disadvantages of using medical clays for oral drug delivery, prolongation of the release and purposefulness of the action of the medicinal substance included in the structure of medical clays, a rational choice of the type of clay as a carrier of medicinal substances are reflected in the works by different scolars [29–32].

The development of carbon sorption drug nanosystems for use in veterinary practice and the study of their physicochemical and toxicological properties are presented in Pyanova et al [33].

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Polymer carriers, in addition to their use in gastroenterology, are widely used in ophthalmology and surgery [34]. The method of obtaining films and capsules by alternating adsorption of oppositely charged polyions in order to obtain sorption medicinal systems based on chitosan, alginates and albumin are presented in Makarevich et al [35–36].

The sorption drug system has been developed in the form of a contact lens with a prolonged therapeutic effect with the use of computer programming. The dynamics of adsorption and desorption of the drugs have been considered, the time of saturation and therapeutic effect of the lens, i.e. the wearing time during which the drug was transported from the lens and exerted therapeutic effect has been determined [37–41].

The technology of creating the polymer-doxorubicin drug sorption system is presented in [42–43]. Sorbed doskorubitsin on the surface of the polymer stent slows down crystallization of bile. Polycaprolactone polymer is a carrier of doxorubicin and is effective in the treatment of cancer.

Researchers from Helsinki University and the Institute of Theoretical and Experimental Biophysics of the Russian Academy of Sciences have created a bone carcass based on hydroxyapatite, gelatin, polypyrrole and mesoporous silica. The carcass contains adsorbed antibiotics which suppress the infection in the damaged bone and help cells-reducers to work. The adsorption of antibiotics allows them to stretch their release from the carcass to 4 months [44].

Thus, the use of polymers as bases for sorption drug systems is aimed at obtaining controlled pharmacokinetics of sorbed drugs [45]. The release of drugs is due to the processes of mass transfer of drugs from the surface of the polymer. When creating polymer sorption drug systems, the polymer is chosen on the basis of the following properties: biocompatibility, biodegradability, physical properties, porosity, specific surface, type and pore volume, which make a controlled release of the drug possible. Currently, polymeric sorption drug systems are used in the development of materials for stents, ophthalmic films, as well as other systems with targeted drug delivery [46–51].

The review of the literature has shown the widespread use of sorption processes in the technology of drug delivery systems. The following sorbents are used as carriers for various substances: activated carbon, mineral sorbents (medical clays) synthetic sorbents (polymers and their combinations). Fig. 1 shows the recommended structure of the sorption drug system (*sorption drug system – "SDS"*).

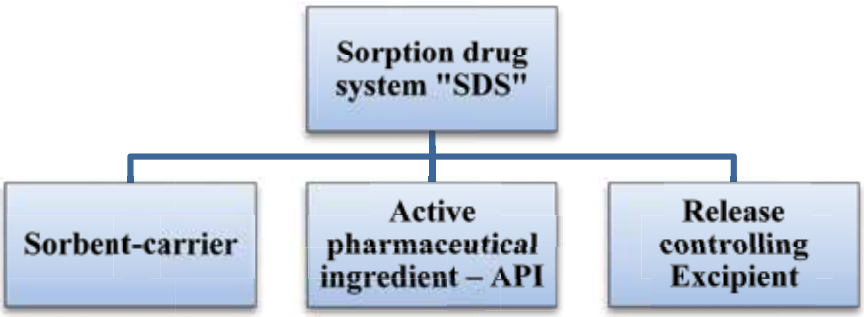

Figure 1. Structure of the sorption drug system

The structure of the sorption drug system consists of a sorbent carrier, an active pharmaceutical ingredient and excipients that provide desorption. Desorption of the active pharmaceutical ingredient may contribute to its modified release. This effect provides a constant therapeutic concentration, the known release rate, elimination of side effects, and stability augmentation [52–54].

Fig. 2 shows the pharmacokinetic model of drug release according to the dose.

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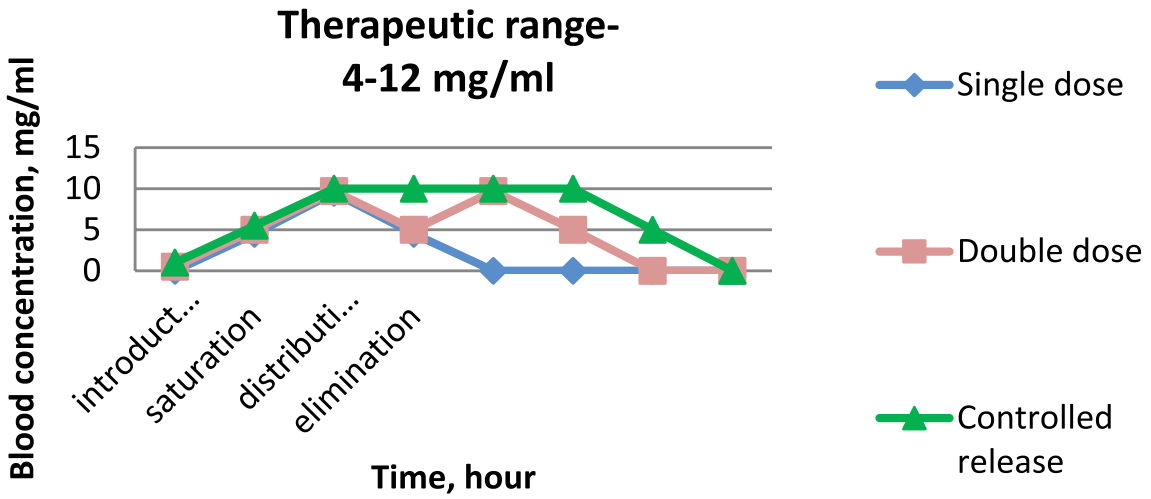

Figure 2. Pharmacokinetic model of drug release

As Figure 2 shows, the dosage form with controlled release is rational, as the active substance is able to have a clinical effect with a pharmacokinetic profile that is different from the one achieved with the use of the dosage form with immediate release at a single or double dosage.

Fig. 3 shows the mechanism of the sorption drug system action of the use *per os*.

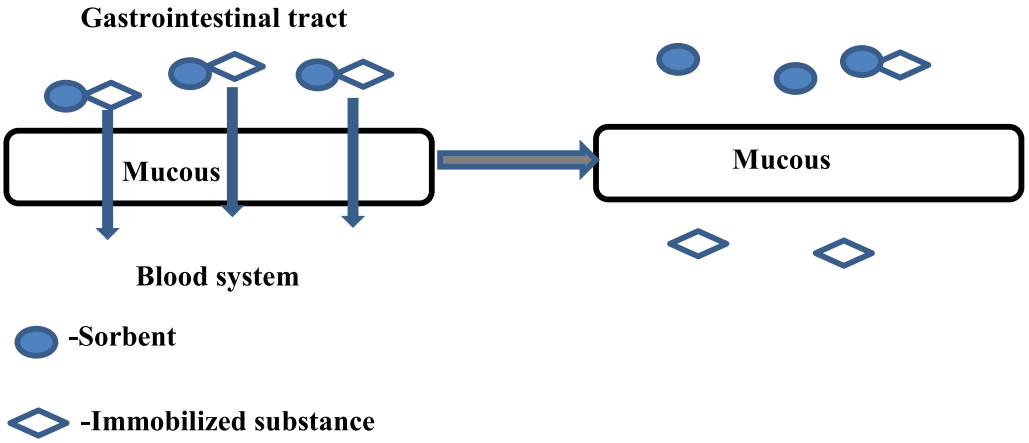

Figure 3. Mechanism of the sorption drug system action of the use per os

As Fig. 3 shows, the sorption drug system desorbs immobilized drug, which, accumulating near the intestinal wall, is absorbed into the blood when ingested into the gastrointestinal tract. The use of carrier sorbents will allow modification of drug release. The following factors may influence the release of drugs: the surface structure of the sorbent carrier, the concentration of drugs in the carrier, type of dosage form, surface area, pore size, adsorption activity.

In the technology of obtaining sorption medicinal systems for use *per os,* it is possible to use only registered pharmaceutical substances with an adsorption pharmacological action. Based on the analysis, the following groups of pharmaceutical substances permitted for use in medical practice, were identified (Table 1) [55–56].

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Table 1. Characteristics of the main groups of sorbents

№	Group	Characteristic	Specific surface area
1	2	3	4
1	Activated Carbon	Obtained from stone coals by cleaning and steaming to increase porosity. It has a highly developed microporous surface	1,5–200 m²/g
2	Dioctahedral Smectite	Prepared from mineral raw materials by purification. It has a mesoporous surface. Due to the structure of the crystal lattice it has an ion-exchange ability	up to 600 m²/g
3	Colloidal Silicon dioxide	Prepared from highly dispersed silica. It shows sorption properties on the surface in the places of silicon oxide bonding with hydroxyl groups	up to 400 m²/g
4	Hydrolyzed Lignin	Obtained by alkaline processing of lignin. It has a macroporous structure	up to 20 m²/g
5	Polyvinylpyrrolidone	Prepared synthetically from the monomer vinylpyrrolidone. Possesses ion-exchangeable capability	200–400 m²/g
6	Polymethylsiloxane-polyhydrate	Prepared by polycondensation of methylsilicic acid hydrogel. It has a silicone porous structure. Sorbs medium molecular substances.	180–300 m²/g

As Table 1 shows, the 6 groups of pharmaceutical substances are registered in Russia and used in the manufacture of enterosorbents. The following sorbents can be used as carriers: activated carbon, dioctahedral smectite, colloidal silicon dioxide, polyvinylpyrrolidone and polymethylsiloxane polyhydrate. Sorbents have different specific surface indicators. This indicator characterizes the amount of sorption processes on their surface, they can occur simultaneously. Hydrolyzed lignin is obtained in the form of secondary raw material after hydrolysis of hardwood and softwood. Lignin has a macroporous structure.

Medical refined clays are used as carriers, using surface hydroxyl groups for sorption of organic substances, as well as active centers inside the crystal lattice for sorption of inorganic substances. Currently, developed polymer sorption drug systems have limitations *in vivo* due to the biodegradation processes, unexplored metabolism, low solubility or insolubility and toxicity. To solve these problems, additional studies of polymers as carriers for sorption drug systems are required.

The technology for obtaining sorption medicinal systems requires further study and development of modeling methods, searching for experimental pharmacological models and technological methods, which allow obtaining a sorption dosage form with modified release.

CONCLUSION

The review of sorption processes in the technology of drug delivery systems has been carried out. The possibility of using sorption processes in drug delivery systems has been established. Sorbents act as carriers for various medicinal substances when taken *per os*, as well as dispensers of various compounds in the human body when using polymer delivery systems in the form of eye films and stents. A model of the sorption drug system, consisting of a sorbent carrier, an active pharmaceutical ingredient and excipients that provide desorption, has been developed. Desorption of the active pharmaceutical ingredient may contribute to its modified release.

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Conflict of interest

The authors declare no conflict of interest.

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WORKING OUT QUALITY STANDARDS OF MODEL COMPOSITION SAMPLES OF GRANULATED DOSAGE FORM WITH GLUTATHIONE RESTORED

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Biologically active sulfur-containing compounds (BASC) exhibit pronounced antioxidant properties. Glutathione reduced (GSH) occupies a particular position among these compounds. It represents a key link in the 3 antioxidant systems of the body from the existing four. Based on the foregoing, a GSH-based dosage form with antioxidant properties was proposed. The aim of this study is to work out a model granulated dosage form based on GSH and methods of its analysis by means of pre-column derivatization with ortho-phthalic aldehyde. ***Materials and methods.*** *GSH and granulated dosage form based on GSH obtained by wet granulation were used as the object of the study. Quantitative evaluation of GSH content in the obtained granules was carried out using pre-column derivatization by the method of reversed-phase high-performance chromatography (RP HPLC). Ortho-phthalic aldehyde was used as a derivatizing agent. A diode-array detector was used to detect the resulting derivative. Ortho-phthalic aldehyde was used as a derivatizing agent. A diode-matrix detector was used to find out the resulting derivative.* ***Results.*** *In the course of the work, a model dosage form was created – granules based on GSH. By reference to the recommendations on the dosage of the drug, the concentration of the active substance was selected. Lactose was chosen as an auxiliary component. Physical and technological characteristics of a model sample of granules with GSH and lactose as a filler were studied. A method of quantitative determination of GSH in granules using pre-column derivatization with ortho-phthalic aldehyde was developed and validated by HPLC. The method of quantitative determination of GSH in granules with the use of pre-column derivatization by ortho-phthalic aldehyde by HPLC was developed and validated.* ***Conclusion.*** *The developed granulated dosage form meets the requirements given in the pharmacopoeial item "Granules" according to the analyzed indicators. Using the validation evaluation it was established, that the developed methods for the quantitative determination of GSH in granules is correct, precise and specific.*

Keywords: *reduced glutathione, ortho-phthalic aldehyde, granules, reverse phase high performance liquid chromatography, derivatization, validation*

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РАЗРАБОТКА НОРМ КАЧЕСТВА ОБРАЗЦОВ МОДЕЛЬНОГО СОСТАВА ГРАНУЛИРОВАННОЙ ЛЕКАРСТВЕННОЙ ФОРМЫ С ГЛУТАТИОНОМ ВОССТАНОВЛЕННЫМ

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Биологически активные серосодержащие соединения (БАСС) проявляют ярко выраженные антиоксидантные свойства. Особое положение из числа данных соединений занимает глутатион восстановленный (GSH). Он представляет собой ключевое звено 3-х антиоксидантных систем организма из существующих четырех. Исходя из вышесказанного, нами была предложена лекарственная форма на основе GSH, обладающая антиоксидантными свойствами. ***Целью*** *данного исследования является разработка модельной гранулированной лекарственной формы на основе GSH и методики ее анализа с помощью предколоночной дериватизации орто-фталевым альдегидом.* ***Материалы и методы.*** *В качестве объекта исследования использовали GSH и гранулированную лекарственную форму на основе GSH, полученную методом влажного гранулирования. Оценку количественного содержания GSH в полученных гранулах, проводился с помощью предколоночной дериватизации методом обращено-фазной высокоэффективной хроматографии (ОФ ВЭЖХ). В качестве дериватизурующего агента использован орто-фталевый альдегид. Для обнаружения образовавшегося деривата был применен диодно-матричный детектор.* ***Результаты.*** *В ходе работы была создана модельная лекарственная форма – гранулы на основе GSH. Исходя из рекомендаций по дозировке препарата подобрана концентрация действующего вещества. В качестве вспомогательного компонента была выбрана лактоза. Изучены физические и технологические характеристики модельного образца гранул с GSH и лактозой в качестве наполнителя. Разработана и отвалидирована методика количественного определения GSH в гранулах с использованием предколоночной дериватизации орто-фталевым альдегидом методом ВЭЖХ.* ***Заключение.*** *Разработанная гранулированная лекарственная форма по анализируемым показателям соответствует требованиям, приведенным в ОФС «Гранулы». При помощи валидационной оценки установлено, что разработанная методика количественного определения GSH в гранулах является правильной, прецизионной и специфичной.*

Ключевые слова: *глутатион восстановленный, орто-фталевый альдегид, гранулы, обращённо-фазная высокоэффективная жидкостная хроматография, дериватизация, валидация*

INTRODUCTION

Reduced glutathione (GSH) is Tripeptide containing amino acid residues of L-glutamic acid, glycine and L-cysteine. GSH is one of the non-enzymatic components involved in the antioxidant protection of living organisms. It acts as an effective antiradical agent and plays a key role in the life cycle of cells, causing their protection from free radicals, hydroperoxides and xenobiotics [1, 2]. GSH status is an indicator of cell functionality and viability [3, 4]. The exhaustion or change of its level inside the cell provokes a number of diseases such as cancer, neurodegenerative, cardiovascular [5, 6]. *In vitro* and *in vivo* studies confirm that GSH deficiency can cause cell death and mitochondrial damage due to an increase in the number of toxic forms of oxygen and an increase in the number of free radicals [7]. GSH is able to prevent the destruction of cells by conjugation with toxic substances and their metabolites. Glutathione conjugation occupies one of the central places in the mechanisms of biotransformation of a number of xenobiotics [8–10]. Nowadays, more than forty types of chemical compounds that react with GSH are known. The mating factor of such reactions is the presence of an electrophilic center capable of reacting with the SH-group of GSH [11]. Consequently, the xenobiotic detoxification system involving GSH plays a key role in the formation of the body's resistance to various influences and is one of the most important defense mechanisms of the cell. Hereby, GSH conjugates with xenobiotics are less reactive and more hydrophilic than the initial substances, so they are less toxic and eliminate from the body faster [12, 13]. GSH is also able to prevent the introduction of lipophilic

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toxicants into lipid bilayer membranes [14]. GSH has a membrane stabilizing activity effect on the hepatocyte, increases the activity of enzymes and promotes detoxification and regenerative activity of the liver through the neutralization of free radicals [15]. Taking into account the spectrum of glutathione pharmacological activity, we have proposed a dosage form with GSH, which has pronounced antioxidant properties.

MATERIALS AND METHODS

As the object of the study, glutathione reduced (CAS No. 70-18-8, EC No. 2007254, Applichem, Germany) and the excipient, lactose, were used The choice of this component is due to the possibility of its application as a filler, a reagent and an agent that regulates some technological characteristics of the granules (stability, disintegration, etc.).

Advantages of lactose as an excipient [16]:

– inert material, high purity, neutral color;

– moisture resistance;

– physical and chemical stability;

– it is well subjected to grinding and sieving;

– its high degree of crystallization, low degree of amorphism.

The wetting angle of GSH with water is less than 90°, it is 45±5°, i.e. GSH is a hydrophilic substance, so purified water was used as a humidifier in the model sample of granules.

To obtain a model sample of granules, a wet granulation method was used. The procedure for the formation of granules occurs as a result of pushing the moistened mass through a perforated sieve.

The technological characteristics of the obtained granules were determined on the basis of the following techniques.

The degree of the granules flowability was established by the method of the State Pharmacopoeia of Russian Federation (SPh), XIV-th edition (pharmacopoeial item "Powder Flow") using a V-12A (VZZTO) vibrating device. The sizes of the obtained granules were determined using the sieve analysis in accordance with SPh, XIV-th edition (pharmacopoeial item "Wet sieve residue test"). The test of "disintegration" was carried out on the "Swinging basket" disintegration tester in accordance with SPh, XIV-th edition (pharmacopoeial item "Granules"). The results of the "Dissolution" test were noted on the "Impeller" dissolution tester in accordance with pharmacopoeial item 1.4.2.0014.15.

Quantitative determination of GSH in a granular dosage form was performed by method of reverse phase high-performance chromatography using pre-column derivatization of this therapeutic agent by ortho-phthalic aldehyde [17].

The analysis was performed on the chromatograph "Agilent Technologies 1200 Infinity" with automatic sampler, vacuum microdoser, gradient pump and thermostat. The gradient chromatography conditions were: mobile phase (A) – 1% aqueous solution of formic acid, (B) – ethyl alcohol 95%; column: *Ascentis express* C18 2.7 dm × 100 mm × 4.6 mm; mobile phase speed – 0.5 ml/min.; column temperature was +35±0.01°C; sample volume – 1 µl.

GSH lacks the chromophores suitable for its analysis by UV spectroscopy and HPLC with diode array detection. In this regard, we proposed the analysis of GSH by HPLC using chemical transformation with a derivatizing agent – ortho-phthalic aldehyde. As a result of the reaction, the obtained derivative of GSH and ortho-phthalic aldehyde acquires a chromophore label, which is fixed during chromatographic analysis using diode-matrix detection.

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Derivatization methods of glutathione-based granules

1 ml of a 0.1% solution of glutathione granules in a 0.05 M of aqueous solution of sodium tetraborate was placed in a 10 ml analysis bottle, 1 ml of a 0.35% solution of o-phthalaldehyde in ethanol was added, shaken thoroughly and immediately chromatographed.

Preparation of model test solutions for validation assessment methods

The characteristics of correctness and precision were investigated on model samples of the preparation with glutathione concentrations that correspond to 80%, 85%, 90%, 95%, 100%, 105%, 110%, 115% and 120% of their content relative to the nominal value. For the preparation of model samples of the drug a volumetric flasks with a capacity of 25 ml was used. The batches were taken directly from the components of the drug. The mass of the batches and the concentration of the resulting solutions of glutathione are shown in Table 2.

RESULTS AND DISCUSSION

On the base of the experimental data obtained, a model dosage form – granules based on glutathione – was created. The concentration of active substances was selected by reference to the recommendations of the

dosage of the drug (0.05–0.5 g). The composition of the granules in a single-dose package (3.0 g) is as folows: glutathione 0.1 g, lactose 2.9 g. At the next stage, organoleptic, physical and technological parameters of granules based on glutathione were determined. The results of the study are presented in Table 1.

Table 1. Pharmaceutical and technological characteristics of granulated model dosage form based on GSH

Indicator under study	Methods of determination	Values obtained experimentally	Reference values
Granule size	Pharmacopoeial item 1.1.0015.15	1.0–1.2 mm	Coarse fraction:> 1.2 mm Average fraction: 1.2 mm Fine fraction: 1.0 mm
Granule form	Pharmacopoeial item 1.2.1.0009.15	Anisodiametric (elongated)	Elongated: >3:1 Lamellar: 3:1 Equiaxed: 1:1
Tapped density	Pharmacopoeial item 1.4.2.0016.15	460.10 ±1.31 kg/m3 (Light)	Very heavy: >2000 kg/m3 Heavy: 1100–2000 kg/m3 Medium: 600–1100 kg/m3 Light: < 600 кг/м3
Flowability	Pharmacopoeial item 1.4.2.0016.15	7.14 ±0.25 g/sec (Good)	Excellent: 8.6–12.0 g/sec Good: 6.6–8.5 g/sec Satisfactory: 3.0–6.5 g/sec Permissible: 2.0–3.0 g/sec Poor: 1.0–2.0 g/sec Very poor: <1,0 g/sec
Disintegration	Pharmacopoeial item 1.4.2.0013.15	4±2 min.	Up to 15 min.
Stability	Pharmacopoeial item 1.4.2.0004.15	98.5±0.5%	Mot less than 97%
Glutathione release	Pharmacopoeial item 1.4.2.0014.15	99.9±0.50% (during 15 min.)	75% (during 45 min.))
Water solubility of granules	Pharmacopoeial item 1.2.1.0005.15	Soluble (1:30)	Highly water soluble: up to 1 ml/g Freely soluble: 1–10 ml/g Soluble: 10–30 ml/g; Sparingly soluble: 30–100 ml/g Slightly soluble: 100–1000 ml/g; Very slightly soluble: 1000–10000 ml/g Practicaly insoluble: more than 10000 ml/g
Weight loss on drying	Pharmacopoeial item 1.2.1.0010.15	1.5±1%	
Uniformity of dosing	Pharmacopoeial item 1.4.2.0008.15	In progress	
Glutathione content, g	Reverse phase highly efficient liquid chromatography 100.0±0.39%	100.09±0.39%	

According to the data of Table 1, glutathione-based granules are elongated particles of 1.0-1.2 mm. They belong to light grains and have a satisfactory flowability. According to the indicators given in the pharmacopoeial item “Granules” (disintegration, uniformity of dosage, dissolution) this dosage form meets the requirements. Glutathione-based granules can be used to fill capsules, as well as an independent dosage form. The worked out granules are soluble and highly soluble in warm water. Glutathione-based granules are an oral dosage form with preliminary dissolution in liquid.

For the purpose of quantitative determination of glutathione recovered in a granulated dosage form, a method for pre-column derivatization with ortho-phthalic aldehyde has been developed. Derivatization with the specified modifier usually occurs within 2-3 minutes. The resulting product is easily detected using a diode array or a fluorimetric detector. The wavelength of the resulting derivative is usually λ – 337 nm. The molar ratios of o-phthalaldehyde and glutathione are 3.5: 1. When chromatographing OPHA-derivative of glutathione, there is one peak with a retention time of 18.066 minutes. The UV spectrum of the derivative exhibits several absorption maxima, the most specific of which is λmax – 336 mn. At the same time, the peak of the derivatizer itself is not visible, due to a different maximum absorption of the derivative (Fig. 1).

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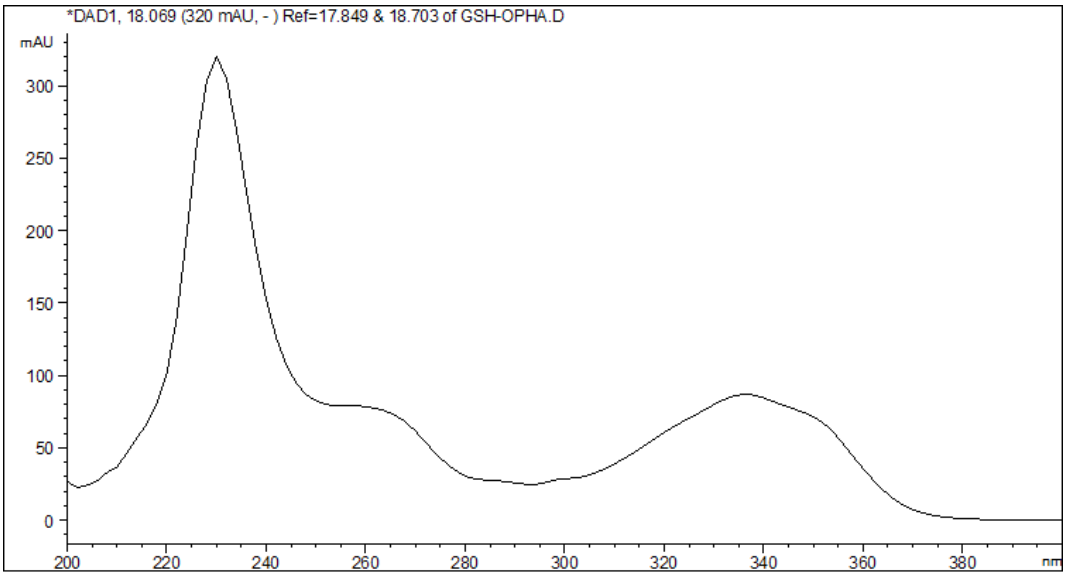

Figure 1. UV spectrum of o-phthalaldehyde derivative with GSH at the wavelength of 336 nm in a granulated dosage form

To confirm the possibility of using the proposed method of identification and quantitative determination of glutathione by the method of pre-column derivatization with ortho-phthalic aldehyde in granules, a validation assessment was carried out according to the characteristics: specificity, linearity, convergence (precision) and correctness [18].

Validation parameters of correctness, precision and linearity were studied on the preparation solutions with glutathione concentrations in the range of 80–120% of the nominal content of in granulated glutathione. This covers the whole range of concentrations and determines the minimum permissible concentration of the methods for quantitative determination [19].

The acceptance criteria were calculated for b=10%, therefore, the maximum value of the total uncertainty of the results of the methods (Δ_{As}) should not exceed the value of B×0.32=3.2% [20].

Table 2. Mass of the batches and concentrations of glutathione reduced in model granulated samples

Number of model sample	Mass of the preparation batches – granules with glutathione, g	Content of glutathione reduced in the batch, g	Concentration of glutathione reduced relative to nominal, %
1	0/6022	0.02000	80.41
2	0/6360	0.02120	85.42
3	0.6760	0.02250	90.85
4	0.7125	0.02370	95.07
5	0.7512	0.02500	100.02
6	0.7875	0.02620	105.20
7	0.8265	0.02755	110.03
8	0.8630	0.02876	114.96
9	0.9012	0.03000	119.92

The results of chromatography, the values of certain concentrations of glutathione in model samples and the calculation of the metrological characteristics of the methods are presented in Table 3.

From the information given in Table 3 it follows, that the methods of quantitative determination of glutathione does not have a statistically significant systematic error.

Thus, validation tests of the methods of quantitative determination of glutathione in granules by the method of pre-column derivatization with ortho-phthalic aldehyde showed that the validation parameters correspond to the accepted quantitative criteria for correctness, convergence in the concentration range from 80% to 120% of the nominal glutathione content in the granulated dosage form.

Table 3. Validation results of the methods for quantitative determination of glutathione in model solutions of granules

Solution number	% of Glutathione taken, compared to the concentration of the reference solution (X,%)	% of Glutathione found, compared to the concentration of the reference solution (Y,%)	Glutathione found to Glutathione taken ratio Z = 100*(Y/X)
1	80.41	80.39	99.98
2	85.42	85.49	100.01
3	90.85	90.78	99.92
4	95.07	95.23	100.17
5	100.02	100.21	100.19
6	105.20	105.01	99.82
7	110.03	110.18	100.14
8	114.96	115.15	100.17
9	119.92	120.36	100.37
Average, Z, %			100.09
Relative standard deviation, S_z, %			0.17%
Relative confidence interval, Δ, % = t (95%, 7)*S_z			0.39
Critical value for convergence of results, maxΔ_{As}, %, at B±10%			3.2
Systematic error, δ = Z_{cp} –100			0.09
Criterion of insignificance of the system error δ≤Δ/3			In progress
General conclusion about the methods			Correct

CONCLUSION

In the course of the work, a model dosage form was created – glutathione-based granules. Physical and technological characteristics of the model sample of granules with GSH and lactose as a filler have been studied. Methods of quantitative determination of GSH in granules using pre-column derivatization with ortho-phthalaldehyde by HPLC has been worked out and validated. The results of the validation assessment showed that this methods complies with all the validation parameters: it is correct, precise, specific and linear in the analytical field.

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Conflict of interest

The authors declare no conflict of interest.

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RESEARCH ARTICLE

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EVALUATION OF THE MITOCHONDRIA RESPIROMETRIC FUNCTION IN THE CONDITIONS OF PATHOLOGIES OF VARIOUS GENESES

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The aim *of the paper is to assess the change in the mitochondrial respirometric function under conditions of various pathologies.* ***Materials and methods.*** *The study was performed on male Wistar rats. Experimental focal cerebral ischemia, traumatic brain injury, coronary occlusive myocardial infarction and muscle dysfunction were used as pathological models. Focal ischemia was reproduced by the method of irreversible thermocoagulation of the middle cerebral artery. Traumatic brain injury was modeled by the method of free fall of the load. Experimental myocardial infarction was reproduced by ligating the descending branch of the left coronary artery. Muscle dysfunction was modeled by the method of «forced swimming with a 20% burden». The respiratory function of mitochondria was assessed by the method of respirometry by the change in oxygen consumption when introducing mitochondrial respiration into the medium: Oligomycin, Rotenone and FCCP. Additionally, we evaluated the intensity of the glycolysis process and the activity of respiratory complexes I, II, IV and V. In order to comprehensively assess the respiratory function, an ELISA study was conducted to determine the concentration of ATP, mitochondrial ATP synthetase, cytochrome C oxidase and NADP-Oxidase 4.* ***Results.*** *In the course of the study it was established that under conditions of experimental cerebral ischemia, traumatic brain injury, myocardial infarction and muscle dysfunction, the ATP-generating ability of mitochondria the maximum breathing and respiratory capacity deteriorated, herby the decrease in overall respiratory function was accompanied by an increase in glycolysis, which was uncompensated, as well as dysfunction of mitochondrial complexes I, II, IV and V, confirmed by an increase in NADPH oxidase 4 activity and a decrease in cytochrome C oxidases and ATP synthetase. As a result, the observed changes in mitochondrial respiration function contributed to a decrease in ATP concentration under conditions of cerebral ischemia - by 3.2 times ($p < 0.05$), traumatic brain injury – by 2.6 times ($p < 0.05$), myocardial infarction – by 1.8 times ($p < 0.05$) and muscle dysfunction – by 4 times ($p < 0.05$).* ***Conclusion.*** *Basing on the data obtained, we can assume that in conditions of cerebral ischemia, traumatic brain injury, myocardial infarction and muscle dysfunction, there is deterioration of the mitochondrial respirometric function with inhibition of ATP synthesis and increased glycolysis.*

Keywords: *cerebral ischemia, myocardial infarction, traumatic brain injury, muscle dysfunction, respirometry*

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ОЦЕНКА РЕСПИРОМЕТРИЧЕСКОЙ ФУНКЦИИ МИТОХОНДРИЙ В УСЛОВИЯХ ПАТОЛОГИЙ РАЗЛИЧНОГО ГЕНЕЗА

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Цель исследования – *оценить изменение респирометрической функции митохондрий в условиях различных патологий.* ***Материалы и методы.*** *Исследование выполнено на крысах самцах линии Wistar. В качестве модельных патологий в работе использовали экспериментальную фокальную ишемию головного мозга, черепно-мозговую травму, коронарооккклюзионный инфаркт миокарда и мышечную дисфункцию. Фокальную ишемию воспроизводили методом необратимой термокоагуляции средней мозговой артерии. Черепно-мозговую травму моделировали методом свободного падения груза. Экспериментальный инфаркт миокарда воспроизводили лигированием нисходящей ветви левой коронарной артерии. Мышечную дисфункцию моделировали методом «принудительного плавания с 20% отягощением». Дыхательную функцию митохондрий оценивали методом респирометрии по изменению потребления кислорода при внесении в среду разобщителей митохондриального дыхания: олигомицин, ротенон и FCCP. Дополнительно оценивали интенсивность процесса гликолиза и активность дыхательных комплексов I, II, IV и V. С целью комплексной оценки респирометрической функции проводили ИФА-исследование с определением концентрации АТФ, митохондриальной АТФ-синтетазы, цитохром-с-оксидазы и НАДФ-оксидазы 4.* ***Результаты.*** *В ходе проведения исследования установлено, что в условиях экспериментальной ишемии головного мозга, черепно-мозговой травмы, инфаркта миокарда и мышечной дисфункции отмечено ухудшение АТФ-генерирующей способности митохондрий, максимального уровня дыхания и респираторной емкости, при этом снижение общей респирометрической функции сопровождалось усилением процессов гликолиза, которое носило некомпенсированный характер, а также дисфункцией митохондриальных комплексов I, II, IV и V, подтверждаемой увеличением активности НАДФ-оксидазы 4 и снижением активности цитохром-с-оксидазы и АТФ-синтетазы. В итоге наблюдаемые изменения респирометрической функции митохондрий способствовали уменьшению концентрации АТФ в условиях церебральной ишемии – в 3,2 раза ($p<0,05$), черепно-мозговой травмы – в 2,6 раза ($p<0,05$), инфаркта миокарда – в 1,8 раза ($p<0,05$) и мышечной дисфункции – в 4 раза ($p<0,05$).* ***Заключение.*** *Основываясь на полученных данных можно предположить, что в условиях ишемии головного мозга, черепно-мозговой травмы, инфаркта миокарда и мышечной дисфункции наблюдается ухудшение респирометрической функции митохондрий с угнетением синтеза АТФ и усилением процессов гликолиза.*

Ключевые слова: *ишемия головного мозга, инфаркт миокарда, черепно-мозговая травма, мышечная дисфункция, респирометрия митохондрий*

INTRODUCTION

Mitochondria are cellular organelles, the main sources of energy in the cell, which also play a significant role in regulating the processes of caspase-dependent and caspase-independent pathways of apoptosis and redox signaling of the cell [1]. In accordance with this, three leading mitochondrial functions are distinguished: respirometric, i.e. ensuring the synthesis of macroergs in the process of redox reactions in the electron-transport mitochondrial respiratory chain [2]; apoptosis-regulating, i.e. regulation of the initiation and progression of the apototic signal [3] and antioxidant, i.e. inactivation of free radicals [4]. At the same time, the main function of mitochondria is respirometric, which provides the relationship between the redox state of the cell and the activation of proapoptotic molecules [5]. Currently, it has been established that the number of "mitochondrial diseases", the pathogenesis of which is associated with impaired functional activity of mitochondria, comprises ischemic stroke, Alzheimer's disease, traumatic brain injury, ischemic heart disease and myocardial infarction, muscle fatigue [6]. In the scientific literature it is reported that in the pathogenesis of these diseases, one of the central roles is assigned to the energy deficit that occurs when there is mitochondrial dysfunction [7]. At the same time, the reduction in the formation of

macroergic compounds is inseparably linked with the disruption of electron transport in the respiratory chain of mitochondria and the dissociation of the reactions of subcomplexes I, II, IV and V, which leads to the activation of glycolysis and a significant decrease in ATP synthesis [8]. In addition, dysfunction of complexes I and II contributes to the redistribution of oxygen flow towards the formation of prooxidants, in particular the superoxide radical [9] and a decrease in the formation of ATP leads to the activation of the caspase-dependent pathway of apoptosis [10]. At the same time, the intensification of anaerobic oxidation processes leads to the accumulation of non-oxidized products of metabolism. That shifts the intracellular pH value in the acidic direction. Under current conditions, activation of pro-apoptotic signaling molecules (proteins of the Bid / Bax family) is noted, triggering a caspase-independent pathway of apoptosis, which enhances cellular destruction [11]. Thus, the assessment of the change in mitochondrial respiration function under conditions of various pathologies may be the basis for the development of mitochondrial disease treatment strategies, which can eliminate energy deficit and associated apoptosis and oxidative modification of cellular structures.

MATERIALS AND METHODS

Biological model

The study was performed on 50 male Wistar rats weighing 220–240 grams, obtained from the nursery of laboratory animals "Rappolovo". The contents and all animal manipulations complied with the requirements of the European Convention for the Protection of Vertebrate Animals used for experiments and other scientific purposes (Strasbourg, 1986). The rats were housed in macrolon cages, where granulated wood faraction was used as litter at the relative humidity of 60 ± 5% and the air temperature of 22 ± 20°C. The feed and water were received by the animals in the free access. During the study, the following experimental groups were formed: intact animals (n = 10), the rats with reproduced cerebral ischemia (n = 10), TBI (n = 10), myocardial infarction (n = 10) and muscle dysfunction (n = 10).

Model of focal cerebral ischemia

Focal cerebral ischemia was modeled by irreversible right-sided thermocoagulation of the middle cerebral artery under chloral hydrate anesthesia (350 mg / kg). The area below and to the right of the eye was depilated, an incision was made. The soft tissues were moved apart, exposing the process of the zygomatic bone, which was removed. Then a trephine opening was burred and the middle cerebral artery was burned through by a thermocoagulator under its intersection with the olfactory tract. Later on , the topography of soft tissues was restored as far as possible. The suture was treated with a 5% iodine solution [12]. The biomaterial was sampled on the 4th day after the reproduction of focal ischemia.

Model of experimental traumatic brain injury

Traumatic brain injury was modeled by the method of a free fall of load of 150 g from a height of 50 cm to the parietal region of the brain of rats. The animals were placed in a special installation, which is a hollow cylinder with backing and retainers, in which the head of rats was fixed. After hat the load was released [13]. The biomaterial was sampled on the 4th day after the reproduction of the TBI.

Muscle Dysfunction Model

Muscle dysfunction was reproduced by the method of "forced swimming with 20% weight" after determining the initial value of the swimming time, the animals were subjected to training tests for 28 days (the swimming time was 20% of the initial index). On days 7, 14, 21, and 28, the rats were subjected to the exhausting test — swimming until they completely abandoned the struggle for life, after which the animals were taken out of from the water. The biomaterial was taken on day 28 [14].

Model of acute myocardial infarction

In animals under conditions of chloral hydrate anesthesia (350 mg / kg) and artificial ventilation of the lungs, the skin on the previously depilated area was cut in the sternum area and the muscles were dissected. Next, the IV rib was isolated, and the chest was opened. The myocardium was separated from the epicardium and the heart was led into the wound. Subsequently, the ligation of the descending branch of the left coronary artery with silk thread was carried out. The wound was sutured in layers. The biomaterial was taken 24 hours after the operation [15].

Biomaterial sampling and sample preparation

Brain, myocardium and muscle tissue (*m.quadriceps femoris*) of the rats were used as biomaterial. The animals were decapitated under chloral hydrate anesthesia (350 mg/kg), their organs were harvested. After that the biomaterial was homogenized in a mechanical homogenizer in a selection medium (1 mmol EDTA, 215 mmol mannitol, 75 mmol sucrose, a 0.1% BSA solution, 20 mmol HEPES, with a pH of 7.2). The cell population was obtained by differential centrifugation, for which the obtained biogenic homogenate was centrifuged in the mode of 1.400 g → 3 min. at 40°C. After that the supernatant was transferred into 2 ml tubes. Next, the resulting supernatant was centrifuged at 13000 g → 10 min and the supernatant (the culture contains native mitochondria) was removed for analysis [16].

Respirometric analysis

The analysis of the state of the mitochondrial respiratory function was carried out by the method of respirometry using the AKPM1-01L laboratory respirometer system (Alfa Bassens, Russia). The mitochondrial respi-

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ratory function was assessed by the change in the oxygen consumption in the medium against the introduction of mitochondrial respiratory uncouplers. The latest in the experiment were: Oligomycin 1 µg / ml; 4 – (trifluoromethoxy) phenyl) hydrazono) malononitrile (FCCP-1 µM); rotenone – 1 µM; sodium azide – 20 mmol. The oxidation substrates were: glucose – 15 mmol; pyruvic acid – 10 mmol; malate – 1 mmol; succinate – 10 mmol; ascorbate – 2 mmol; ADP – 1 mmol; N, N, N ‘, N’-tetramethyl-1,4-phenylenediamine (TMPD- 0.5 mmol). The overall assessment of mitochondrial function was determined by the level of oxygen consumption in the medium after sequential addition of oligomycin, FCCP and rotenone to the medium, and the ATP-generating ability was determined (by the difference in oxygen consumption after the addition of FCCP and oligomycin); the maximum level of respiration (according to the difference in oxygen consumption after the addition of FCCP and rotenone) and the respiratory capacity (according to the difference in oxygen consumption after the addition of FCCP and the basal level of oxygen consumption). The activity of glycolysis processes was evaluated when glucose was used as an oxidation substrate during the registration of oxygen consumption under the conditions of sequential addition of glucose, oligomycin and sodium azide to the medium. The intensity of glycolysis was determined according to the difference in oxygen consumption after adding glucose and the basal level of oxygen consumption; the intensity of glycolytic capacity was determined according to the difference in oxygen consumption after adding oligomycin and glucose; and the intensity of glycolytic reserve was determined according to the difference in oxygen consumption after adding glucose and sodium azide. Additionally, the activity of complexes I, II, IV, and V of the mitochondrial respiratory chain was evaluated. The activity of complex I was determined by the difference in oxygen consumption after adding the malate / pyruvate and rotenone mixture to the medium. The activity of complex II was evaluated by the difference in oxygen consumption after adding succinate and oligomycin to the medium. The activity of complex IV was determined by the difference in oxygen consumption after adding the mixture of rotenone / TMPD / ascorbate and sodium azide to the medium. The activity of complex V was evaluated by the difference in oxygen consumption after adding rotenone and ADP to the medium. During the analysis, the biosample volume was 275 µl, and 25 µl of injected analyzers. The oxygen consumption was determined in ppm [19].

ELISA – study

In this study, the concentration of ATP, mitochondrial ATP synthetase-(mATP), cytochrome C oxidase (CoX), and NADP oxidase 4 (NOX4) were determined by ELISA in the supernatants of the myocardial, brain and muscle tissues. We used species-specific sets of reagents produced by *Cloud clone corp.* (USA). The sample preparation and the course of the analysis corresponded to the instructions attached to the enlistment.

Statistical analysis methods

Statistical analisys of the obtained results was performed using the stat-analysis package STATISTICA 6.0. The data were presented as M ± SEM. The comparison of medium groups was performed using the ANOVA method with the post-test of Newman-Keuls at $p < 0.05$.

RESULTS

During the overall assessment of the mitochondrial respiratory function under conditions of various pathologies, it was found out that in rats with TBI and cerebral ischemia (Fig. 1), compared with the intact animals, there was a decrease in ATP-generating ability of mitochondria by 1.75 times ($p < 0, 05$) and by 4.6 times ($p < 0.05$), respectively. A decrease in the maximum level of respiration and respiratory capacity relative to intact rats was also noted in animals with cerebral ischemia by 2.85 times ($p < 0.05$) and by 2.13 times ($p < 0.05$), respectively. Against the background of the experimental traumatic brain injury, the animals compared to intact rats, showed a decrease in the maximum level of respiration by 1.77 times ($p < 0.05$) and respiratory capacity by 3.92 times ($p < 0.05$).

Under conditions of myocardial infarction (Fig. 2) in rats, there was a decrease in ATP-generating activity, the maximum level of respiration and respiratory capacity relative to the group of intact animals by 2.27 times ($p < 0.05$); by 2.98 times ($p < 0.05$) and by 2.78 times ($p < 0.05$), respectively.

In rats, against the background of muscle dysfunction (Fig. 3) compared with intact animals, a decrease in the maximum level of respiration, ATP-generating activity and respiratory capacity was observed by 3.28 times ($p < 0.05$); by 4.62 times ($p < 0.05$) and by 2.13 times ($p < 0.05$), respectively.

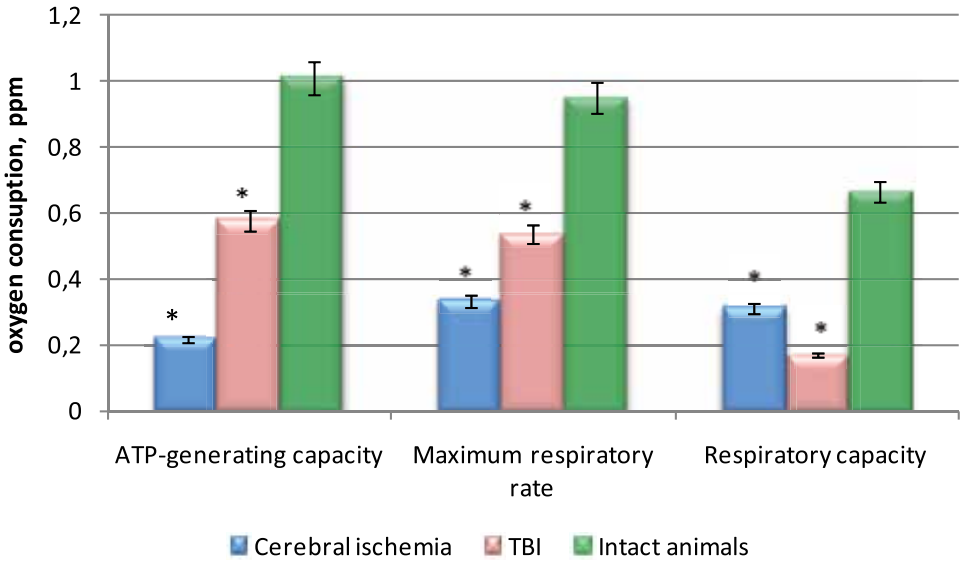

Figure 1. General assessment of mitochondrial respiration function under conditions of cerebral ischemia and traumatic brain injury

*Note: * – statistically significant relative to the group of intact animals (Newman-Keuls test, p <0.05)*

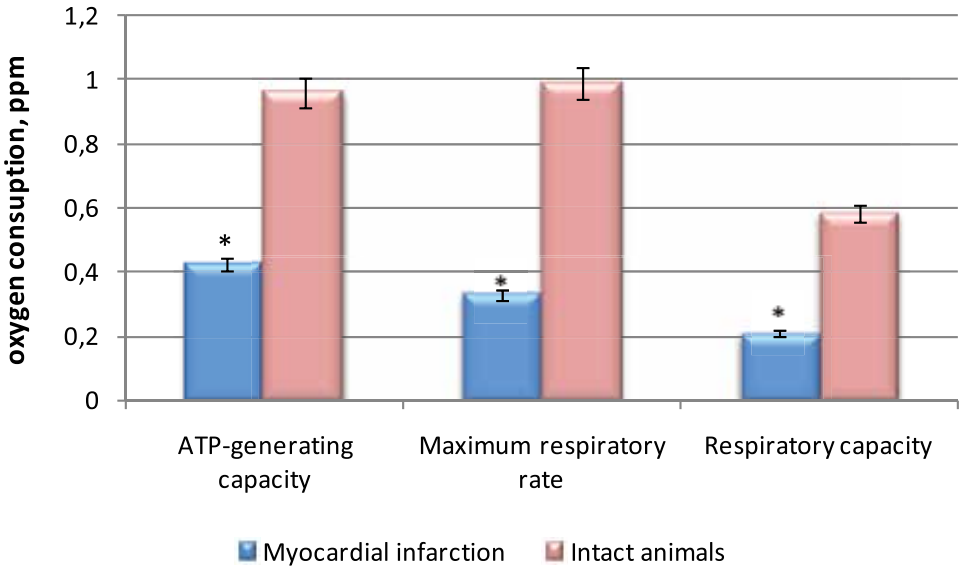

Figure 2. General assessment of the mitochondrial respiration function in experimental myocardial infarction

*Note: * – statistically significant relative to the group of intact animals (Newman-Keuls test, p <0.05)*

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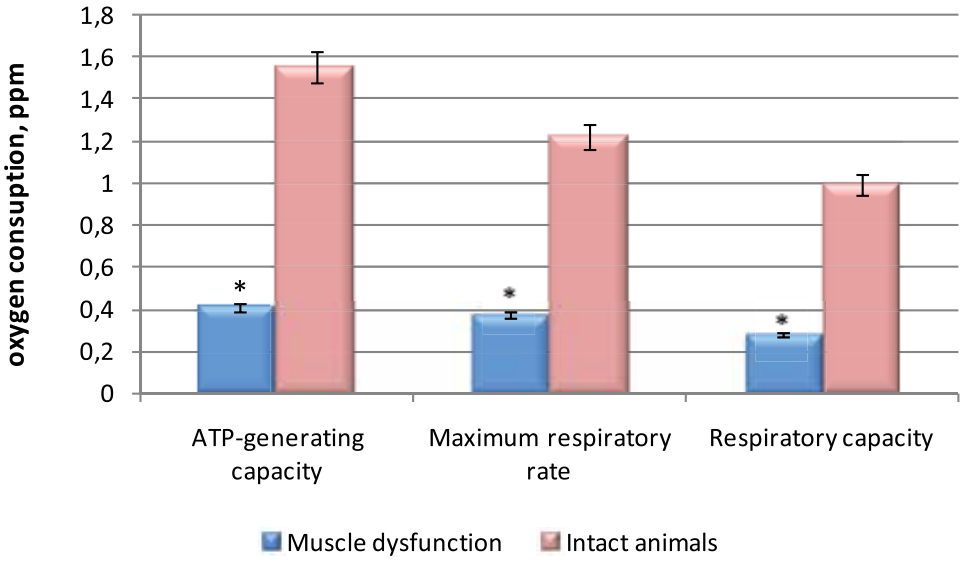

Figure 3. General assessment of mitochondrial respiration function under conditions of muscle dysfunction

*Note: * – statistically significant relative to the group of intact animals (Newman- Keuls test, p <0.05)*

When assessing glycolytic processes under conditions of various pathologies it was found out that in animals with TBI and cerebral ischemia (Fig. 4) there was an increase in glycolysis intensity compared to the group of intact animals by 18.04 times (p <0.05) and by 23.89 times (p <0.05), respectively. At the same time, in rats with experimentally reproduced cerebral ischemia, a decrease in glycolytic capacity relative to the group of intact animals was observed by 4 times (p<0.05), and the level of glycolytic reserve got a negative value (Fig. 2). Against the background of TBI in rats, in comparison with the intact group of animals, the glycolytic capacity and glycolytic reserve decreased by 22.6 times (p <0.05) and by 6 times (p <0.05), respectively.

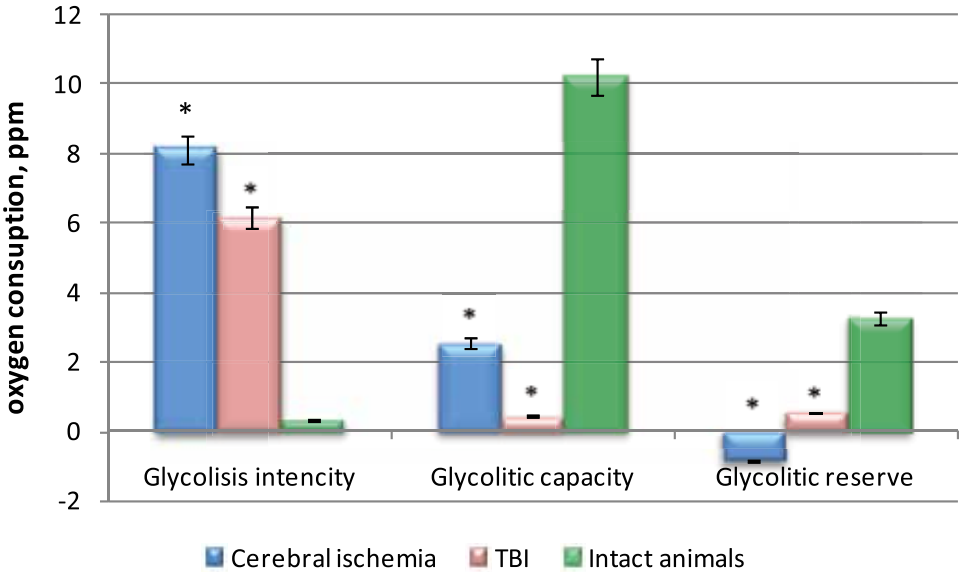

Figure 4. Assessment of changes of the glycolysis process in experimental cerebral ischemia and traumatic brain injury conditions

*Note: * – statistically significant relative to the group of intact animals (Newman- Keuls test, p <0.05)*

Under conditions of myocardial infarction in experimental animals (Fig. 5), the intensity of glycolysis processes exceeded that of the intact group of animals by 17.3 ($p < 0.05$) times, against the background of a decrease in glycolytic capacity and glycolytic reserve by 9.25 times ($p < 0.05$) and by 37.28 times ($p < 0.05$), respectively.

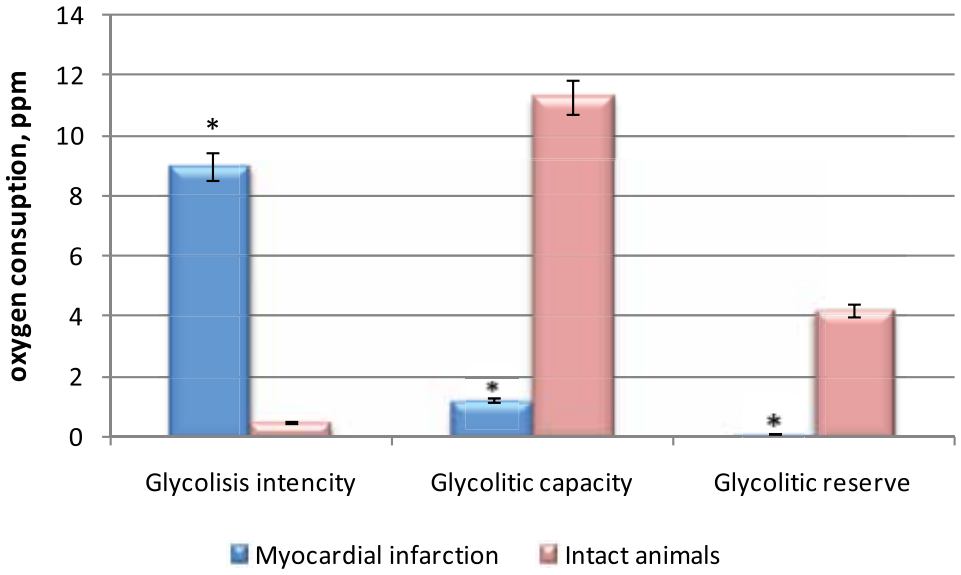

Figure 5. Assessment of changes in the glycolysis process in myocardial infarction conditions

*Note: * – statistically significant relative to the group of intact animals (Newman- Keuls test, $p < 0.05$)*

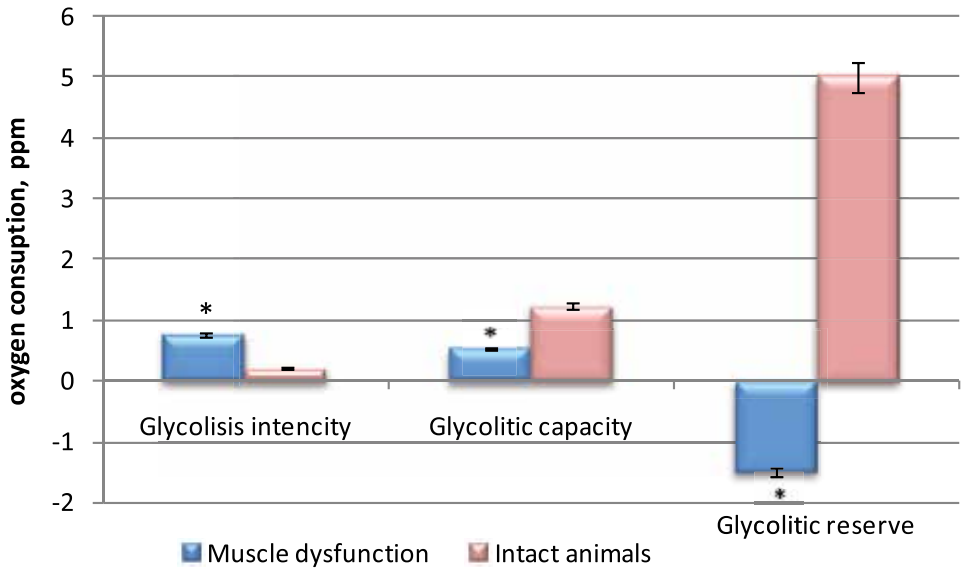

Figure 6. Assessment of changes in the glycolysis process in muscle dysfunction conditions

*Note: * – statistically significant relative to the group of intact animals (Newman- Keuls test, $p < 0.05$)*

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In rats, against the background of muscle dysfunction (Fig. 6), in comparison with intact animals, an increase in glycolysis intensity was observed, as well as a decrease in glycolytic capacity by 3.55 times (p <0.05) and by 2.35 times (p <0.05) , while the value of the glycolytic reserve took a negative value.

Evaluating the change in the activity of the mitochondrial respiratory chain complexes, it was found out that in rats under conditions of cerebral ischemia (Fig. 7) a decrease in the activity of mitochondrial complexes I, II, IV and V was observed in comparison with the intact group of rats by 4.8 (p <0.05 a) times; by 4.6 times (p <0.05); by 13.4 times (p <0.05) and by 9.33 times (p <0.05, respectively. Against the background of experimentally modeled TBI (Fig. 7), in animals relative to the intact group of rats, a decrease in the activity of complex I by 2.17 times (p <0.05), complex II – by 4.8 times (p <0.05), complex IV – by 11.1 times (p <0.05) and complex V – 8.1 by times (p <0.05) was observed.

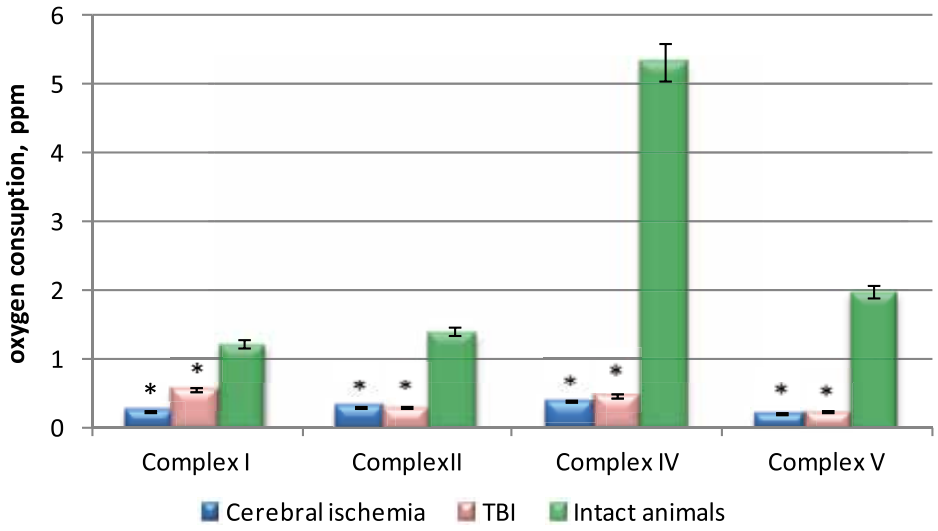

Figure 7. Evaluation of changes in the activity of the mitochondrial respiratory chain complexes under conditions of experimental cerebral ischemia and traumatic brain injury

*Note: * – statistically significant relative to the group of intact animals (Newman- Keuls test, p <0.05)*

Under conditions of myocardial infarction (Fig. 8), the animals showed a decrease in the activity of mitochondrial complexes I, II, IV and V in comparison with intact animals by 3.3 times (p <0.05); by 3.4 times (p <0.05); by 11.1 times (p <0.05) and by 7.5 times (p <0.05), respectively.

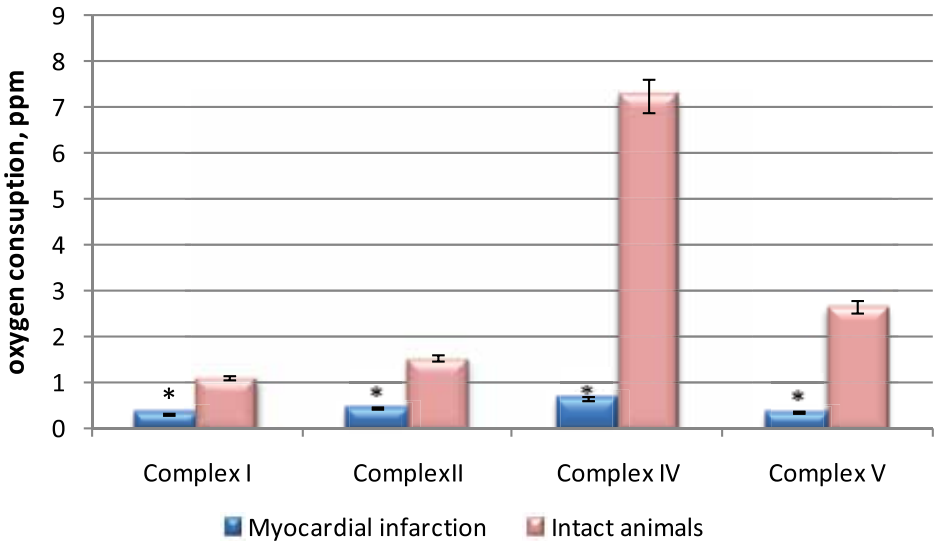

Figure 8. Evaluation of changes in the activity of the mitochondrial respiratory chain complexes under conditions of experimental myocardial infarction

*Note: * – statistically significant relative to the group of intact animals (Newman- Keuls test, p <0.05)*

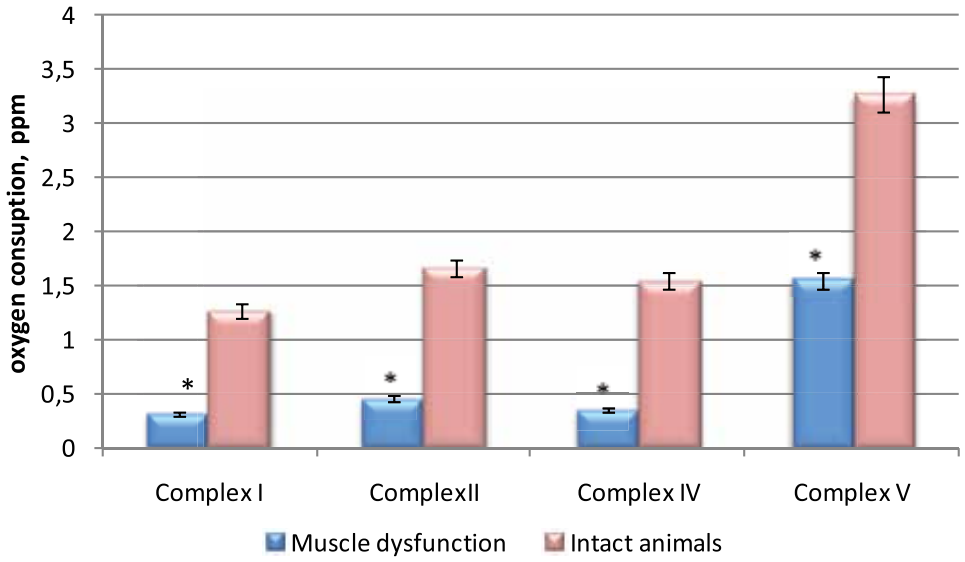

Figure 9. Evaluation of changes in the activity of the mitochondrial respiratory chain complexes under conditions of experimental muscle dysfunction

*Note: * – statistically significant relative to the group of intact animals (Newman- Keuls test, p <0.05)*

In animals with muscle dysfunction (Fig. 9), the activity of the respiratory complexes I, II, IV and V was 4 times lower in comparison with the intact rats (p <0.05); 3.6 times (p <0.05); 4.3 times (p <0.05) and 2.1 times (p <0.05), respectively.

Assessing the change in the concentration of enzyme complexes characterizing mitochondrial function (Table 1), it was found out that NOX4 activity increases in groups of animals with model pathologies: cerebral ischemia, TBI, myocardial infarction and muscle dysfunction compared to the group of the intact rats by 15.8 times (p <0.05); by 10.2 times (p <0.05); by 9.2 times (p <0.05) and by 6.1 times (p <0.05), respectively. In animals with experimentally reproduced cerebral ischemia, a decrease in CoX and mATP activity was also observed relative to the group of the intact rats by 2.9 times (p <0.05) and by 3.4 times (p <0.05), respectively.

Table 1. Change in the concentration of mitochondrial function markers under conditions of various pathologies (ELISA study)

Group	NOX4, ng/ml	CoX, ng/ml	mATP, ng/ml	ATP ng/ml
Intact animals (Brain)	1.2±0.014	46.97±0.695	98.62±2.631	1172.34±10.291
TBI	12.23±0.237*	26.4±0.896*	36.3±1.917*	453.1±8.614*
Cerebral ischemia	18.1±0.331*	16.35±0.417*	29.1±1.118*	364.61±7.924*
Intact animals (Myocardium)	1,6±0.028	43.94±0.792	101.2±2.939	1233.1±9.144
Myocardial infarction	14.75±0.542*	28.6±0.991*	43.2±1.249*	662.4±5.271*
Intact animals (Muscle tissue)	2.65±0.634	48.91±0.541	109.24±1.712	1536.2±8.176
Muscle dysfunction	16.2±0.524±0.743*	27.5±0.335*	18.6±2.364*	379.65±6.928*

*Note: * – statistically significant relative to the group of intact animals (Newman-Keuls test, p <0.05)*

At the same time, in animals with TBI, the content of Cox and mATP, in comparison with intact rats decreased by 1.8 times (p <0.05) and 2.7 times (p <0.05), , respectively. Under the conditions of myocardial infarction (Table 1), the rats showed a decrease in CoX and mATP activity relative to the intact group of animals by 1.5 times (p <0.05) and by 2.3 times (p <0.05), respectively. Besides, against the background of experimental

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muscle dysfunction (Table 1), the content of these enzymes also decreased (compared to the intact group of rats: CoX – by 1.8 times ($p < 0.05$); mATP – by 5.9 times ($p < 0,05$)). It is quite important that the observed negative changes in mitochondrial function under conditions of cerebral ischemia, TBI, myocardial infarction and muscle dysfunction were accompanied by a decrease in ATP concentration relative to intact rats by 3.2 times ($p < 0.05$); by 2.6 times ($p < 0.05$); by 1.8 times ($p < 0.05$) and by 4 times ($p < 0.05$), respectively.

DISCUSSION

Currently, it has been established that a significant number of pathologies are associated with the development of mitochondrial dysfunction [18]. Mitochondrial dysfunction is an integral part of the etiopathogeneses of various diseases, however, mitochondrial dysfunction plays the most important role in the development and progression of pathologies of the brain, heart and skeletal muscles – most energy-intensive organs, functioning of which requires a constant sum of macroergs [19–21]. The present study focused on the evaluationt of mitochondrial respiration function under conditions of ischemic genesis pathologies, in which there is a significant energy deficit that directly characterizes the activity of mitochondria – focal ischemia, brain injury, myocardial infarction and muscle dysfunction [22]. The study has shown that under conditions of model pathologies, there is a significant deterioration in the ATP-synthetic ability of mitochondria, which reflects a decrease in the maximum level of respiration, respiratory capacity and ATP-generating ability of mitochondria in comparison with intact animals [23]. At the same time, it is important that the decrease in the ATP-synthesizing function of mitochondria was accompanied by the intensification of glycolysis processes which was not compensated, and had a maximum permissible nature. It can be judged by a significant decrease in glycolytic capacity, glycolytic reserve and ATP concentration in the animals with model pathologies relative to the intact rats [24]. In addition, dysfunction of mitochondrial complexes I, II, IV and V was observed in animals against the background of the pathological processes of the brain, myocardium and muscles, which, ultimately, had a negative effect on the process of electron transfer in the mitochondrial respiratory chain [25]. NOX4 indicates a significant decrease in the electron transport potential of complexes I and II, with an increasing prooxidant potential of the cell [26]. It is known that when it is impossible to directly transport oxygen in the mitochondrial respiratory chain, the oxidizer is metabolized in an alternative way with activation of NADPH oxidase and in particular NOX4, resulting in a significant increase in the intracellular concentration of the superoxide radical that triggers oxidative stress [27]. Subsequently, the termination of electron transfer in complexes IV and V (confirmed by a decrease in CoX and mATP activity) hinders the conversion of ADP to ATP, and as a result, the total pool of high-energy compounds decreases, requiring an increase in glycolysis processes, which has been also established by this study and is consistent with the literature data [28].

CONCLUSION

Based on the data obtained, a significant deterioration of the mitochondrial respirometric function under conditions of ischemic brain, myocardial and skeletal musculature, accompanied by dissociation of electron transfer in the mitochondrial respiratory chain (dysfunction of complexes I, II, IV and V), decrease in ATP-synthesizing ability of mitochondria and the reinforcement of the glycolysis processes of a limiting nature can be supposed. In this case, probably, the correction of the mitochondrial respiratory function may be a new strategy for the treatment of ischemic conditions, which allows the targeted therapeutic effect to level the energy deficit and the mechanisms of cellular damage associated with it under ischemia.

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Conflict of interest

The authors declare no conflict of interest.

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RESEARCH ARTICLE

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INVESTIGATION OF MEDICALLY INDUCED SKIN REACTIONS BASED ON THE ANALYSIS OF REPORTS OF ADVERSE DRUG REACTIONS IN THE REPUBLIC OF CRIMEA (FROM 2009 TO 2016)

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Drug hypersensitivity reactions are among the most important problems that arise when using drugs. The occurrence of such reactions in the population is at least 7% and tends to a constant increase. The most frequent manifestations of drug hypersensitivity reactions are medically induced skin lesions. ***The aim*** *of this research was to study and analyze the cases of development of skin drug reactions on the basis of the reports on the adverse reactions (ADRs) of the drugs, registered in the Republic of Crimea in the period from 2009 to 2016.* ***Materials and methods.*** *The objects of the research were report cards about the adverse reactions, registered in the regional base (registry) of spontaneous messages called ARCADe (Adverse Reactions in Crimea, Autonomic Database) for the period from 2009 to 2016. During the analysis of the report cards, 2,698 cases of the development of skin drug reactions arising in response to the use of drugs in patients were selected. The study of the frequency of occurrence of skin drug reactions in the application of various groups of drugs was carried out taking into account the codes of the Anatomical Therapeutic Chemical (ATC) Classification System of drugs of the World Health Organization (WHO).* ***Results.*** *Of the study showed that the development of skin drug reactions was most often associated with the use of antimicrobial agents for internal use, nonsteroidal anti-inflammatory drugs (NSAIDs), drugs for the treatment of diseases of the gastrointestinal tract and agents that affect the nervous system. Among the clinical manifestations of skin drug reactions, generalized and localized rashes prevailed, and itching and hyperemia of the skin were much less common in patients. The analysis of age categories showed that the most frequently medically induced reactions occurred in children from birth to 3 years, as well as in the age group of patients from 46 to 60 years. The risk factors identified in the course of the analysis, were female gender, early childhood and old age, as well as the presence of aggravated drug allergy history.* ***Conclusion.*** *Drug hypersensitivity reactions create certain difficulties in clinical practice related to the diagnosis, treatment and prophylaxis, and may also cause danger to health or life of patients. In this connection, the study of such adverse reactions is the most important task of practical health care and requires direct participation of doctors of all specialties.*

Keywords: *medicines, adverse reactions, drug hypersensitivity, medicinal skin lesions*

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ИЗУЧЕНИЕ ЛЕКАРСТВЕННО-ИНДУЦИРОВАННЫХ КОЖНЫХ РЕАКЦИЙ НА ОСНОВАНИИ АНАЛИЗА КАРТ-ИЗВЕЩЕНИЙ О НЕЖЕЛАТЕЛЬНЫХ РЕАКЦИЯХ ЛЕКАРСТВЕННЫХ СРЕДСТВ В РЕСПУБЛИКЕ КРЫМ ЗА 2009–2016 ГГ.

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Реакции лекарственной гиперчувствительности (РЛГ) являются одними из наиболее важных проблем, возникающих при применении лекарственных препаратов. Встречаемость подобных реакций в популяции составляет не менее 7% и имеет тенденцию к постоянному росту. Наиболее частыми проявлениями РЛГ являются лекарственно-индуцированные поражения кожи. ***Целью настоящего исследования*** *явилось изучение и анализ случаев развития кожных лекарственных реакций (КЛР) на основании карт-извещений о нежелательных реакциях (НР) лекарственных средств (ЛС), зарегистрированных в Республике Крым за 2009–2016 гг.* ***Материалы и методы.*** *В работе были использованы данные карт-извещений о НР ЛС, зарегистрированных в региональной базе (реестре) спонтанных сообщений ARCADe (Adverse Reactions in Crimea, Autonomic Database) за период 2009–2016 гг. При проведении анализа карт-извещений было отобрано 2698 случаев развития КЛР, возникающих в ответ на применение у пациентов лекарственных препаратов. Изучение частоты встречаемости КЛР при применении различных групп ЛС проводилось с учетом кодов Анатомо-терапевтически-химической (АТХ) классификации лекарственных средств Всемирной Организации Здравоохранения (ВОЗ).* ***Результаты.*** *Развитие кожных лекарственных реакций наиболее часто было связано с применением противомикробных средств для внутреннего применения, нестероидных противовоспалительных средств (НПВС), препаратов для лечения заболеваний желудочно-кишечного тракта и средств, влияющих на нервную систему. Среди клинических проявлений кожных лекарственных реакций преобладали генерализованные и локализованные сыпи, значительно реже у пациентов наблюдались зуд и гиперемия кожных покровов. Анализ возрастных категорий показал, что наиболее часто лекарственно-индуцированные реакции возникали у детей с рождения до 3 лет, а также в возрастной группе пациентов от 46 до 60 лет. Выявленными в ходе проведения анализа факторами риска развития КЛР были женский пол, ранний детский и пожилой возраст, а также наличие у пациентов отягощенного лекарственного аллергологического анамнеза.* ***Заключение.*** *Реакции лекарственной гиперчувствительности создают определенные трудности в клинической практике, связанные с их диагностикой, лечением и профилактикой, а также могут представлять собой угрозу здоровью и жизни пациентов. В связи с чем, изучение таких НР является важнейшей задачей практического здравоохранения и требует непосредственного участия врачей всех специальностей.*

Ключевые слова: *лекарственные средства, нежелательные реакции, лекарственная гиперчувствительность, лекарственные поражения кожи*

INTRODUCTION

Drug hypersensitivity reactions are among the most important problems that arise when drugs are used. The frequency of such reactions in the population is about 7% and tends to a constant increase [1, 2]. Clinical manifestations of drug hypersensitivity reactions are allergic reactions of varying severity, which can occur directly to active ingredients or to auxiliary components of the drug [3, 4].

The most frequent manifestations of drug hypersensitivity reactions are medically induced skin lesions. According to the terminology worked out by the World Health Organization, a medically induced skin reaction is defined as any unintended and harmful morphological skin change that occurred during systemic or local use of the drug in usual doses for the purpose of prevention, treatment and diagnosis [4]. According to the literature data, the frequency of occurrence of such reactions is in the range of 2–3% in hospitalized patients [5], and among all detected adverse drug reaction (ADR) – from 19 to 48% [6,7]. At the same time, the relevance of studying skin drug reactions is caused not only by the high frequency of their development, but also by the severity and unpredictability of such reactions, which can cause a significant danger to health or life of patients.

THE AIM of this investigation was to study and analyze the cases of development of skin drug reactions on the basis of report cards of ADRs to the drugs regis-

tered in the Republic of Crimea in the period from 2009 to 2016, the identification of pharmacological groups of the drugs that cause skin drug reactions most often, and also the allocation of risk factors for the development of such adverse reactions.

MATERIALS AND METHODS

The objects of the research were the report cards about the adverse reactions, registered in the regional base (registry) of spontaneous messages called ARCADe (Adverse Reactions in Crimea, Autonomic Database) for the period from 2009 to 2016. When analyzing the report cards, 2,698 cases of development of skin drug reactions were selected in response to the use of drugs in patients. The criterion for the selection of cases of ADR was an indication of the skin manifestations of ADRs in the section "Standardized description of the reaction".

The study of the frequency of occurrence of skin drug reactions in the application of various groups of drugs was carried out taking into account the codes of the Anatomical Therapeutic Chemical (ATC) Classification System of drugs of the World Health Organization (WHO) [8].

The research methodology (the analysis of the registry data) did not imply making comparisons and determining the data correlations among themselves. ADRs frequency determination was performed in MS Excel 2016 Microsoft Office.

RESULTS AND DISCUSSION

For the analysis of cases of skin drug reactions arising in response to the use of different groups of drugs, 2,698 report cards were selected from the regional database – ARCADe – of spontaneous messages, which accounted for 43.1% of the total number of the registered ADRs for the corresponding period (6254 report cards).

The first stage of the research was to study the frequency of cases of the development of skin drug reactions when using drugs of different pharmacological groups. The analysis of the report cards showed that the most frequent skin drug reactions occurred when using drugs of the group of antimicrobial agents for systemic use (1215 cases), which accounted for 45% of the total number of medically induced skin reactions. Such adverse reactions occurred much less often when using drugs that affect the digestive system and metabolism (277 cases, 10.27%), drugs that affect the nervous system (239 cases, 8.85%), and drugs that affect the musculoskeletal systems, including nonsteroidal anti-inflammatory drugs (NSAIDs) (229 cases, 8.49%). The frequency of occurrence of skin drug reactions when using drugs of other pharmacotherapeutic groups in accordance with the WHO Anatomic Therapeutic Chemical Classification of Drugs is presented in Fig. 1.

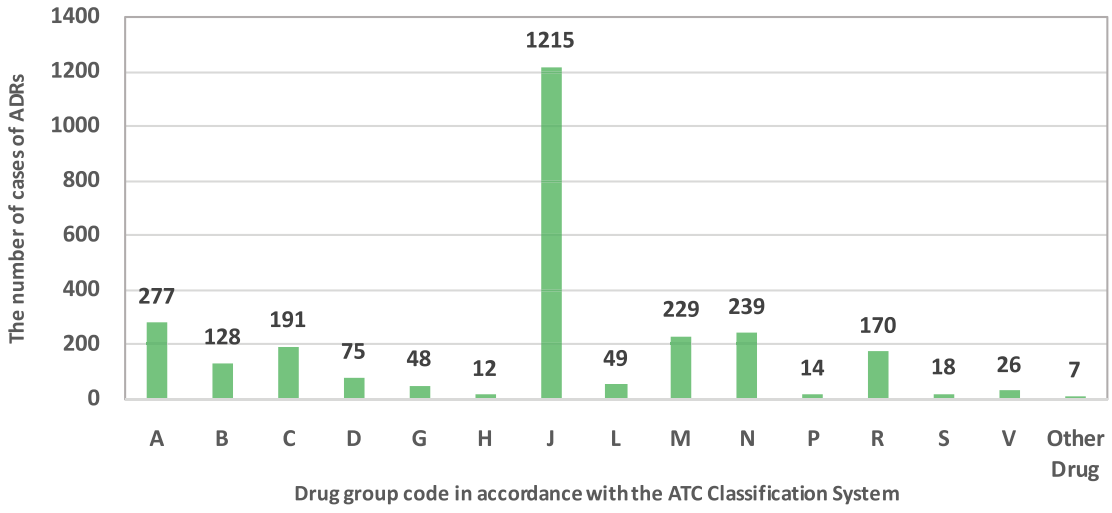

Figure 1. Distribution of drugs causing skin drug reactions, in accordance with the WHO ATC Classification System of Drugs

Studying the frequency of adverse reactions to individual groups of drugs that cause medically induced skin reactions showed that among the group of antibacterials for systemic use, such adverse reactions were most often associated with the use of J01 group (antibacterials for systemic use) – 1065 cases (87, 65% of the total number

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of cases of adverse reactions in this group). Less commonly, skin drug reactions were associated with the use of antivirals for systemic use (J05 – 72 cases, 5.9%) and antimycobacterials (J04 – 43 cases, 3.54%) (Fig. 2).

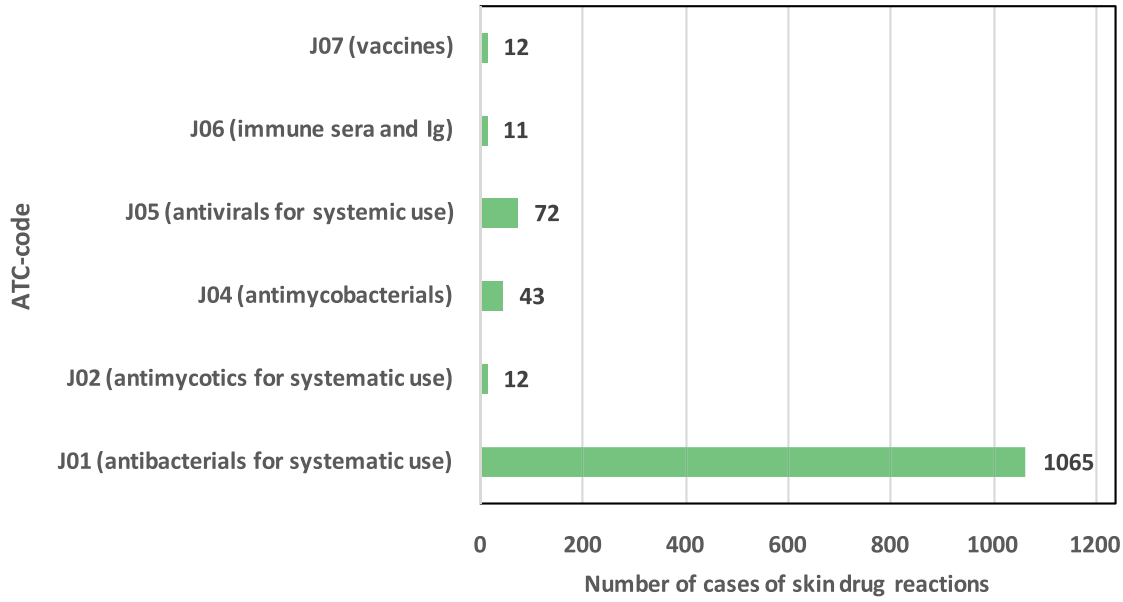

Figure 2. Distribution of cases of skin drug reactions in the group of antibacterials for systemic use

The analysis of skin drug reactions of individual groups for system use, confirms the known data on the high incidence of allergic reactions when using antibacterial drugs, including β-lactam antibiotics of the cephalosporins and penicillins groups [9–11]. In our case, the frequency of development of skin drug reactions when using these groups of drugs was 557 (52.3% of the total number of cases of adverse reactions to antimicrobial agents) and 207 cases (19.4%), respectively. The frequency of occurrence of drug-induced skin reactions of individual members of the β-lactamantibiotic groups is presented in Table 1. Other groups of antimicrobial agents caused allergic reactions much less frequently: fluoroquinolones – 122 cases of ADRs (11.5%), macrolides and azalides – 65 cases (6.1%), aminoglycosides – 40 cases (3.8%). Levofloxacin (45 cases of ADRs) and Ciprofloxacin (42 cases) became the "leaders" in the development of drug-induced reactions in the group of fluoroquinolones. In the group of Macrolide antibiotics, the development of skin drug reactions was most frequently recorded with Azithromycin (34 cases of ADRs) and Clarithromycin (13 cases). The high incidence of development of skin drug reactions in the above mentioned groups of drugs is most likely due to their frequent prescription by health care professionals, as well as the use of drug data by patients during self-treatment. [12].

The greatest number of cases of skin drug reactions in the use of antiviral drugs was caused by the use of drugs effective against HIV (non-nucleoside reverse transcriptase inhibitors (NNRTI) – 28 cases of ADRs, nucleoside reverse transcriptase inhibitors (NRTI) – 6 cases, protease inhibitors – 4 cases). Combined antiviral drugs that are effective against HIV, caused the development of ADRs in 9 cases.

Table 1. Frequency of occurrence of drug-induced skin reactions when using drugs of the ß-lactam antibiotics group (penicillins, cephalosporins)

Representatives of antimicrobial groups	The number of cases of skin drug reactions, the absolute value	The number of cases of skin drug reactions,% (relative to the total number of cases of skin drug reactions)
Penicillins		
Ampicillin (J01CA01)	5	0.2%
Amoxicillin (J01CA04)	72	2.7%
Benzylpenicillin (J01CE01)	4	0.15%
Ampicillinandbeta-lactamaseinhibitor (J01CR01)	15	0.6%
Amoxicillinandbeta-lactamaseinhibitor (J01CR02)	111	**4.1%**
Cephalosporins		
First-generation		
Cefalexin (J01DB01)	14	0.5%
Cefazolin (J01DB04)	13	0.48%
Second-generation		
Cefuroxime **(**J01DC02)	53	2%
Third-generation		
Cefotaxime (J01DD01)	152	**5.6%**
Ceftazidime (J01DD02)	12	0.4%
Ceftriaxone (J01DD04)	230	**8.5%**
Cefixime (J01DD08)	33	1.2%
Cefoperazone (J01DD12)	5	0.18%
Cefpodoxime (J01DD13)	16	0.6%
Ceftibuten (J01DD14)	1	0.04%
Ceftriaxone, combinations (J01DD54)	6	0.2%
Cefoperazone and beta-lactamase inhibitor (J01DD62)	9	0.3%
Fourth-generation		
Cefepime (J01DE01)	8	0.3%

Among the anti-TB drugs, the development of medically induced skin reactions in most cases (28 cases, 65% of the total number of cases for anti-TB drugs) was associated with the use of Pyrazinamide. Much less frequently, skin drug reactions occurred with the use of Isoniazid, Ethambutol and Rifampicin.

Special attention should be paid to the high incidence of medically induced skin reactions that occur when using NSAIDs (186 cases, which accounted for 6.9% of the total number of cases of skin drug reactions). In this pharmacological group, the most frequently similar ADRs were associated with the use of drugs derived from Propionic acid (106 cases) and Acetic acid (51 cases). Analysis of the frequency of skin drug reactions in the application of individual representatives of the groups of NSAIDs is presented in Fig. 3.

The largest number of skin drug reactions was registered with the use of Ibuprofen (99 cases, 53.22%), Diclofenac (31 cases, 16.7%), Ketorolac (18 cases, 9.7%) and Nimesulide (12 cases, 6.45 %).

The study of drug-producing countries that cause skin drug reactions revealed that in 911 cases the development of ADRs was associated with the use of drugs manufactured in Ukraine, in 309 cases in the territory of the Russian Federation. The development of ADRs against the background of the use of drugs produced by foreign pharmaceutical companies was observed slightly less. Thus, drugs produced in India caused 291 cases of skin drug reactions, in the UK –210 cases, in Germany – 177 cases of ADRs.

A study of the clinical manifestations of medically induced skin reactions showed that the most common forms were: generalized skin rashes (1673 cases of ADRs, 62%), localized skin rashes (713 cases of ADRs, 26.4%), localized and generalized skin itching (128 and 95 cases of ADRs, respectively), as well as skin redness (78 cases, 2.9%). Indicators of the occurrence of such manifestations in patients are presented in Table 2.

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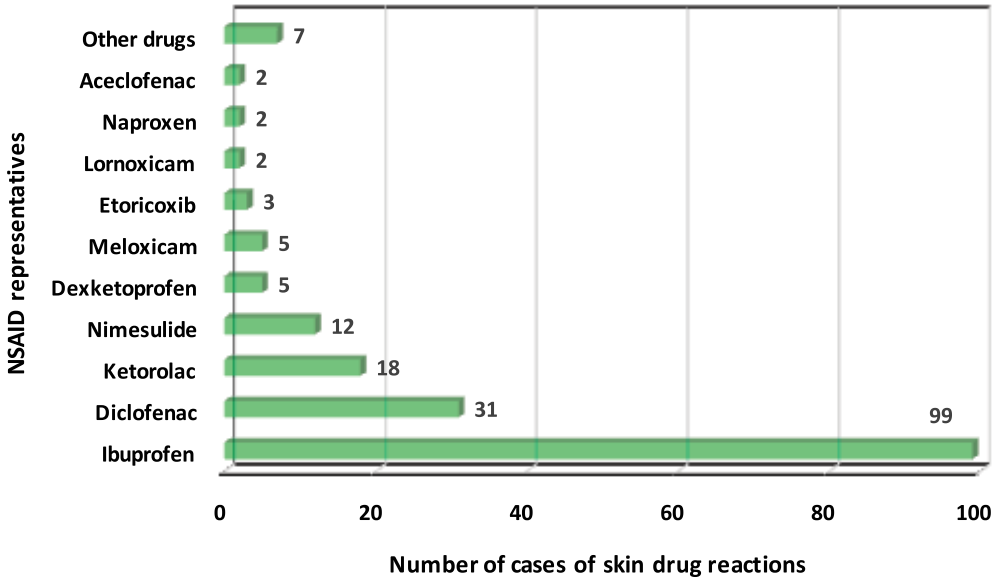

Figure 3. Frequency of skin drug reactions when using NSAIDs

Table 2. Frequency of various clinical manifestations of skin drug reactions

Clinical manifestations of skin drug reactions	Number of reported cases of ADRs	
	Number	%
Generalized rash	1673	62
Localized rash	713	26.4
Generalized pruritus	95	3.5
Localized pruritus	128	4.8
Skin hyperemia	78	2.9
Allergic toxic dermatitis	9	0.3
Photodermatitis	2	0.1

The analysis of the age periods for which the occurrence of ADRs in the form of skin manifestations was typical, showed the following results (Fig.4): the most frequent complications of pharmacotherapy were observed before the age of 3 years – 766 cases (from birth to 28 days – 42 cases of ADRs, 28 days – 1 year – 392 cases of ADRs, from 1 to 3 years – 342 cases) and in the age category «46-60 years» – 441 cases. It is worth noting that a rather high incidence of skin drug reactions was observed in the age periods of "19–30 years", "31–45 years" and "61–75 years" – 349, 354 and 331 cases of ADRs, respectively.

A study of the gender of the patients revealed that the most frequent skin drug reaction occurred in females – 1613 (60%) cases, in the remaining 1085 cases ADRs (40%) was observed in males.

Our further analysis was aimed at studying the routes of administration of drugs that cause medically induced skin reactions. So, the most frequent way of administering such drugs was their ingestion (per os) – 1326 cases (49.15%), less often ADRs occurred against the background of parenteral administration of drugs: intramuscularly – 535 cases (19.8%), intravenously – 510 cases (18.9%), subcutaneously – 32 cases (1.19%). Attention is drawn to the fact that in 139 cases (5.15%) skin drug reactions occurred with the external use of drugs, and in 52 cases (1.9%) with rectal administration.

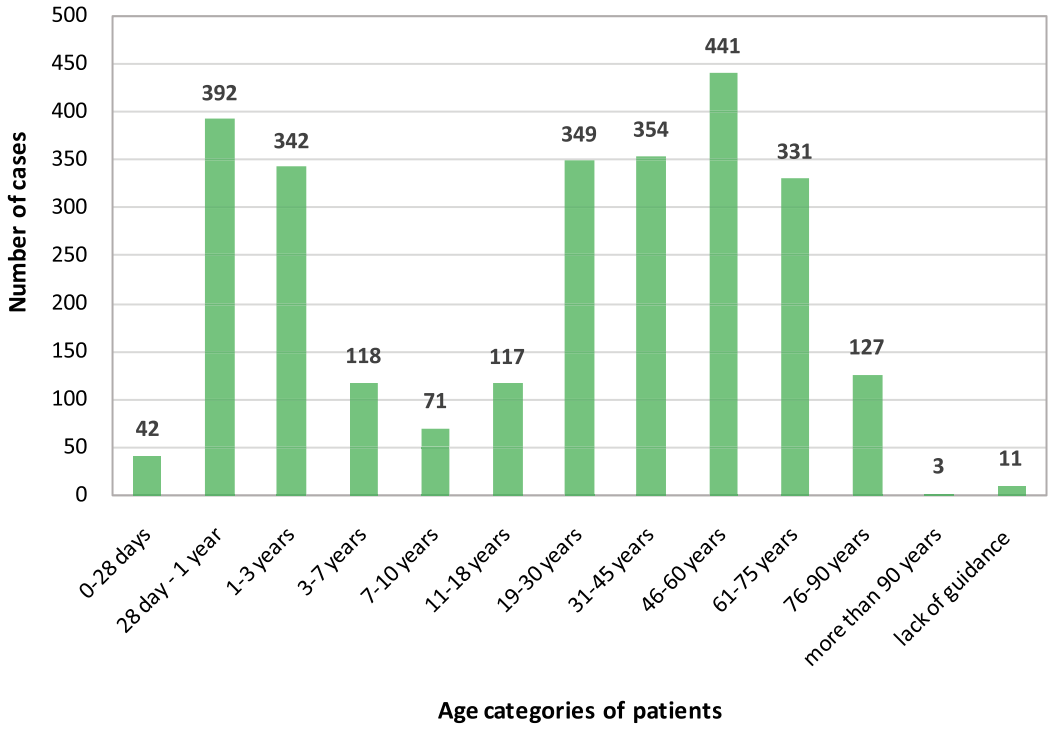

Figure 4. Distribution of patients with medically induced skin reactions by age

One of the most important factors determining the likelihood of adverse reactions of an allergic nature is the patient's history of hypersensitivity to drugs, food components, contact home allergens and other factors. A study of the allergic history in patients with skin drug reactions showed that in most cases it was calm (2399 cases, 88.92%), while in the other cases an aggravated allergic history was observed: drug allergy – 156 cases of ADRs (5.8%), food allergy – 80 cases of ADRs (3%), household (contact allergy) – 35 cases (1.3%), in 28 cases patients had mixed allergies (Fig. 5).

In addition, one of the significant factors necessary for assessing the cause-effect relationship between taking a suspected drug and the skin drug reaction that occurs is the number of drugs prescribed simultaneously with it. In our case, the frequency of polypragmasy (simultaneous administration of 5 or more drugs) amounted to 320 cases of development of a skin drug reaction. At the same time, simultaneous prescription of 4 drugs was observed in 162 cases (6%), 5 drugs – in 83 cases (3.1%), 6 drugs – in 40 cases (1.5%). The prescription of 7 or more drugs was observed in 35 cases, which could significantly increase the risk of adverse reactions (*Fig*. 6).

The course of immediate-type allergic reactions, the appearance of affected skin lesions are characterized by a high rate of development and unpredictability. In this respect, treatment is reduced, as a rule, to the abolition of the drug that caused an adverse reaction, and to the conduct of symptomatic therapy. The analysis of cases of skin drug reactions in the Republic of Crimea showed that the cancellation of suspected drugs was carried out in 2651 cases (98.26%), hereby in 2266 cases the cancellation of the suspected drug was accompanied by the disappearance of adverse reactions.

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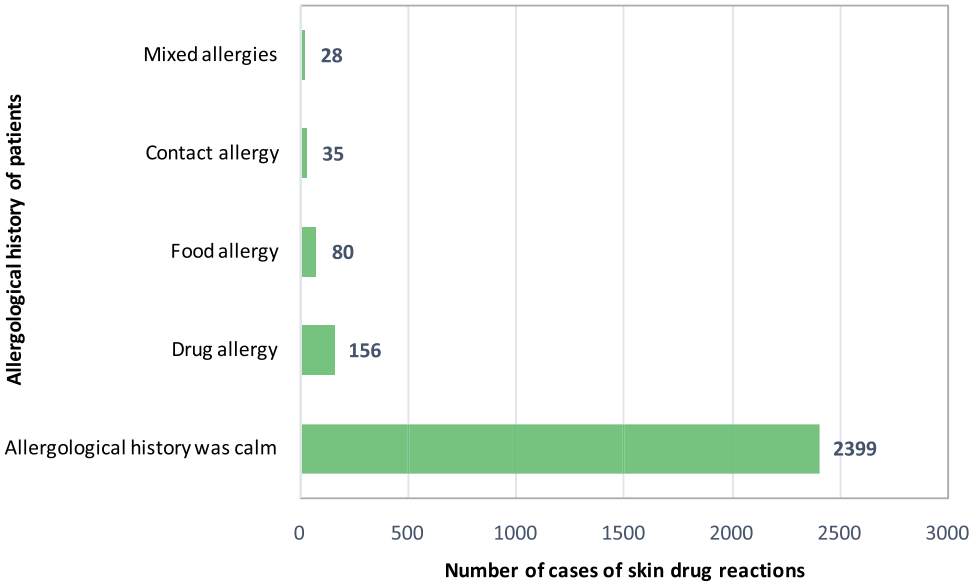

Figure 5. Analysis of the allergological history of patients with manifestations of skin drug reactions

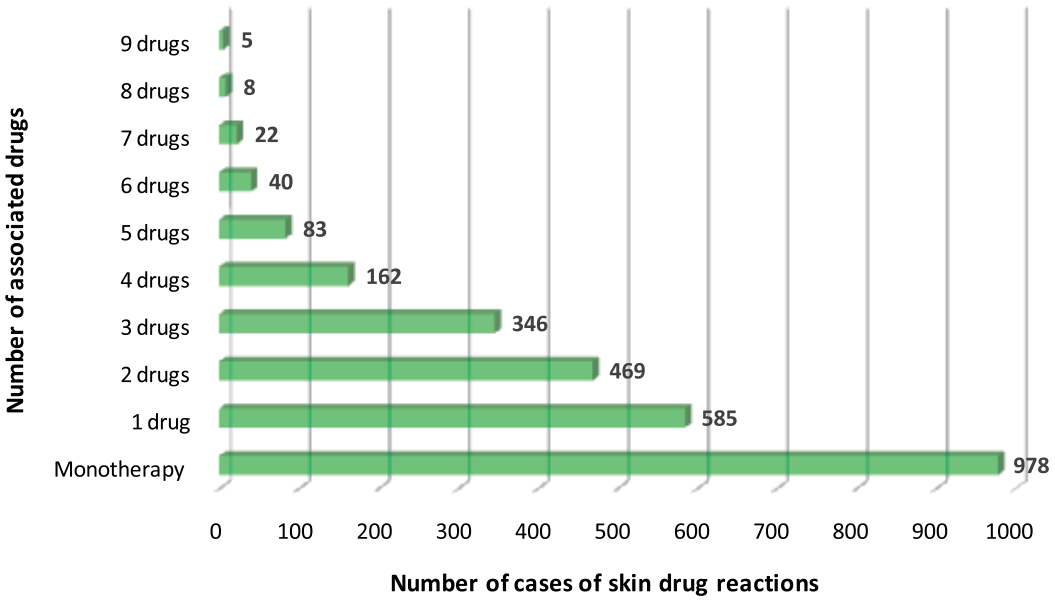

Figure 6. Analysis of report cards with manifestations of skin drug reactions by the number of associated drugs

Symptomatic therapy for the relief of skin drug reactions was observed in 2242 cases (83.1%), in other cases the patient did not need any additional pharmacotherapy.

The next stage of the study of skin drug reactions was aimed at analyzing the outcome of undesirable drug reactions. The results of the analysis showed that in most cases, skin drug reactions were not serious and did not cause pronounced consequences for patients (2009 cases, 74.5%). However, it is worth noting the frequency of serious consequences of ADRs for patients:

a life-threatening condition was observed in 32 cases (1.19%), hospitalization and extension of hospitalization of the patient's terms were required in 261 (9.7%) and 153 cases (5.7%) respectively. In 242 cases, the development of a skin drug reaction led to the patient's temporary disability (9%).

Thus, the results obtained during the analysis confirmed a high frequency of occurrence, severity and unpredictability of the occurrence of drug-induced skin reactions [13, 14]. Among all pharmacological groups of drugs, the most frequent skin drug reactions were associated with the use of antibacterials for systemic use, namely drugs of the cephalosporins and penicillins groups. Such high rates of development of ADRs when using these groups of drugs, could be associated not only with the presence of a β-lactam ring in their structure, which can covalently bind to serum proteins and the cell wall and cause the development of allergic reactions, but also with a significant frequency in clinical practice [15]. The risk factors for the development of skin drug reactions identified in the course of the analysis, were female gender (60% of cases), early childhood and old age, as well as the presence of burdened drug allergological history in patients.

CONCLUSION

At present, one of the priorities of the health care system is to monitor the effectiveness, safety and quality of drugs used in the stages of diagnosis, prevention and treatment of patients.

Sufficient knowledge of health professionals in the field of pharmacological safety of drugs will allow them not only to be prepared for the relief of serious unforeseen manifestations of skin drug reactions in the shortest possible time, but also to the possible prevention of life-threatening conditions associated with the use of drugs.

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Conflict of interest

The authors declare no conflict of interest.

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RESEARCH ARTICLE

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STIMULATION OF REPARATION IN A LINEAR WOUND MODEL IN RATS BY BISCHOFIT GEL

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The aim *of the article is to evaluate Bischofit gel reparative activity in a linear wound model in rats.* ***Materials and Methods.*** *The study was conducted on 36 male Wistar rats weighing from 193 to 218 grams. On the 8th day after modeling a linear wound defect 50±1 mm long, the reparative effect of bischofite, Actovegin and Contractubex in the gel compositions was evaluated. The evaluation was carried out using: the following methods: 1) studying the physicomechanical characteristics of the wound defect (a wound-tearing machine Metrotest REM-0.2-1); 2) morphological examination of the skin graft taken from the wound area (stained with hematoxylin-eosin and Van Gieson's solution); 3) determining the ratio of collagen types I and III in a polarizing microscope (the picrosirius was red); 4) colorimetric analysis of the hydroxyproline concentration in the wound surface tissues.* ***Results.*** *On the 8th day, the wound defects sampled from the bischofite treated animals, were characterized by the most pronounced strength (the average force at the rupture moment was 13.70 N), which was significantly higher ($p<0.01$) than in the control group (11.76 N). Actovegin showed less influence on this parameter (12.60 N), and the use of Contractubex led to its decrease (8.10 N). The effect of the drugs on the morphological state of the skin tissue was similar. The hydroxyproline concentration in the studied groups' samples was: Bischofit 13.23±1.68; Actovegin 15.89±1.37; Contractubex 17.61 ± 0.67; the Control was 16.59±1.08. According to the impact on the ratio of collagen in types I and III, the studied drugs were arranged in the following sequence: Bishofit (0.73±0.023) > Actovegin (0.67±0.017) > Control (0.56±0.012) > Contractubex (0.38 ±0.020).* ***Conclusion.*** *The carried out study showed that Bischofit has a pronounced ability to stimulate the regeneration of the skin wound defect. Hereby, the reference drug Actovegin showed less activity, and Contractubex worsened wound healing.*

Keywords: *bischofite, regeneration, Actovegin, Contractubex, hydroxyproline, collagen*

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СТИМУЛЯЦИЯ РЕПАРАЦИИ В МОДЕЛИ ЛИНЕЙНОЙ РАНЫ У КРЫС ГЕЛЕМ С БИШОФИТОМ

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Цель *– оценка репаративной активности геля с Бишофитом на модели линейной раны у крыс.* ***Материалы и методы.*** *Исследование было проведено на 36 самцах крыс линии Wistar массой 193–218 г. На 8-е сутки после моделирования линейного раневого дефекта длиной 50±1 мм оценивали репаративное действие Бишофита, Актовегина и Контрактубекса в составе гелей. Оценка проводилась с помощью: 1) изучения физико-механических свойств раневого дефекта (механический раноразрыватель Метротест РЭМ-0.2-1); 2) морфологического исследования тканей кожного лоскута, взятого из области раны (окраска гематоксилин-эозин и Ван Гизон); 3) определения соотношения коллагена I и III типов в поляризационном микроскопе (окраска пикросириус красный); 4) колориметрического анализа концентрации гидроксипролина в тканях раневой поверхности.* ***Результаты.*** *На 8-е сутки наибольшей прочностью характеризовались раневые дефекты, полученные от животных с применением Бишофита (среднее усилие на момент разрыва 13,70 Н), что достоверно выше (p<0,01), чем в контрольной группе (11,76 Н). Актовегин повлиял на данный параметр в меньшей степени (12,60 Н), а Контрактубекс привел к его снижению (8,10 Н). Влияние препаратов на морфологическую картину тканей кожи было аналогичным. Содержание гидроксипролина в образцах исследуемых групп составило: Бишофит – 13,23±1,68; Актовегин – 15,89±1,37; Контрактубекс –17,61±0,67; Контроль – 16,59±1,08. По влиянию на соотношение коллагена I и III типов исследуемые препараты располагались в следующей последовательности: Бишофит (0,73±0,023) > Актовегин (0,67±0,017) >Контроль (0,56±0,012) > Контрактубекс (0,38±0,02).* ***Заключение.*** *Проведенное исследование показало, что Бишофит обладает выраженной способностью стимулировать регенерацию раневого дефекта кожи. При этом препарат сравнения Актовегин продемонстрировал меньшую активность, а Контрактубекс ухудшил ранозаживление.*

Ключевые слова: *Бишофит, регенерация, Актовегин, Контрактубекс, гидроксипролин, коллаген*

INTRODUCTION

Despite the rapid development of streamlined synthesis, the emergence of highly selective drugs and biological therapy, simpler, multitarget compounds do not lose their relevance [1]. One of these tools is a gel based on Bischofit. Its natural mineral resource is presented in the territory of the Lower Volga region. For a long time, Bischofit has been used in clinical practice to treat a wide range of pathologies. The pharmacological activity of this mineral, including the gel form, has been studied in detail for several decades [2, 3]. Bishofit has proved to have anti-inflammatory and immunomodulatory activity, as well as accelerating regenerative processes [4–7].

MATERIALS AND METHODS

Animals

The study included 36 male Wistar rats weighing from 193 to 218 grams. The rats obtained from the mouse bank of "Stolbovaya" (Moscow region) were used as laboratory animals. All manipulations performed on the individuals were performed in accordance with international norms of experimental ethics (European Convention for the Protection of Vertebrate Animals used for experimental and other scientific purposes (Strasbourg, 22 June, 1998)) and with the requirements of good laboratory practices (GLP). The animals were placed in macrolon cells with lattice steel lids and a forage well. The litter material was non-coniferous sawdust. During the experiment all the rats were kept in standard vivarium conditions (humidity 65 ± 5%, temperature 22 ± 2ºC). Individuals were under natural light with free access to food and water. The cages, bedding and drinkers changed as they became soiled.

Study design

Under anesthesia (chloral hydrate 300 mg/kg) after preliminary depilation (80×45 mm) and treatment with an antiseptic (70% solution of ethyl alcohol) in the dorsal area, a linear wound 50±1 mm long was modeled by cutting the skin along the paravertebral line with a blade with a depth limiter of 2 mm, after which the edges of the wound were brought together by imposing three sutures with sterile threads [8].

Then the animals were divided into 4 equal groups:

I – *Control group* – imitation of rubbing the drug on the shaved area 10 minutes after the wound modeling and for the next 6 days (once a day)

II – *Bischofit* – rubbing 500 mg of gel with Bischofit on the wound area and adjacent tissues 10 minutes after modeling the wound and for the next 6 days (once a day)

III – *Actovegin* – rubbing 500 mg of Actovegin gel on the wound area and adjacent tissues 10 minutes after modeling the wound and for the next 6 days (once a day)

IV – *Contractubex* – rubbing 500 mg of Cotractubex gel on the wound area and adjacent tissues 10 minutes after modeling the wound and for the next 6 days (once a day)

After natural predrying of the application area, the animals were placed in individual cages. In the next 6 days, in addition to applying the gels, the clinical condition, motor activity, feed and food consumption, as well as photographic images of the wound area were assessed. On the 8th day, the animals were removed from the experiment by the method of cranial dislocation under anesthesia, after which 4 skin grafts (the total surface was 25×45 mm) were sampled from the dorsal surface for research (Fig. 1).

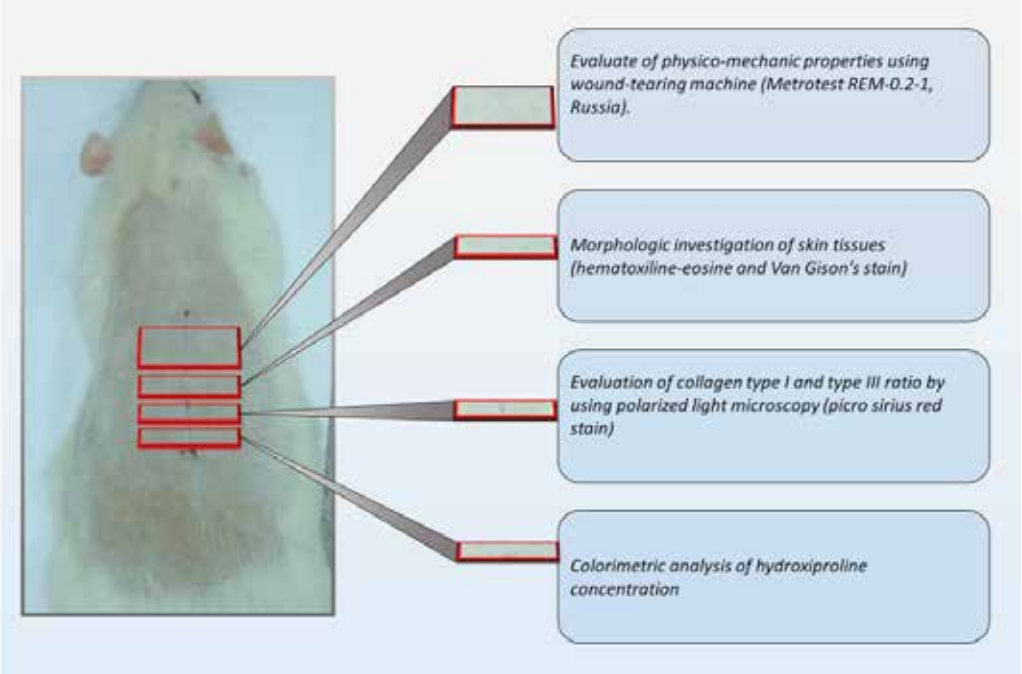

Figure 1. Schematic representation of skin areas sampled for assessment of the investigated drugs reparative effect

1. The study of physical and mechanical characteristics of the wound defect was performed using a wound-tearing machine. The cut skin fragment was fixed in a special installation with the help of threads and metal spokes. After launching the device, the force (discreteness=0.1 N), necessary for tearing tissues along the wound line, was monitored. The ultimate deformation data (stretching at the rupture moment) of the skin flap were also obtained. This parameter represents the elasticity of the wound defect.

2. Calorimetric analysis of hydroxyproline concentration in the wound defect tissues. To assess the degree of reparative reaction in the tissues, the concentration of hydroxyproline (HP) as the basic amino acid of collagen was determined. HP is formed as a result of the cotranslational hydroxylation of proline by the enzyme proline-hydroxylase, which occurs even before the synthesis of the polypeptide chain is completed [9].

To determine the HP concentration in the samples, a calorimetric method of detecting the reaction products of oxidized HP and Ehrlich reagent [10] was used. In the process of the sample preparation, round skin areas without underlying tissues with a diameter of 5 mm and including all the layers were taken from the euthanized animals using the Dermal Punch tool (USA). The samples were frozen in liquid nitrogen by immersion for 1–2 seconds and stored at minus 72°C in sealed Eppendorf tubes.

On the day of the study, the samples were thawed for 3–5 hours in the open air at the room temperature. The samples were weighed and cut so that the weight of one of the fragments was about 20 mg. Then hydrolysate was prepared from the samples. To determine HP, 1 ml of chloramine B was added to 1 ml of hydrochloric acid solution 36%, shaken and kept for 20 minutes at the room temperature. 1 ml of perchloric acid was added, shaken again and 1 ml of a 20% solution of Ehrlich reagent was

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added. The tubes were shaken again and placed into a water bath (60°C) for 20 minutes. Then the reaction was terminated by immersing the tubes into an ice bath and adding 5 ml of ethyl cellosolve. The optical density was determined at the wavelength of 557 nm. For the preparation of standards, crystal HP manufactured by Sigma-Aldrich (USA) was used.

3. Morphological investigation of the skin graft tissues taken from the wound area were carried out in a standard way. The samples were fixed with 10% buffered formalin. The cut-sections were stained with hematoxylin and eosin and Van Gieson's solution. Staining with hematoxylin and eosin makes it possible to carry out a general assessment of the histological picture, and staining by Van Gieson's solution makes it possible to carry out a detailed study of the connective tissue architectonics, differentiating between mature and immature collagen. Then the received preparations were assigned code names for an independent assessment by an expert commission consisting of 5 doctors of the pathoanatomical bureau from the Belgorod regional clinical hospital n. a. Saint Joseph (Russia, Belgorod). The assessment was made according to the specially developed scale (Table 1).

Table 1. Scale for assessing the reparative activity of the studied drugs using the histological picture of the wound defect area

Qualitative character	Points and their characteristics			
Cytoarchectonics disruption	0 –None	1 – Low-grade	2 – Obvious	3 – Florid
Architectonics disruption of intracellular matrix	0 – None	1 – Low-grade	2 – Obvious	3 – Florid
Hemorrhage, enlarged vessels	0 – None	1 – Low-grade	2 – Obvious	3 – Florid
Violation of epithelialization	0 – None	1 – Low-grade	2 – Obvious	3 – Florid
Leukocyte infiltration	0 – None	1 – Low-grade	2 – Obvious	3 – Florid

4. Assessment of the ratio in collagen I and III types in a polarizing microscope

To assess the viability of the reparative process, the ratio in collagen types I and III was determined, since the predominance of mature (I) collagen over the immature one (III) indicates a normal regeneration of the wound. To quantify the ratio of mature (I) and immature (III) types of collagen, the sections were stained with picrosirius red, then microscoped in a polarization microscope and photographed. For each cut-section, 10 fields of view were photographed at x400 magnification. The color ratio of the differential coloration was established by automatically analyzing color histograms for each of the microphotographs using the image J program and subsequent statistical processing. A lower ratio indicates a higher proportion of immature type III collagen [11].

Statistical processing of the obtained data was performed using STATISTICA 10.0 software. Descriptive statistics was applied to all the data.

The normality of distribution was determined using Shapiro-Wilk and Kolmogorov-Smirnov criteria. The statistical significance of the differences was carried out using Newman-Keuls test depending on the nature of the data was carried out using the Student's and Mann-Whitney tests with the Bonferroni correction. The differences were recognized statistically significant at p£0.05.

RESULTS AND DISCUSSION

After recovery and on further days of the study, the animals were active, the consumption of feed and food was within the normal range. There were no purulent complications, hemorrhages, excoriations and other unwished effects. By day 7, the greatest visual differences had been observed between the animals treated with Bischofit gel and the Control group (Fig. 2).

A ***B*** ***C***

Figure 2. General view of the animals immediately before euthanasia

Note:
A – a group of animals treated with Actovegin gel;
B – a group of animals treated with Contractubex gel;
C – a group of animals treated with Bischofit gel

Determination of physicomechanical characteristics of the wound defect

When determining the force at the rupture point using a wound-tearing machine (Metrotest REM-0.2-1, Russia), it was found out that the average force required to rupture a skin flap along the wound defect in the control group was 11.76±0.71 N. The greatest strength of the wound defect can be positioned as follows (in descending order): gel with Bischofit (13.70±0.76 N, p<0.01); Actovegin (12.60±0.63 N, p<0.05); Contractubex (9.65±0.59 H, p<0.01) (Fig. 3).

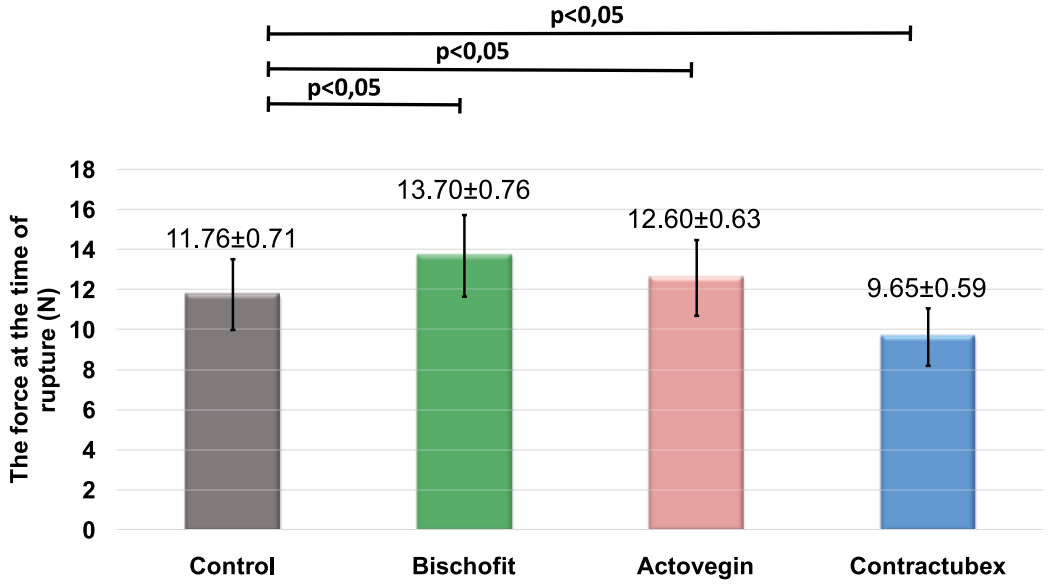

Figure 3. The results of determining physicomechanical characteristics of the wound defect. The force at the rupture point (N) in assessing the strength of a wound defect using a mechanical wound breaker (M±m)

When analyzing the ultimate deformation of the skin flap, it was detected that the increase in the length of the skin flap in the Control group at the rupture point was 8.0±1.7%. According to the effect of the preparations on the elasticity of the wound defect, they can be arranged as follows (in descending order): gel with Bischofit (10.7±2.3%); Actovegin (8.2±1.9%); Contractubex (8.1±1.7%). The statistical processing showed that this parameter (p<0.05) reliably differs from the Control group only in the group that received Bischofit gel (Fig. 4).

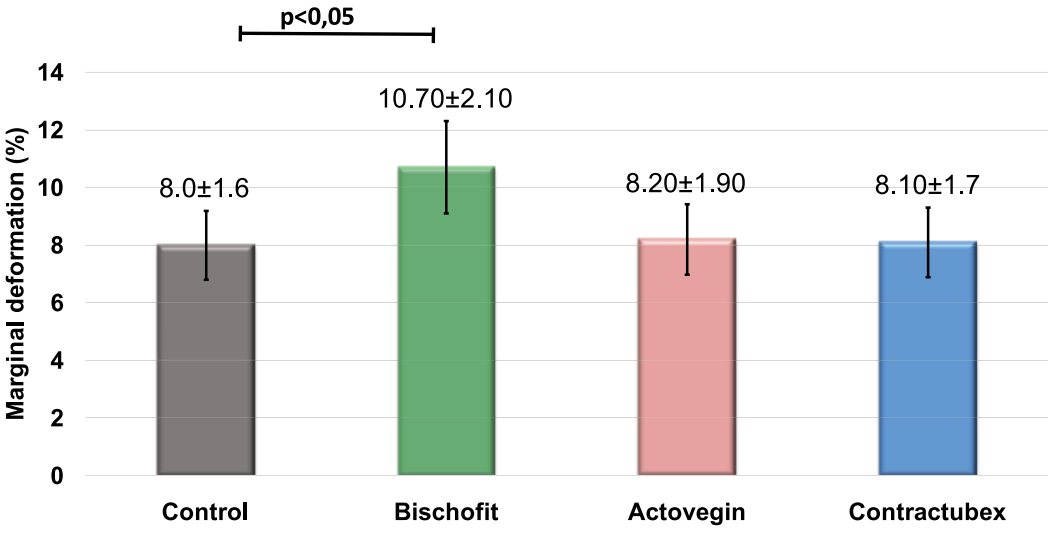

Figure 4. Results of determining physicomechanical characteristics of the wound defect. Elastic limit deformation (%) when evaluating elasticity of a wound defect using a wound-tearing machine (M±m)

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Colorimetric analysis of hydroxyproline concentration in the tissues of the wound defect

In colorimetric analysis it was found out that the highest concentration of hydroxyproline was in the tissues of wound defects in the animals treated with Contractubex. However, there was no statistically significant difference with the Control group. In comparison with the control concentration of HP ($p<0.05$), the tissues of the modeled wounds in the animals treated with Bischofit gel (79.7% of the control) contained significantly lower concentration of HP (Table 2).

Table 2. Concentration of hydroxyproline (HP) in tissue samples of wound defects obtained on day 8 after starting the experiment (M±m)

Group	Control	Bischofit	Actovegin	Contractubex
Concentration of HP, mg/g	16.59±1.08	13.23±1.68	15.89±1.37	17.61±0.67

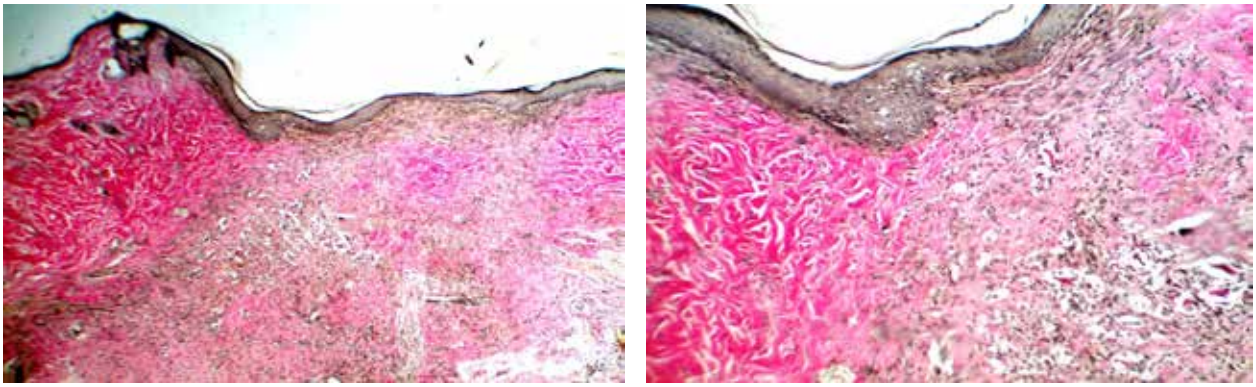
*Note: * – the presence of statistical significant differences when compared with the control group upon Mann-Whitney criterion ($p\leq0,05$)*

Taking into account the data obtained in determining the physicomechanical characteristics of the wound defect, the probable cause of an increase in the concentration of HP in the tissues of the animals treated with Contractubex is safekeeping of the inflammatory reaction, the prolongation of the remodeling processes of the newly formed connective tissue and the growth of granulation tissue.

On the other hand, a decrease in the concentration of HP in the wound defects of the group treated with Bischofit gel, indicates a decrease in secondary alteration and an accelerated repair.

Morphological study of the tissues of the skin flap taken from the wound area

Control group. In the Control group, a newly-formed connective tissue scar occupies a wide area, and the areas of uneven maturation of the connective tissue are visualized. The regenerated epidermis covering the wound is 3–4 times thicker than the epidermis of the intact skin lying next to it (Fig. 5A).

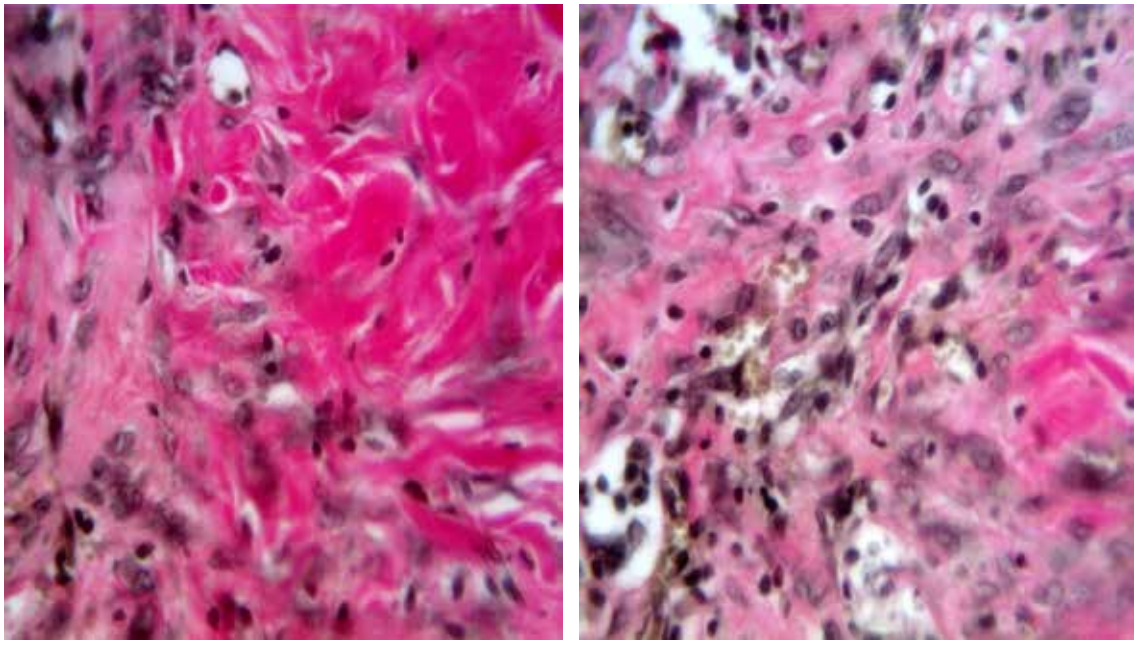


A ***B***

Figure 5. Photomicrograph of the skin cut in the wound area in the group of control observations

Note: stained by Van Gieson's solution × 100 (A); × 200 (B)

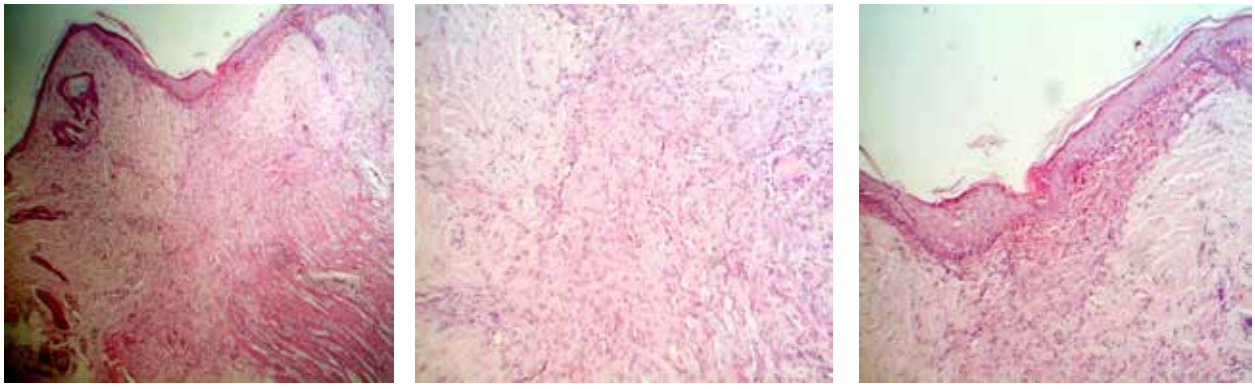
In the thickness of the epidermis against the background of mitotic dividing cells of the basal layer, epithelial cells with pycnomorphic nuclei and phenomena of karyolysis have been visualized. The heterogeneity of the structure of the connective tissue scar should also be notified (Fig. 5B). The fibrous component in the scar area is represented by thin multidirectional collagen fibers. The cellular component prevails over the fibrous one. It should be notified that in the area of the scar there are no hair follicles and sebaceous glands Fig. 6).



A ***B***

Figure 6. Photomicrograph of the skin cut in the wound area in the group of control observations. Stained by Van Gieson's solution. × 400

Bischofit. On histological cut-sections of the skin of the animals treated with Bischofit gel, a thin connective tissue scar is visualized in the wound area. A complete regeneration of the epidermis is determined. It is several times larger than in the adjacent wound of the epidermis. In the scar zone, no derivatives have been detected (Fig. 7A). Directly under the epidermis, a wide band of connective tissue containing blood-filled vessels with local hemorrhages into the surrounding tissue is visualized. (Fig. 7B, C).



A ***B*** ***C***

Figure 7. Photomicrograph of the skin cut in the wound area in the group of control observations with the use of Bischofit gel

Note: the thickened newly formed epidermis (A, B) and newly formed granulation tissue with a large number of blood vessels (C) are well visualized. Stained with hematoxylin and eosin. × 100 (A). × 200 (B, C)

Regarding the spatial organization of the newly formed connective tissue scar, the violation of the layered structure of the skin should be notified. On the part of the newly formed connective tissue, germination occurs in the underlying hypodermis and muscle tissue (Fig. 8A).

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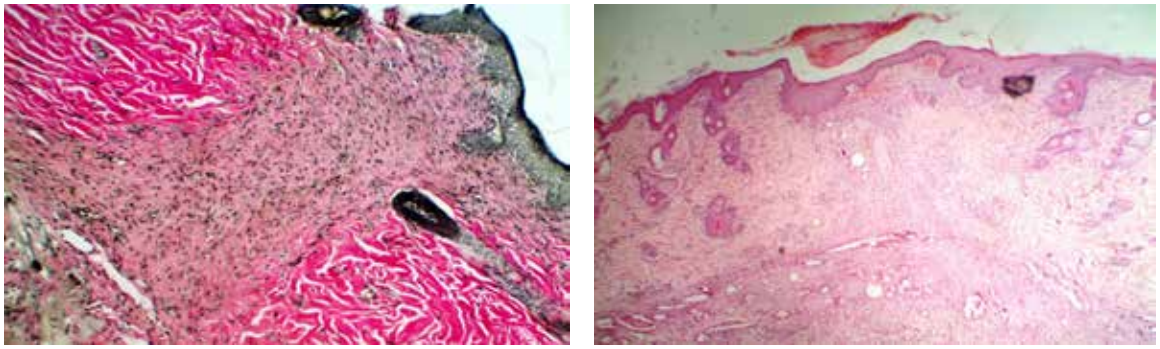
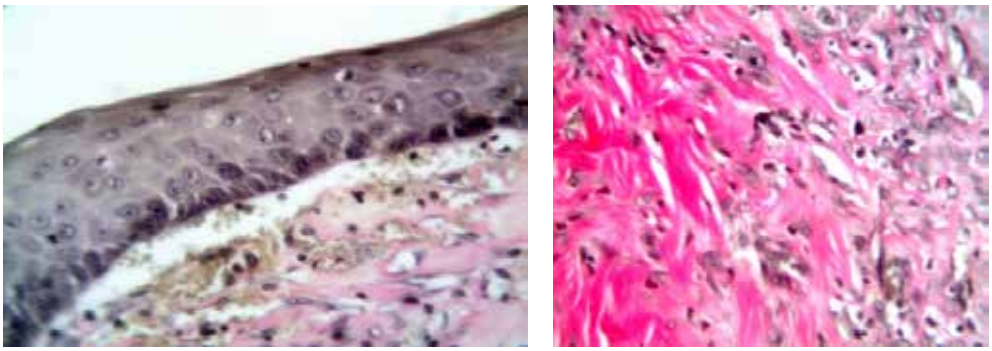


A B

Figure 8. Photomicrograph of the wound area skin section in the group

Note:
A – Bischofit gel. Stained by Van Gieson's solution. × 200 (A);
B – Actovegin. Stained with hematoxylin and eosin. × 100 (B)

Actovegin. When treated with preparations, there is a fully formed connective tissue scar of a wedge-shaped form. A complete closure of the wound defect with a stratified squamous epithelium is observed (Fig. 8B). A large number of mitoses are visualized in the basal and spinous layers of epidermis (Fig. 9A).



A B

Figure 9. Photomicrograph of the skin cut in the wound area in the group of observations with the use of Actovegin

Note:
stained by Van Gieson's solution. × 400;
A – in the field of view fibroblasts and lymphocytes, single fibrocytes dominate. The absence of a well-formed papillary layer should be notified;
B – in the areas adjacent from the scar, dermis, the fibers are thick structured and have all the normal functional criteria of dense unformed connective tissue

The cellular component prevails over the fibrous one.

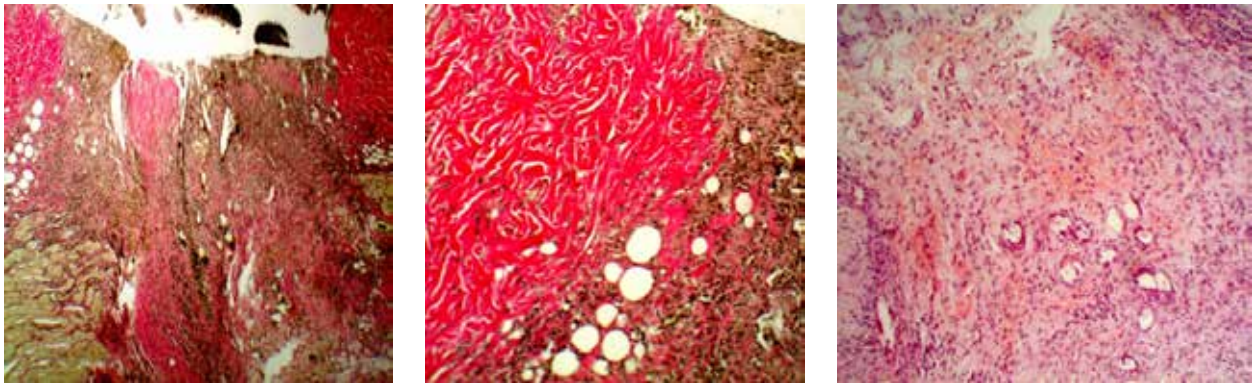
At the base of the wedge-shaped connective tissue scar, the cellular component predominates over the fibrous one. In the field of view fibroblasts and lymphocytes, single fibrocytes prevail. He absence of a well-formed papillary layer should be notified.. In sight, mature brightly oxyphilic collagen fibers placed randomly, dominate.

Hereby, in the areas adjacent from the scar, dermis, the fibers are thick structured and have all the normal functional criteria for dense unformed connective tissue (Fig. 9B).

Contractubex. In the study of the skin preparations of the animals treated with Contractubex, a wide area of the scar and complete filling of the wound defect with granulation tissue are visualized (Fig. 10A).

Over the entire surface of the scar there is a detachment of the newly formed thickened epithelium. The presence of heterogeneity in the spatial organization of the connective scar draws the attention. Local areas with a high degree of maturity of the newly formed connective tissue take place. Hereby, collagen fibers located chaotically, not tightly, alternate with portions of single-positioned fat cells with different diameters that are not prone to fusion (Fig. 10B).

At the base of the newly formed connective tissue scar, which continues in the deep layers of the dermis, the hypodermis and the muscular layer of the skin, a large number of dilated blood vessels with a tendency to hemorrhage into the surrounding tissue are found (Fig. 10C).



A ***B*** ***C***

Figure 10. Photomicrograph of the skin cut in the wound area in the group of observations with the use of Contractubex

Note:

A – complete filling of the wound defect with granulation tissue. Stained by Van Gieson's solution;× 100;

B – an alternation of areas with a high degree of maturity of the newly formed connective tissue with areas of single fat cells with different diameters that are not prone to fusion. Stained by Van Gieson's solution; × 100;

C – in the deep layers of the dermis, hypodermis and muscular layer of the skin, a large number of dilated blood vessels with a tendency to hemorrhage into the surrounding tissue are detected. Stained by hematoxylin and eosin;× 200

Comparative quantitative assessment

In the questionnaire survey by the expert committee, the average score was determined in each group (Table 3). A lower score indicates a more consistent histological pattern of specimens obtained from the groups.

Table 3. Results of the scoring microscopic skin samles by the expert committee (M±m)

Qualitative character	Quantitative assessment (in points)			
	Control	Bishofit	Actovegin	Contractubex
Cytoarchitectonics disruption	1.71±0.18	1.34±0.21	1.49±0.15	1.49±0.15
Intracellular matrix architectonics disruption	1.32±0.21	1.12±0.09	1.21±0.10	1.31±0.11
Hemorrhage, enlarged vessels	1.91±0.19	1.24±0.11	1.54±0.16	1.39±0.15
Violation of epithelialization	1.72±0.21	1.52±0.15	1.51±0.19	1.79±0.21
Leukocyte infiltration	1.84±0.23	1.32±0.31	1.29±0.12	2.0±0.21
Average score	1.70±0.20	1.31±0.21*	1.41±0.15*	1.60±0.16

Note:

a lower score indicates a more consistent histological pattern;

** – p <0.05 when compared with the Control*

From the data presented in Table 3 it can be seen that less pronounced morphological changes are observed in the groups treated with Bischofite gel and gel with Actovegin.

Evaluation of the ratio in collagen types I and III in a polarizing microscope

When assessing the ratio in collagen types I and III in the tissues of the wound defect when dyeing with picrosirius red, it was established that, by the number of mature collagen fibers, the studied groups can be arranged in the following sequence (descending): Bishofit > Actovegin > Control > Contractubex (Table 4, fig. 11).

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Table 4. Ratio in collagen* types *I and III in tissue samples of modeled wounds received on day 8 after starting the experiment (M±m)

Group	Control	Bishofite	Actovegin	Contractubex
Ratio in collagen types I and III	0.56±0.012	0.73±0.023*	0.67±0.017	0.38±0.02*

*Note: * – p≤0.05 when compared with the control group*

Statistically significant differences were found out in the Bischofite and Contractubex groups. In the group treated with Bischofite, the differences are unidirectional in nature, relative to the content of the type III of collagen, which indicates a higher degree of scar organization. In the group that received Contractubex, there is an increased relative content of collagen type III, which indicates a delay in the maturation of collagen and the tendency to form the scar tissue.

A ***B*** ***C***

Figure 11. Microscopic picture of the modeled skin wound area

Note:
polarization microscopy. Sirius Redstain. × 400;
Control group (A);
Bishofit (B);
Contractubex (C)

CONCLUSION

The study showed that the best results had been obtained when using Bischofit gel. The wound defect in this group was characterized by the greatest strength, elasticity, a good histological pattern. Judging by the low concentration of hydroxyproline and collagen type III, it is less prone to scar formation. Actovegin has a less significant, but pronounced reparative effect on this model. Actovegin gel showed a positive effect on the macro- and microscopic picture of the wound defect, as well as the strength of the wound and the preventive effect on the excessive formation of the scar tissue. Less satisfactory results were obtained when applying Contractubex. Without having a significant impact on the physicomechanical characteristics of the wound, Contractubex increased the content of HP and reduced the content of mature collagen (type III). The similar results show that Contractubex has reduced the reparative potential of tissues, increasing the growth of granulation tissue and slowing down its recovery. This conclusion is confirmed by the results of histological examination of the animals treated with Contractubex and can be explained in terms of the available information on the pharmacodynamics of this drug.

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Conflict of interest

The authors declare no conflict of interest.

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USING QUANTUM-CHEMICAL PARAMETERS FOR PREDICTING ANTI-RADICAL (HO·) ACTIVITY OF RELATED STRUCTURES CONTAINING A CINNAMIC MOLD FRAGMENT. I. DERIVATIVES OF CINNAMIC ACID, CHALCON AND FLAVANON

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45 compounds uniting 3 groups of derivatives of cinnamic acid, chalcone and flavanone, have been studied. Each of them includes 15 substances. The analyzed compounds contain a common structural fragment, which is a cinnamic acid residue (cinnamoyl fragment). ***The aim*** *is to study the quantum-chemical parameters of the listed groups of the compounds in order to predict possible ways of their interaction with the most aggressive and dangerous of the active oxygen species (ROS) – a hydroxyl radical.* ***Materials and methods****. For the analyzed structures, the Mulliken charges (a.u.), bond numbers (Nμ), unsaturation index (IUA), and electron density values on all 9-carbon atoms of the cinnamoyl fragment have been determined. The calculations have been carried out on a workstation with an Intel Xeon E5-1620 3.5 GHz processor, 20 GB of RAM. The semi-empirical method PM7 was used (WinMopac 2016 program). The ORCA 4.1 program was used to calculate the energies of homolytic cleavage of the O – H bond.* ***Results****. The analysis of Mulliken charges (a.u.), bonded numbers (Nμ), unsaturation indices (IUA), and electron density revealed a number of regularities on the basis of which it can be concluded, that taking into account the nature of the substituent, the most probable for addition in the aryl residueare positions C-1, C-2, C-3, C-4 and C-5. In the propenone fragment, the radical HO· first attacks position 8, then 7. For the hydroxy-substituted, the energy of the homolytic breaking of the H – O bond has been determined and it has been established that the spatial difficulty of phenols (compounds 13k, 13x, 13f, 14k, 14x, 14f) H-O bonds are the smallest and on average are -160.63 kJ/mol. It has also been established that the higher the positive Mulliken charge on the carbon atom with which the phenolic hydroxyl is bound, the lower the energy of the homolytic breaking of the H – O bond and the more stable the resulting phenoxy radicalis.* ***Conclusion.*** *The carried out quantum chemical calculations allow us to conclude that the studied classes of compounds can be used to bind the hydroxyl radical formed in the body, causing various kinds of mutations, leading, among other things, to the development of oncological diseases.*

Keywords: *hydroxyl radical, cinnamic acid derivatives, chalcones, flavanones, Mulliken charges, bond numbers, unsaturation index, electron density*

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ИСПОЛЬЗОВАНИЕ КВАНТОВО-ХИМИЧЕСКИХ ПАРАМЕТРОВ ДЛЯ ПРОГНОЗИРОВАНИЯ АНТИРАДИКАЛЬНОЙ (HO·) АКТИВНОСТИ РОДСТВЕННЫХ СТРУКТУР, СОДЕРЖАЩИХ ЦИННАМОИЛЬНЫЙ ФРАГМЕНТ. I. ПРОИЗВОДНЫЕ КОРИЧНОЙ КИСЛОТЫ, ХАЛКОНА И ФЛАВАНОНА

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Изучено 45 соединений, объединяющих 3 группы производных коричной кислоты, халкона и флаванона, каждая из которых включает по 15 веществ. Анализируемые соединения содержат общий структурный фрагмент, представляющий собой остаток коричной кислоты (циннамоильный фрагмент). ***Цель работы –*** *изучение квантово-химических параметров перечисленных групп соединений с целью прогнозирования возможных путей их взаимодействия с наиболее агрессивным и опасным из числа активных форм кислорода (АФК) гидроксильным радикалом.* ***Материалы и методы.*** *Для анализируемых структур определены Малликеновские заряды (а.е.), связевые числа (Nμ), индекс ненасыщенности (IUA) и величины электронной плотности на всех 9-атомах углерода циннамоильного фрагмента. Расчеты осуществлены на рабочей станции с процессором IntelXeonE5-1620 3,5 ГГц, 20 Гб оперативной памяти. При этом использован полуэмпирический метод PM7 (программа WinMopac 2016). Для расчетов энергий гомолитического расщепления связи O–H использована программа ORCA 4.1.* ***Результаты.*** *Анализ величин Малликеновских зарядов (а.е.), связевых чисел (Nμ), индексов ненасыщенности (IUA) и электронной плотности позволил выявить ряд закономерностей, на основании которых можно делать выводы о том, что с учетом природы заместителей наиболее вероятными для присоединения в арильном остатке являются положения C-1, C-2, C-3, C-4 и C-5. В пропеноновом фрагменте радикал HO* в первую очередь атакует положение 8, затем 7. Для гидроксизамещенных определена энергия гомолитического разрыва связи H-O и установлено, что у пространственно затрудненных фенолов (соединения 13к, 13х, 13ф, 14к, 14х, 14ф) энергия разрыва связи H-O наименьшая и в среднем составляет – 160,63 кДж/моль. Установлено также, что,чем выше положительный Малликеновский заряд на атоме углерода, с которым связан фенольный гидроксил, тем ниже энергия гомолитического разрыва связи H-O и тем более устойчив образующийся феноксильный радикал.* ***Заключение.*** *Проведенные квантово-химические расчеты позволяют сделать вывод о том, что изучаемые классы соединений могут быть использованы для связывания образующегося в организме гидроксильного радикала, вызывающего различного рода мутации, приводящие, в том числе, к развитию онкологических заболеваний.*
Ключевые слова: *гидроксильный радикал, производные коричной кислоты, халконы, флаваноны, Малликеновские заряды, связевые числа, индекс ненасыщенности, электронная плотность*

INTRODUCTION

Currently, experimental biochemistry and clinical pharmacology have accumulated extensive material indicating the relationship of free radical oxidation processes involving reactive oxygen species (ROS) and many diseases. It is known that in violation of the mechanisms of antioxidant protection in the body there is an accumulation of ROS, of which the HO-radical is the most dangerous. It is able to interact with the nitrogenous bases of DNA and RNA, which contributes to the formation of various types of mutations [1, 2]. It also interacts with phospholipids of cell membranes, increasing the level of their peroxidation and resulting in reperfusion tissue damage, carcinogenesis and other pathological processes [2, 3].

In case of disturbances in the equilibrium processes involving ROS, natural compounds — derivatives of cinnamic acid, chalcones and flavanones, containing a common cinnamoyl fragment, are getting more and more become important. In flavonoids it is the main conjugate chain and, in essence, it represents the residue of cinnamic acid. These three groups of compounds are interconnected by biogenetic transformations [4–6].

The listed representatives of polyphenolic compounds are characterized by a broad spectrum of pharmacological activity, which is probably due to their high antiradical activity.

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acteristics of cinnamic acid derivatives, chalcones and flavanones containing substituents in the aryl moiety conjugation of the main chain to predict their possible interactions with the hydroxyl radical HO.

MATERIALS AND METHODS

The objects of the study were hydroxy and methoxy substituted cinnamic acid, chalcone and flavanone derivatives in the aryl residue of the cinnamoyl moiety, 45 compounds in total. Quantum-chemical parameters of the analyzed structures were calculated on a workstation with an Intel Xeon E5-1620 3.5 GHz processor, 20 GB of RAM.

The hydroxyl radical HO, whose life expectancy in a biological medium is about 10^{-9} seconds, represents the greatest danger among the active oxygen species (ROS).

One of the ways of formation of a hydroxyl radical in the body can be the Fenton reaction or the oxidation of Fe^{2+} to the Fe^{3+} hypochlorite anion, which, in turn, is formed in phagocytes.

It has been proved that the yield of hydroxyl radical OH· in the second case is higher than in the Fenton reaction [8].

The same radical can be formed by the reaction of Haber-Weiss [8].

The consequences of these reactions involving bivalent iron are obvious: the "extraction" of Fe^{2+} cation from the systems containing it and its subsequent oxidation to Fe^{3+}, which is extremely dangerous in itself, since it contributes to the destruction of blood heme and iron-containing endogenous substances.

On the other hand, the hydroxyl radical, interacting with the amino acid fragments of proteins, causes denaturation of the latter and subsequent inactivation of enzymes.

There is an opinion that the OH· radical·is able to selectively accumulate near the DNA [9].

Possessing sufficiently high electrophilic properties, it can not only hydroxylate the nitrogenous bases of nucleic acids, but also contribute to the subsequent breaking of both carbohydrate bridges between nucleotides and hydrogen bonds of "interlaced" polynucleotide chains [2]. It is clear that further processes will mutate or damage genes.

In the lipid layer of cell membranes, the HO· initiates a chain reaction of lipid oxidation by a radical mechanism, which leads to cell damage and cell death.

Biochemical processes involving ROS in the physiological norm are controlled by both enzyme and non-enzyme components of cells. In case of disturbances in the equilibrium processes involving ROS, natural antioxidants, such as polyphenolic compounds as cinnamic acid derivatives, as well as flavonoids (chalcones, flavanones, flavones and flavonols), become important.

Due to the structural diversity, as well as the totality of the manifested pharmacological effects, they occupy a special place among natural antioxidants.

It is known that cinnamic acid is directly involved in the biosynthesis of flavonoids [4]. Comparing the structures of cinnamic acid and flavonoids, it is easy to verify that the common structural fragment in all the compounds is the cinnamoyl fragment, which is essentially a cinnamic acid residue (Fig. 1).

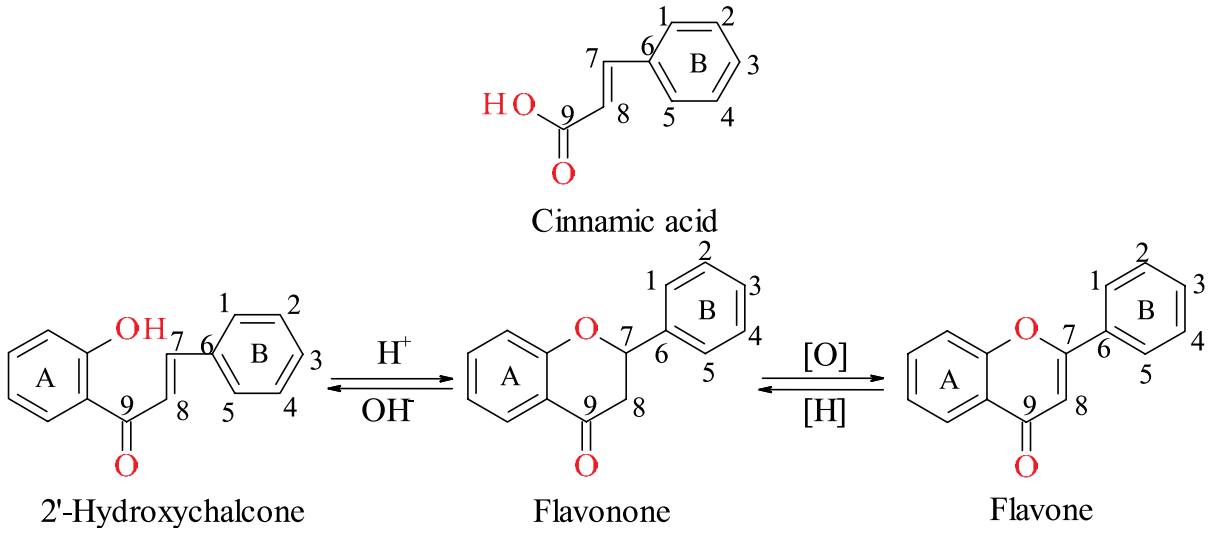


Figure. 1. Structural features of chalcone, flavanone and flavone

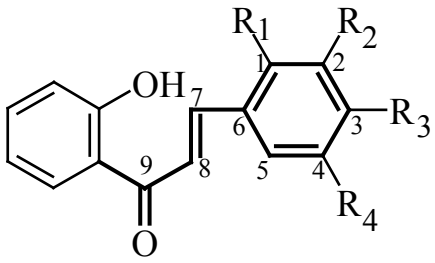
It should be noted that with slight changes in the pH-environment, the chalcones become flavanones and vice versa: flavanone prevails in the acidic environment, and chalcone prevails in the alkaline one. This circumstance is important from the point of view of the biological activity of chalcones and flavanones.

It is clear from the presented structures, that the main conjugation chain is formed thanks to the cynnamoyl fragment, and the transfer of electronic effects exerted by substituents in ring B, occurs through this chain.

In continuation of our earlier studies [10–13], and also taking into account the structural proximity of cinnamon

acids, chalcones and flavanones (the presence of a cinnamoyl fragment), we found it advisable to a priori examine the activity of the chalcones and flavanones in relation to the hydroxyl radical OH· using such quantum-chemical parameters as Mulliken charges (a.u.), bond numbers (Nμ), theoretical valence (Vμ), unsaturation index (IUA), and electron density. Table 1 presents the analyzed compounds, which are designated respectively 1k–15k (derivatives of cinnamic acid), 1x–15x (derivatives of chalcone), 1f–15f (derivatives of flavanone).

Table 1. Derivatives of cinnamic acid (k), chalcone (x) * and flavanone with substituents in the aromatic core of the main conjugation chain



No.			Position of substituents			
K	x*	f	1	2	3	4
1k	1x	1f	H	H	H	H
2k	2x	2f	OH	H	H	H
3k	3x	3f	CH_3O	H	H	H
4k	4x	4f	H	OH	H	H
5k	5x	5f	H	CH_3O	H	H
6k	6x	6f	H	H	OH	H
7k	7x	7f	H	H	CH_3O	H
8k	8x	8f	H	OH	OH	H
9k	9x	9f	H	CH_3O	OH	H
10k	10x	10f	H	OH	CH_3O	H
11k	11x	11f	H	CH_3O	CH_3O	H
12k	12x	12f	H	OH	OH	OH
13k	13x	13f	H	CH_3O	OH	CH_3O
14k	14x	14f	H	$C(CH_3)_3$	OH	$C(CH_3)_3$
15k	15x	15f	H	CH_3O	CH_3O	CH_3O

** Note: chalcone derivatives containing an OH group in the ortho-position to the carbonyl are considered, since in its absence the chalcone-flavanone transition is impossible*

Previously, using semi-empirical quantum-chemical methods, we studied the reactivity indices of cinnamic acid derivatives with respect to the hydroxyl radical [10]. This made it possible to identify the most reactive centers in the cinnamoyl fragment: the interaction of cinnamic acid with the OH-radical electrophilic in properties occurs primarily at the C-8 position, since this atom is characterized by the lowest degree of "saturation" (the lowest bond number), the highest electron density, and the greatest negative charge compared to its two nearest atoms. Further interaction of cinnamic acid with the formation of the corresponding adducts is possible according to the C-7, C-6, C-1 and C-5 positions[1].

Taking into account the trends revealed in cinnamic acid, we considered it expedient to determine the most probable centers of primary attack by the OH· radical of chalcone and flavanone.

The quantum chemical characteristics listed above were calculated using the PM7 semi-empirical method (WinMopac 2016 program) for chalcones and flavanones containing hydroxy and methoxy groups in the aryl moiety of the main conjugation chain.

Tables 2, 3, 4 present the distribution of Mulliken charges (a.u.), bond numbers (Nμ), unsaturation index (IUA) and electron density on the carbon atoms of the cinnamoyl fragment of the two derivatives of cinnamic acid (6k and 7k), chalcone (6x and 7x) and flavanone (6f and 7f).

[1] Henceforward, numbering of atoms in the analyzed structures is given not in accordance with the IUPAK rules, but in accordance with the calculation programs. For cinnamic acid, the carbon numbering generated by the programs is shown. To make it easier and more convenient to compare the results obtained, authors have kept this numbering for chalcones and flavanones.

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Table 2. Values of Mulliken charges (a.u.), bond numbers (Nμ), unsaturation index (IUA) and electron density on the carbon atoms of the cinnamoyl fragment of compounds 6k and 7k, 6x and 7x, 6f and 7f

	1		2		3		4		5		6		7		8	
	6k	7k	6k	7k	6k	7k	6k	7k	6k	7k	6k	7k	6k	7k	6k	7k
a.u.	-0.044	-0.043	-0.262	-0.269	0.317	0.217	-0.326	-0.317	-0.042	-0.046	-0.134	-0.138	0.002	0.008	-0.316	-0.321
Nμ	3.810	3.813	3.789	3.782	3.774	3.774	3.777	3.774	3.811	3.810	3.836	3.832	3.866	3.864	3.790	3.787
IUA	0.154	0.151	0.167	0.174	0.132	0.149	0.173	0.176	0.154	0.156	0.148	0.150	0.079	0.080	0.157	0.158
el.d.	4.044	4.043	4.262	4.270	3.683	3.722	4.326	4.317	4.042	4.046	4.134	4.134	3.997	3.991	4.316	4.321

	6x	7x	6x	7x	6x	7x	6x	7x	6x	7x	6x	7x	6x	7x	6x	7x
a.u.	-0.047	-0.046	-0.265	-0.272	0.317	0.277	-0.322	-0.314	-0.044	-0.047	-0.134	-0.138	0.009	0.016	-0.337	-0.342
Nμ	3.810	3.814	3.788	3.783	3.773	3.775	3.777	3.774	3.809	3.810	3.836	3.835	3.851	3.849	3.805	3.803
IUA	0.156	0.152	0.169	0.173	0.133	0.148	0.173	0.177	0.156	0.156	0.147	0.148	0.091	0.091	0.147	0.147
el.d.	4.047	4.046	4.265	4.272	3.683	3.722	4.322	4.314	4.044	4.047	4.134	3.983	3.990	3.983	4.337	4.342

	6f	7f	6f	7f	6f	7f	6f	7f	6f	7f	6f	7f	6f	7f	6f	7f
a.u.	-0.068	-0.069	-0.248	-0.253	0.306	0.265	-0.316	-0.305	-0.050	-0.057	-0.161	-0.162	0.138	0.141	-0.441	-0.440
Nμ	3.826	3.222	3.786	3.780	3.783	3.785	3.777	3.774	3.826	3.826	3.821	3.819	3.838	3.839	3.818	3.820
IUA	0.140	0.139	0.172	0.179	0.126	0.142	0.175	0.179	0.139	0.140	0.160	0.162	0.064	0.162	0.115	0.114
el.d.	4.068	4.070	4.248	4.253	3.693	3.735	4.316	4.305	4.050	4.057	4.161	4.162	3.861	3.859	4.441	4.440

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Table 3. Values of Mulliken charges (a.u.) of bond numbers (Nμ), index unsaturation (IUA) and the electron density on the carbon atoms of cinnamoyl moiety 8k and 9k compounds 8h and 9h, 8f and 9F

	1		2		3		4		5		6		7		8	
	8k	9k	8k	9k	8k	9k	8k	9k	8k	9k	8k	9k	8k	9k	8k	9k
a.u.	-0.105	0.105	-0.216	-0.217	0.229	0.225	0.126	0.094	-0.212	-0.206	-0.060	-0.066	-0.008	-0.004	-0.303	-0.306
Nμ	3.803	3.802	3.786	3.788	3.763	3.755	3.761	3.766	3.767	3.762	3.839	3.839	3.868	3.867	3.794	3.792
IUA	0.168	0.169	0.176	0.174	0.172	0.178	0.172	0.183	0.203	0.207	0.152	0.152	0.08	0.08	0.156	0.156
el.d.	4.105	4.105	4.216	4.218	3.770	3.775	3.873	3.905	4.212	4.206	4.060	4.066	4.008	4.004	4.303	4.306
	8x	9x	8x	9x	8x	9x	8x	9x	8x	9x	8x	9x	8x	9x	8x	9x
a.u.	-0.110	-0.109	-0.218	-0.225	0.228	0.192	0.129	0.141	-0.213	-0.280	-0.059	0.061	-0.02	0.004	-0.324	-0.328
Nμ	3.804	3.806	3.788	3.780	3.763	3.760	3.761	3.760	3.766	3.763	3.841	3.838	3.854	3.853	3.809	3.807
IUA	0.169	0.166	0.175	0.183	0.172	0.189	0.172	0.175	0.203	0.206	0.151	0.153	0.090	0.090	0.147	0.147
el.d.	4.110	4.109	4.218	4.225	3.771	3.807	3.870	3.858	4.214	4.220	4.059	4.061	4.022	3.996	4.324	4.328
	8f	9f	8f	9f	8f	9f	8f	9f	8f	9f	8f	9f	8f	9f	8f	9f
a.u.	-0.196	-0.198	0.206	0.165	0.147	0.163	-0.232	-0.242	-0.137	-0.134	-0.073	-0.080	0.130	0.133	-0.437	-0.435
Nμ	3.765	3.760	3.777	3.776	3.748	3.746	3.799	3.797	3.815	3.815	3.833	3.833	3.840	3.841	3.818	3.820
IUA	0.197	0.205	0.162	0.178	0.183	0.187	0.169	0.170	0.156	0.156	0.159	0.158	0.064	0.062	0.115	0.114
el.d.	4.196	4.198	3.793	3.835	3.852	3.837	4.232	4.242	4.137	4.134	4.073	4.080	3.869	3.866	4.437	4.435

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Table 4. Values of Mulliken charges (a.u.) of bond numbers (Nμ), unsaturation index (IUA) and the electron density on the carbon atoms cinnamoyl moiety 10k and 13K compounds, 10x and 13x, 10f and 13f

	1		2		3		4		5		6		7		8	
	10k	13k	10k	13k	10k	13k	10k	13k	10k	13k	10k	13k	10k	13k	10k	13k
a.u.	-0.105	-0.271	-0.223	0.183	0.193	0.102	0.138	0.146	-0.218	-0.268	-0.063	0.008	-0.002	-0.01	-0.308	-0.298
Nμ	3.806	3.728	3.779	3.776	3.760	3.712	3.760	3.763	3.765	3.738	3.837	3.845	3.866	3.867	3.792	3.797
IUA	0.165	0.228	0.184	0.179	0.189	0.234	0.175	0.186	0.205	0.219	0.154	0.149	0.081	0.08	0.156	0.154
el.d.	4.105	4.271	4.223	3.817	3.806	3.897	3.861	3.854	4.218	4.268	4.063	3.991	4.002	4.001	4.308	4.298
	10x	13x	10x	13x	10x	13x	10x	13x	10x	13x	10x	13x	10x	13x	10x	13x
a.u.	-0.109	-0.242	-0.220	0.203	0.224	0.057	0.097	0.171	-0.206	-0.290	-0.066	0.023	0.002	-0.025	-0.327	-0.303
Nμ	3.802	3.733	3.788	3.774	3.755	3.699	3.766	3.770	3.761	3.734	3.840	3.852	3.853	3.852	3.807	3.811
IUA	0.170	0.224	0.174	0.176	0.179	0.235	0.183	0.176	0.208	0.217	0.152	0.142	0.09	0.142	0.147	0.150
el.d.	4.109	4.242	4.220	3.796	3.776	3.943	3.902	3.830	4.206	4.290	4.066	3.976	3.998	3.976	4.327	4.303
	10f	13f	10f	13f	10f	13f	10f	13f	10f	13f	10f	13f	10f	13f	10f	13f
a.u.	-0.194	-0.265	0.200	0.220	0.114	0.05	-0.224	0.176	-0.140	-0.292	-0.076	-0.004	0.132	0.129	-0.438	-0.430
Nμ	3.766	3.746	3.771	3.771	3.752	3.705	3.795	3.765	3.815	3.745	3.832	3.835	3.841	3.838	3.819	3.818
IUA	0.196	0.205	0.167	0.176	0.196	0.227	0.173	0.180	0.157	0.203	0.159	0.157	0.062	0.063	0.114	0.116
el.d.	4.194	4.260	3.800	3.779	3.885	3.949	4.224	3.823	4.140	4.293	4.076	4.004	3.867	3.870	4.438	4.430

It is characteristic for the C-7 and C-8 atoms to have the same dynamics of changes in parameters as it had been in cinnamic acid. In the analyzed structures, the carbon atom C-9 is characterized by a significantly lower electron density and a higher positive Mulliken charge, although the bond number is insignificant (third decimal place), it is higher than those for C-8 and C-7. The highest negative Mulliken charge and the electron density, as well as the smallest bond numbers compared to the two nearest atoms, are concentrated on the C-8 atom of all the three types of the structures under consideration. Similar electronic effects are easy to explain, if we take into consideration the fact that with respect to the propenone moiety, the electron-donating hydroxy and methoxy groups at positions 1 and 3 (ortho and para positions with respect to the propene unit) contribute to the enhancement of the polar conjugation and, consequently, to an increase in the Mulliken charge and electron density on C-8 (compounds 2, 3, 6, 7) compared with the parent structure of each group of the analyzed compounds.

If the hydroxy- and methoxy groups are in position 2 of the aryl fragment (compounds 4 and 5), then the electron density and Mulliken charge decrease, but the same parameters increase on the C-1, C-3 and C-5 atoms, that is, in two ortho- (P-1 and P-3) and para-positions (P-5) (table 5). This dependence is repeated in all the three types of the structures under consideration - 4k, 4x, 4f and 5k, 5x and 5f. Such electronic effects are in good agreement with the contribution of the Taft constants [14].

Table 5. Values of Mulliken charges (a.u.), electron density and bond numbers (Nμ) on carbon atoms in o- and p- positions with respect to the substituent for the derivatives of cinnamic acid, chalcone and flavanone, numbered 2, 3 and 4

	Unsubstitute dcinnamic acid			Unsubstitute dchalcone			Unsubstituted flavanone		
Cv	a.u.	el.d.	Nμ	a.u.	el.d.	Nμ	a.u.	el.d.	Nμ
1	-0.118	4.1180	3.812	-0.122	4.122	3.813	-0.141	4.141	3.827
2	-0.159	4.1590	3.852	-0.161	4.162	3.852	-0.145	4.145	3.849
3	-0.125	4.1249	3.836	-0.121	4.126	3.837	-0.135	4.135	3.845
4	-0.159	4.1595	3.854	-0.156	4.156	3.854	-0.151	4.151	3.852
5	-0.126	4.1260	3.816	-0.128	4.128	3.814	-0.132	4.133	3.829
6	-0.054	4.0540	3.858	-0.053	4.054	3.856	-0.081	4.081	3.840
7	-0.020	4.0200	3.869	-0.013	4.013	3.855	0.122	3.877	3.840
8	-0.298	4.9810	3.798	-0.319	4.319	3.814	-0.440	4.440	3.819
		2k			2x			2f	
Cv	a.u.	el.d.	Nμ	a.u.	el.d.	Nμ	a.u.	el.d.	Nμ
8	-0.288	4.288	3.798	-0.364	4.364	3.763	-0.446	4.442	3.812
2	-0.234	4.047	3.852	-0.268	4.268	3.787	-0.229	4.229	3.803
4	-0.149	4.062	3.854	-0.238	4.238	3.842	-0.197	4.197	3.844
1	-0.046	4.322	3.832	0.342	3.658	3.747	0.284	3.715	3.777
		3k			3x			3f	
Cv	a.u.	el.d.	Nμ	a.u.	el.d.	Nμ	a.u.	el.d.	Nμ
8	-0.301	4.301	3.781	-0.368	4.368	3.785	-0.439	4.439	3.819
2	-0.238	4.050	3.838	-0.270	4.270	3.780	-0.309	4.310	3.773
4	-0.161	4.060	3.783	-0.235	4.235	3.842	-0.227	4.228	3.834
1	-0.050	4.314	3.833	0.309	3.690	3.740	0.269	3.730	3.777
		4k			4x			4f	
Cv	a.u.	el.d.	Nμ	a.u.	el.d.	Nμ	a.u.	el.d.	Nμ
8	-0.283	4.283	3.804	-0.299	4.299	3.819	-0.437	4.437	3.818
1	-0.290	4.290	3.740	-0.230	4.230	3.750	-0.240	4.240	3.747
3	-0.228	4.228	3.779	-0.296	4.296	3.769	-0.303	4.303	3.774
5	-0.205	4.205	3.800	-0.210	4.210	3.803	-0.223	4.223	3.813
		5k			5x			5f	
Cv	a.u.	el.d.	Nμ	a.u.	el.d.	Nμ	a.u.	el.d.	Nμ
8	-0.290	4.290	3.802	-0.303	4.303	3.819	-0.436	4.436	3.818
1	-0.278	4.278	3.737	-0.234	4.234	3.743	-0.252	4.252	3.757
3	-0.233	4.233	3.773	-0.285	4.285	3.766	-0.293	4.293	3.770
5	-0.204	4.204	3.798	-0.210	4.210	3.803	-0.215	4.210	3.814

Note: k – cinnamic acid, x – chalcone, f – flavanone

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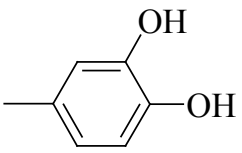
Thus, the primary hydroxyl radical electrophilic attack will take place primarily at position C-8, and then at the C-7 position.

A similar conclusion is valid for the considered types of the analyzed compounds, as shown before [11, 12].

Using a similar approach to interpret the possible attack paths of the monosubstituted derivatives by the OH-radical (compounds 2k, 2x, 2f – 7k, 7x, 7f) taking into account the quantum chemical parameters, it can be assumed that the most likely are the C-2 and C-4 positions for compounds 2 and 3, since they are characterized by the highest IUA values. If the substituent is in position 2, then the attack is likely to occur in C-1, C-3 or C-5 positions of the phenyl fragment of all three types of structures under consideration due to high IUA values. For compounds of types 6 and 7, the attack of a hydroxyl radical is equally probable in the C-2 and C-4 positions for the same reasons as mentioned above (Tables 2, 3, 4, 5).

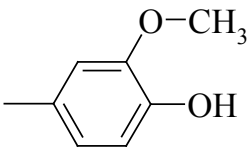
In the case of disubstituted for the aryl fragment, the dynamics of changes in the Mulliken charges, the unsaturation index and the electron density in compounds 8, 9 and 10 of all the three types of the structures under consideration practically coincide and actually make the same electronic contribution to the C-8 propene unit.

It should be emphasized that two hydroxy- or hydroxy- and methoxy-groups in positions 2 and 3 of the aryl fragment have a competitive effect on the conjugation system: the effect of the para-substituent is partially extinguished by the inconsistent influence of the same substituent in position 2. This conclusion can be illustrated by Taft σ-constants for –OH and –OCH3 groups [14]:



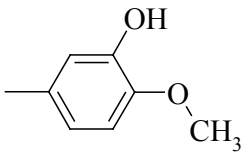
σ = 0.127
σ = -0.370
∑=-0.243

Compounds 8k, 8x, 8f



σ = 0.115
Σ= -0.370
∑=-0.255

Compounds 9k, 9x, 9f



σ = 0.127
σ= -0.268
∑= -0.141

Compounds 10k, 10x, 10f

When interpreting antiradical (HO·)activity of polyhydroxy cinnamic acid derivatives, chalcones and flavanones, one should take into account their ability to bind reactive oxygen species not only with the participation of carbon atoms of the aryl radical, but also due to the homolytic breaking of the H – O bond of the phenolic hydroxy-group to form an intermediate adduct — the phenoxyl radical.

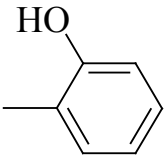
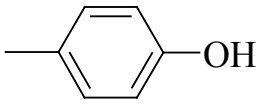
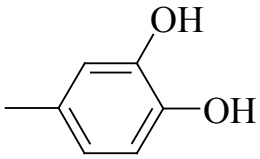
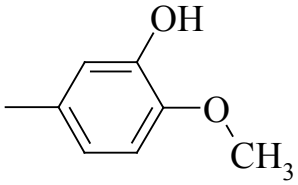
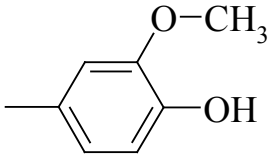
Earlier, when analyzing the antiradical activity of polyhydroxychalcons, we calculated the energies of homolytic breaking of H–O bonds in monohydroxy-compounds in which the hydroxy-group is located at C-3 or C-4, as well as for disubstituted ones, as shown below.

In continuation of these studies using the ab initio method, we calculated the energies of the homolytic breaking of the H O bond with the transition of the hydrogen atom to the hydroxyl radical in the cinnamoyl disubstituted along the aryl residue. The relationship between the breaking energy of the H–O bond and the unsaturation index (IUA) of the carbon atom which the substituent is associated has been revealed. For this program, ORCA 4.1 was used. The optimization of the geometry of molecules was performed using the density functional theory (UB3LYP) method using the set of basis functions 3-21G*. Vibrational analysis, as well as the calculation of thermodynamic functions (enthalpy, entropy, and Gibbs energy) were performed on the basis of the density functional theory (UB3LYP) using the set of basic functions 6-311G** [15, 16]. It has been established that the lower the bond breaking energy, the higher the IUA value is (Table 6).

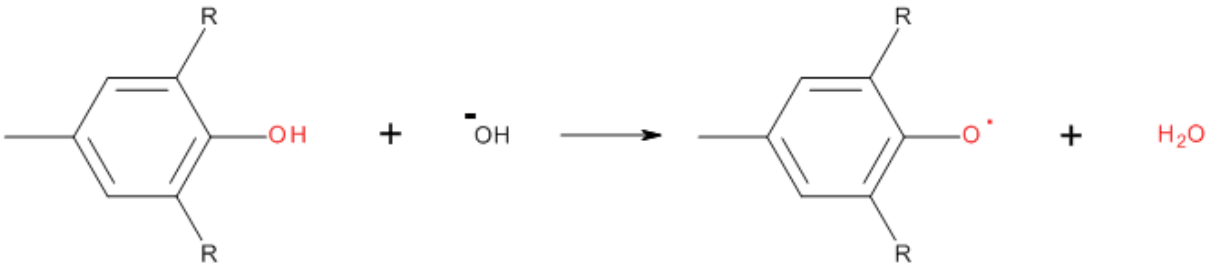
In the presented data, a clear relationship can be traced: the larger the unsaturation index (IUA) of the aryl carbon atom with which the hydroxy group is associated, the lower the energy of the H – O homolytic bond break is. There is a similar relationship for bond numbers (Table 3).

In the list of the compounds subjected to quantum-chemical study, we have considered three types of compounds containing three substituents in positions 2, 3, 4 of the aryl fragment and designated by numbers 12, 13 and 14.

Table 6. Gibbs free energy of homolytic breaking of the H–O bond

Structuralfragment	Breaking energy of the O – H bond	IUA (C-1)	IUA (C-2)	IUA (C-3)
	-150.30	0.135 (2k)	-	-
		0.152 (2x)	-	-
		0.145 (2f)	-	-
	-137 , 70	-	-	0.132 (6k)
		-	-	0.133 (6x)
		-	-	0.126 (6f)
	-173.89	-	0.175 (8k)	0.172 (8k)
		-	0.173 (8x)	0.172 (8x)
		-	0.162 (8f)	0.183 (8f)
	-130.01	-	0.184 (10k)	0.189 (10k)
		-	0.174 (10x)	0.179 (10x)
		-	0.167 (10f)	0.196 (10f)
	-174.26	-	0.174 (9k)	0.178 (9k)
		-	0.183 (9x)	0.189 (9x)
		-	0.178 (9f)	0.187 (9f)

For these compounds, the energies of the homolytic O – H bond have been calculated resulting in corresponding phenoxyl radical in position C-3 and the addition of a hydrogen atom to a hydroxyl radical. In our opinion, it can be represented by the following scheme:



This reaction was simulated by the molecular dynamics method in a 3-21G* force field using the density functional theory (UB3LYP) method for 50 picoseconds. In the process of simulation, a hydroxyl radical attacks a phenolic hydroxyl group, bonding a hydrogen atom with its oxygen. In the course of the oscillation of the phenolic hydroxyl group, covalent bonding of the hydrogen atom of the phenol hydroxide to the oxygen atom of the hydroxyl radical occurs. After that, a free water molecule and a phenol radical are formed. Figure 2 shows a graph of the dependence of potential energy of the simulated system on time.

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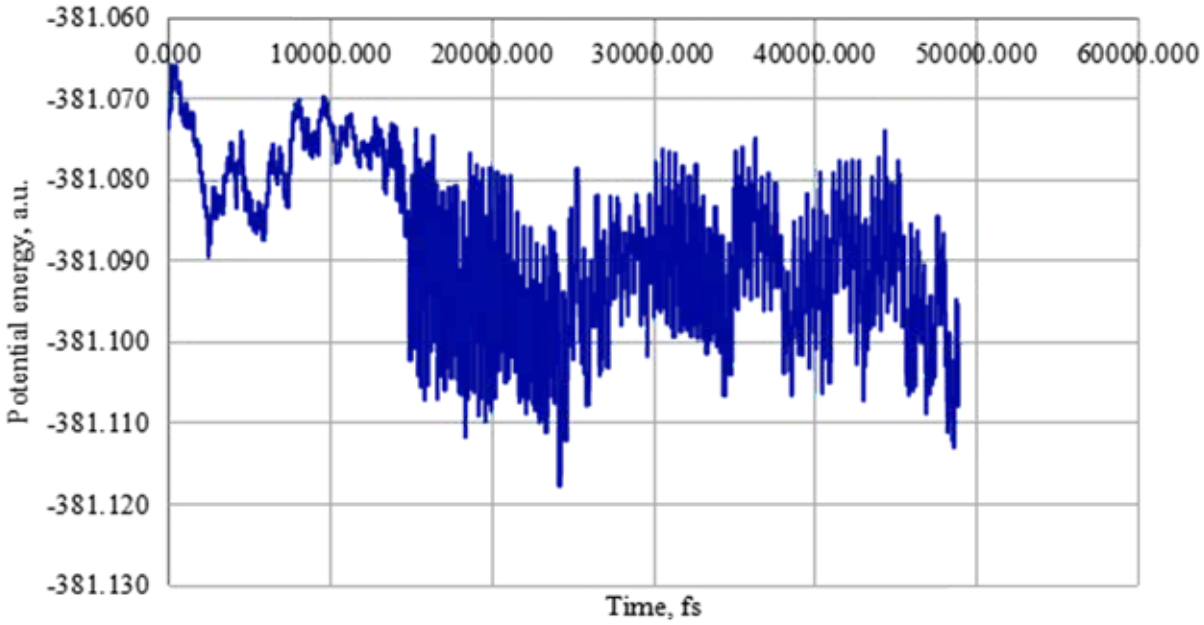
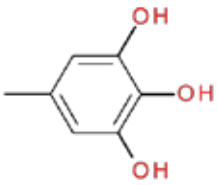
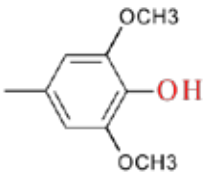
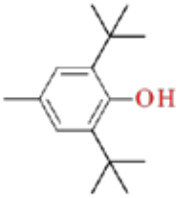


Figure 2. Dynamics of potential energy changes in the process of simulating homolytic cleavage of the OH bond of the phenolic hydroxyl

According to the results of the molecular dynamics simulation, the activation energy of a simulated reaction of the homolytic cleavage of phenolic hydroxyl with the transition of a hydrogen atom to a hydroxyl radical has been determined. The activation energy was 34.918 kJ/mol, which indicates that the reaction proceeds fairly quickly at a human body temperature (310 K).

It should be notified that this phenolic hydroxyl is surrounded by two ortho substituents, which have a shielding effect. The phenoxyl radical formed by the C-3 hydroxyl in structures 12, 13, and 14 belongs to the spatially obstructed types of radicals and is therefore more stable.

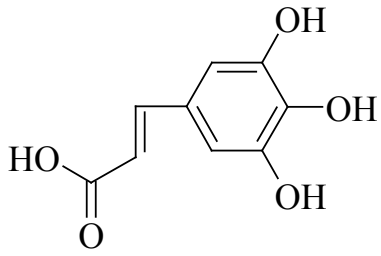
Table 7. Gibbs free energy of homolytic breaking of the H – O bond

Substance	Structuralfragment	Energy of O-Hsplittingbreaking
12x		-171.21
12k		-170.72
12f		-166.79
13x		-164.66
13k		-117.04
13f		-159.40
14x		-178.31
14k		-181.29
14f		-163.08

The same dependence as in the case of disubstituted ones, is observed here, i.e. the lower the energy of the homolytic breaking of the H – O bond in sterically hindered phenols, the higher the IUA values and the positive charge of the carbon atom, phenolic hydroxyl is bound with (in this case C-3). It should be notified that compound 14k (4-hydroxy-3,5-di-tertbutyl-cinnamic acid) was previously synthesized by us in accordance with the forecast [11], since its high activity had been predicted. An experimental study of the pharmacological properties confirmed our prediction: the substance is characterized by cerebroprotective [17], antioxidant [18], endothelioprotective [19] and actoprotective [20] types of activity.

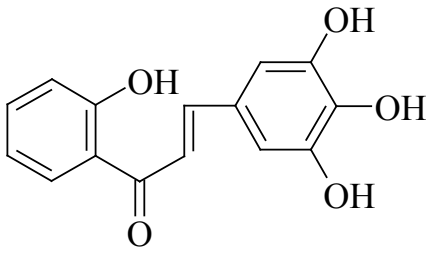
It is possible to predict with high probability the same level of activity for compounds 14x and 14f, since their quantum-chemical characteristics are almost identical with compound 14k.

With regard to the analysis of structures of all three types, we found it expedient to take into account the molar mass in the characterization of bonded numbers (Nμ), unsaturation index (IUA), and electron density. For this purpose, the total value of the listed characteristics was determined for each compound, which was then referred to the molar mass. The partial dividing of the total valuesof Nμ, IUA and electron density by the molar mass, in our opinion, characterizes the specific value of the listed parameters in terms of the mass unit of the molecule. In our opinion, a similar indicator in the future may be useful for the interpretation of biologically active related compounds. The results are presented below:



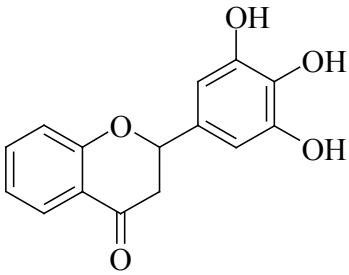
$C_9H_8O_5$ M=196

$$\frac{\sum_{N_\mu}}{M}=\frac{33.99}{196}=0.1734$$
$$\frac{\sum_{IUA}}{M}=\frac{1.41}{196}=0.0072$$
$$\frac{\sum_{el.d.}}{M}=\frac{35.685}{196}=0.1820$$



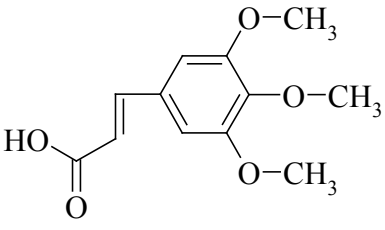
$C_{15}H_{12}O_5$ M=272

$$\frac{\sum_{N_\mu}}{M}=\frac{34.04}{272}=0.1251$$
$$\frac{\sum_{IUA}}{M}=\frac{1.41}{272}=0.0051$$
$$\frac{\sum_{el.d.}}{M}=\frac{35.874}{272}=0.1319$$



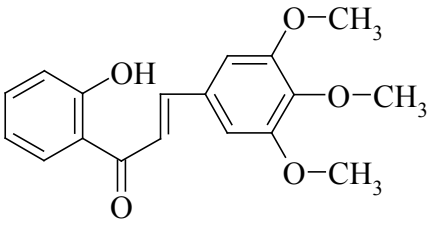
$C_{15}H_{12}O_5$ M=272

$$\frac{\sum_{N_\mu}}{M}=\frac{34}{272}=0.1259$$
$$\frac{\sum_{IUA}}{M}=\frac{1.42}{272}=0.0052$$
$$\frac{\sum_{el.d.}}{M}=\frac{35.205}{272}=0.1294$$



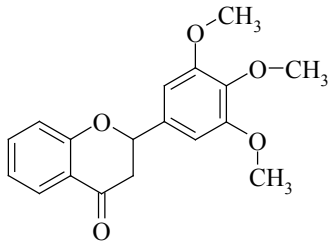
$C_{11}H_{12}O_5$ M=224

$$\frac{\sum_{N_\mu}}{M}=\frac{33.94}{224}=0.1515$$
$$\frac{\sum_{IUA}}{M}=\frac{1.49}{224}=0.0066$$
$$\frac{\sum_{el.d.}}{M}=\frac{35.798}{224}=0.1598$$



$C_{15}H_{16}O_5$ M=276

$$\frac{\sum_{N_\mu}}{M}=\frac{34}{276}=0.1231$$
$$\frac{\sum_{IUA}}{M}=\frac{1.49}{276}=0.0054$$
$$\frac{\sum_{el.d.}}{M}=\frac{35.974}{276}=0.1303$$



$C_{15}H_{16}O_5$ M=276

$$\frac{\sum_{N_\mu}}{M}=\frac{33.95}{276}=0.1230$$
$$\frac{\sum_{IUA}}{M}=\frac{1.51}{276}=0.0054$$
$$\frac{\sum_{el.d.}}{M}=\frac{35.906}{276}=0.1301$$

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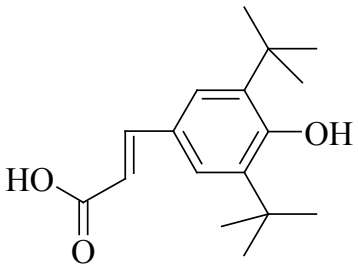
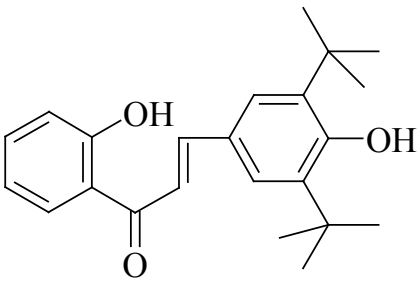


$C_{17}H_{24}O_3$ M=276

$$\frac{\sum_{N_\mu}}{M} = \frac{34.12}{276} = 0.1236$$

$$\frac{\sum_{IUA}}{M} = \frac{1.32}{276} = 0.0047$$

$$\frac{\sum_{el.d.}}{M} = \frac{35.974}{276} = 0.1303$$

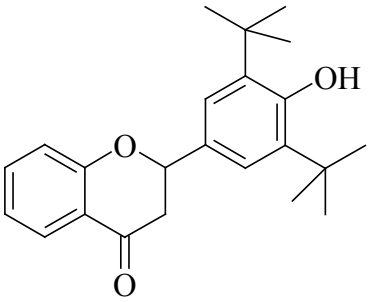


$C_{23}H_{28}O_3$ M=352

$$\frac{\sum_{N_\mu}}{M} = \frac{34.24}{352} = 0.0972$$

$$\frac{\sum_{IUA}}{M} = \frac{1.31}{352} = 0.0037$$

$$\frac{\sum_{el.d.}}{M} = \frac{36.204}{352} = 0.1028$$



$C_{23}H_{28}O_3$ M=352

$$\frac{\sum_{N_\mu}}{M} = \frac{34.13}{352} = 0.0970$$

$$\frac{\sum_{IUA}}{M} = \frac{1.36}{352} = 0.0386$$

$$\frac{\sum_{el.d.}}{M} = \frac{36.116}{352} = 0.1026$$

The attention of the scientistshas been attracted to very close values of specific indicators of bond numbers, indices of unsaturation and electron density for chalcone and flavanone. This fact once again confirms not only the ease of their interconversion, but, apparently, the same level of pharmacological properties.

CONCLUSION

Cinnamic acid differs from chalcone and flavanone in the absence of an aryl residue in its molecule, directly associated with carboxyl carbon, hence there are some differences in the values of quantum-chemical characteristics. But anyhow, all the three types of compounds can be successfully used to bind a hydroxyl radical in order to prevent those detrimental effects that the hydroxyl radical can cause, being, by Vladimirov Yu.A. metaphor, a destroyerradical, a killer radical.

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Conflict of interest

The authors declare no conflict of interest.

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MENTE ET ANIMO
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