Научно-практический журнал
Scientific and Practical Journal

ISSN 2307-9266
e-ISSN 2413-2241

ФАРМАЦИЯ И ФАРМАКОЛОГИЯ

PHARMACY & PHARMACOLOGY

2020 Том / Volume VIII

№ 2

Обзоры, лекции
Reviews, Lectures

Фармакогнозия, ботаника
Pharmacognosy, Botany

Фармацевтическая технология и биотехнология
Pharmaceutical Technology and Biotechnology

Фармацевтическая и токсикологическая химия
Pharmaceutical and Toxicological Chemistry

Фармакология и клиническая фармакология
Pharmacology and Clinical Pharmacology

Информационные технологии в фармации
Information Technologies in Pharmacy

Организация и экономика фармацевтического дела
Organization and Economy of Pharmacy

Экономика и менеджмент медицины
Economy and Management of Medicine

Фармацевтическое образование
Pharmaceutical Education

Краткие сообщения
Brief Reports

Дискуссии, рецензии, юбилеи, научные школы, история фармации и фармакологии
Discussions, Referee Reports, Anniversaries, Schools of Thought, History of Pharmacy and Pharmacology

www.pmedpharm.ru www.pharmpharm.ru

ПМФИ
Филиал ВолгГМУ

Scientific and Practical Journal

PHARMACY & PHARMACOLOGY

Scientific and practical journal
Volume VIII, Issue 2, 2020
The mass media registration certificate:
ПИ №ФС77–67428 от 13.10.2016
ISSN 2307-9266 e-ISSN 2413-2241

Founder: Volgograd State Medical University. 1, Pavshikh Bortsov Sq., Volgograd, Russia, 400131
Editors office address: 11, Kalinin ave., Pyatigorsk, Russia, 357532
Pyatigorsk Medical and Pharmaceutical Institute – branch of Volgograd State Medical University
Phone number: +7(8793) 32-44-74. E-mail: pharmjournal@mail.ru
www.pharmpharm.ru
Union catalogue. Russian Press / Newspapers an journals. Code 94183
A4 size, 1000 issues circulation. Price free

Journal "Pharmacy & Pharmacology" is recommended International Comittee Of Medical Journal Editors and included in Higher Attestation Commission, Scopus, Web of Science (ESCI), Russian citation database, eLibrary, ARISTI (All-Russian Institute of Scientific and Technical Information), RSL (Russian State Library), CyberLeninka, Socionet, EMBASE, Chemical Abstracts (CAS), Directory of Open Access Journals (DOAJ), EBSCO Discovery Service, RNMJ, University of CAMBRIDGE, Ulrich'sWeb, Google Scholar, Biefeld Academic Search Engine (BASE), Directory of Open Access Scholarly Resources (ROAD), Research Bible, Open Archives Initiative, Academic Keys, JournalTOCs, WorldCat, OpenAIRE, University of Oxford, The British Library, Universitait Gent, Université de Montréal, University of Saskatchewan.

Printed in the LLC "Amirit" in accord with provided materials, 410004, Saratov, 88, Chernishevsky Str.

Научно-практический журнал

ФАРМАЦИЯ И ФАРМАКОЛОГИЯ

Периодичность 6 номеров в год

Том 8, Выпуск 2, 2020

Свидетельство регистрации СМИ:
ПИ №ФС77–67428 от 13.10.2016 г.

ISSN 2307-9266 e-ISSN 2413-2241

Учредитель: Федеральное государственное бюджетное образовательное учреждение высшего образования «Волгоградский государственный медицинский университет» Минздрава России.
400131, Россия, г. Волгоград, площадь Павших Борцов, д. 1
Адрес издательства: 357532, г. Пятигорск, пр-т Калинина, 11.
Пятигорский медико-фармацевтический институт – филиал ФГБОУ ВО ВолгГМУ Минздрава России
Телефон: +7 (8793) 32-44-74. E-mail: pharmjournal@mail.ru
www.pharmpharm.ru
Объединенный каталог. Пресса России. Газеты и журналы. Индекс 94183
Формат А4, тираж 1000 экз. Цена свободная.

Журнал «Фармация и фармакология» включен в перечень рецензируемых научных изданий, входящих в международные реферативные базы данных и системы цитирования, и в соответствии с пунктом 5 правил формирования перечня рецензируемых научных изданий, в которых должны быть опубликованы основные научные результаты диссертаций на соискание ученой степени кандидата наук, на соискание ученой степени доктора наук (Перечень ВАК), Scopus, Web of Science (ESCI), РИНЦ, eLibrary, ВИНИТИ, РГБ, Киберленинка, Соционет, EMBASE, Chemical Abstracts (CAS), Directory of Open Access Journals (DOAJ), EBSCO Discovery Service, RNMJ, University of CAMBRIDGE, Ulrich'sWeb, Google Scholar, Biefeld Academic Search Engine (BASE), Directory of Open Access Scholarly Resources (ROAD), Research Bible, Open Archives Initiative, Academic Keys, JournalTOCs, WorldCat, OpenAIRE, University of Oxford, The British Library, Universitait Gent, Université de Montréal, University of Saskatchewan.

Отпечатано в соответствии с предоставленными материалами в ООО «Амирит», 410004, г. Саратов, ул. Чернышевского, 88.

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY

СОДЕРЖАНИЕ / CONTENS

Оригинальные статьи / Research Articles

Pharmacology and Clinical Pharmacology / Фармакология и клиническая фармакология

A.A. Spasov, A.F. Kucheryavenko, K.A. Gaidukova, M.V. Chernikov, O.N. Zhukovskaya

ANTITROMBOTIC ACTIVITY OF A NEW BENZIMIDAZOLE DERIVATIVE WITH A SPATIALLY DIFFICULT PHENOLIC SUBSTITUTE IN ITS STRUCTURE 78

A.V. Kalashnikov, A.A. Vorobiev, S.A. Kalashnikova, D.Sh. Salimov

COMPLEX BIOSTIMULATION OF INTRAPLEURAL ADHESIOGENESIS IN THORACAL SURGERY 86

O.A. Puchenkova, S.V. Nadezhdin, M.A. Zhuchenko, V.O. Soldatov, M.V. Kubekina, D.S. Korshunova, E.N. Korshunov, L.V. Korokina, P.A. Golubinskaya, A.L. Kulikov, V.V. Gureev, V.M. Pokrovskiy, E.A. Patrakhanov, P.R. Lebedev, T.A. Denisyuk, V.S. Belyaeva, E.A. Movchan, E.I. Lepetukha, M.V. Pokrovskiy

STUDY OF ANTIATHEROSCLEROTIC AND ENDOTHELIOPROTECTIVE ACTIVITY OF PEPTIDE AGONISTS OF EPOR/CD131 HETERORECEPTOR 100

А.А. Спасов, А.Ф. Кучерявенко, К.А. Гайдукова, М.В. Черников, О.Н. Жуковская

АНТИТРОМБОТИЧЕСКАЯ АКТИВНОСТЬ НОВОГО ПРОИЗВОДНОГО БЕНЗИМИДАЗОЛА, ИМЕЮЩЕГО В СВОЕЙ СТРУКТУРЕ ПРОСТРАНСТВЕННО ЗАТРУДНЕННЫЙ ФЕНОЛЬНЫЙ ЗАМЕСТИТЕЛЬ 78

А.В. Калашников, А.А. Воробьев, С.А. Калашникова, Д.Ш. Салимов

КОМПЛЕКСНАЯ БИОСТИМУЛЯЦИЯ ВНУТРИПЛЕВРАЛЬНОГО АДГЕЗИОГЕНЕЗА В ТОРАКАЛЬНОЙ ХИРУРГИИ 86

О.А. Пученкова, С.В. Надеждин, М.А. Жученко, В.О. Солдатов, М.В. Кубекина, Д.С. Коршунова, Е.Н. Коршунов, Л.В. Корокина, П.А. Голубинская, А.Л. Куликов, В.В. Гуреев, В.М. Покровский, Е.А. Патраханов, П.Р. Лебедев, Т.А. Денисюк, В.С. Беляева, Е.А. Мовчан, Е.И. Лепетюха, М.В. Покровский

ИЗУЧЕНИЕ АНТИАТЕРОСКЛЕРОТИЧЕСКОЙ И ЭНДОТЕЛИОПРОТЕКТИВНОЙ АКТИВНОСТИ ПЕПТИДНЫХ АГОНИСТОВ ГЕТЕРОРЕЦЕПТОРА EPOR/CD131 100

Informational Technologies in Pharmacy / Информационные технологии в фармации

E.T. Oganesyan, S.S. Shatokhin

USING QUANTUM-CHEMICAL PARAMETERS FOR PREDICTING ANTI-RADICAL (HO·) ACTIVITY OF RELATED STRUCTURES CONTAINING A CINNAMOYL FRAGMENT II. DERIVATIVES OF 2',4'-DIHYDROXYCHALCONE, FLAVANONE AND FLAVONE, CONTAINING A HYDROXY GROUP I N POSITION 7 112

Э.Т. Оганесян, С.С. Шатохин

ИСПОЛЬЗОВАНИЕ КВАНТОВО-ХИМИЧЕСКИХ ПАРАМЕТРОВ ДЛЯ ПРОГНОЗИРОВАНИЯ АНТИРАДИКАЛЬНОЙ (HO·) АКТИВНОСТИ РОДСТВЕННЫХ СТРУКТУР, СОДЕРЖАЩИХ ЦИННАМОИЛЬНЫЙ ФРАГМЕНТ. II. ПРОИЗВОДНЫЕ 2',4'-ДИГИДРОКСИХАЛКОНА, А ТАКЖЕ ФЛАВАНОНА И ФЛАВОНА, СОДЕРЖАЩИЕ ГИДРОКСИГРУППУ В ПОЛОЖЕНИИ 7 112

Reviews, Lectures / Обзоры, лекции

V.M. Kishchenko, V.V. Vernikovsky, I.M. Privalov, A.M. Shevchenko

FILMS IN RUSSIAN MEDICINE AND COSMETOLOGY: DEVELOPMENT HISTORY, CLASSIFICATION, TECHNOLOGY 124

A.A. Orlova, M.N. Povydysh

CHEMICAL CONSTITUENTS OF GEUM RIVALE L. AND THEIR BIOLOGICAL ACTIVITY 133

В.М. Кищенко, В.В. Верниковский, И.М. Привалов, А.М. Шевченко

ПЛЕНКИ В РОССИЙСКОЙ МЕДИЦИНЕ И КОСМЕТОЛОГИИ: ИСТОРИЯ РАЗВИТИЯ, КЛАССИФИКАЦИЯ, ТЕХНОЛОГИЯ 124

А.А. Орлова, М.Н. Повыдыш

ХИМИЧЕСКИЕ КОМПОНЕНТЫ GEUM RIVALE L. И ИХ БИОЛОГИЧЕСКАЯ АКТИВНОСТЬ 133

RESEARCH ARTICLE

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY

ANTITROMBOTIC ACTIVITY OF A NEW BENZIMIDAZOLE DERIVATIVE WITH A SPATIALLY DIFFICULT PHENOLIC SUBSTITUTE IN ITS STRUCTURE

A.A. Spasov[1], A.F. Kucheryavenko[1], K.A. Gaidukova[1], M.V. Chernikov[2], O.N. Zhukovskaya[3]

[1] Volgograd State Medical University
1, Pavshikh Bortsov Square, Volgograd, Russia, 400131
[2] Pyatigorsk Medical and Pharmaceutical Institute – a branch of Volgograd State Medical University, 11, Kalinin av., Pyatigorsk, Russia, 357532
[3] Research Institute of Physical and Organic Chemistry, Southern Federal University
194, Bldg 2, Stachki Av., Rostov-on-Don, Russia, 344090

E-mail: aspasov@mail.ru

Received 10 February 2020 Review (1) 10 April 2020 Review (2) 20 April 2020 Accepted 28 April 2020

The aim of the study was to investigate antithrombogenic properties of compound RU-1144 with previously identified pronounced antiplatelet and antioxidant activities. The thrombosis induced by *Ferric chloride* ($FeCl_3$) was carried out in rats' carotid artery, in comparison with the known antiaggregant drugs – acetylsalicylic acid (ASA) and clopidogrel, as well as with the antioxidant preparation – ethylmethylhydroxypyridine succinate (EMHPS).
Materials and methods. The antithrombotic activity of compound RU-1144 was studied on the model of the rats with carotid artery thrombosis, induced by the application of 50% *ferric chloride* ($FeCl_3$), and the Global Thrombosis Test model (the *Görög Thrombosis Test*). The evaluation of this type of activity was carried out by prolonging the time of a blood clot formation. The studies of the compound RU-1144 effect on the bleeding time parameter were performed in mice. Acetylsalicylic acid, clopidogrel and EMHPS were used as reference drugs.
Results. The antithrombotic effect of the RU-1144 substance revealed in the model of arterial thrombosis induced by the application of *ferric chloride* ($FeCl_3$), exceeded that of both acetylsalicylic acid and clopidogrel by 3.5 times and that of EMHPS by 2.9 times. In the model of the *in vitro* Global Thrombosis Test (the *Görög Thrombosis Test*), compound RU-1144 reduced the thrombogenic potential of the blood equally with acetylsalicylic acid and clopidogrel. The assessment of "the bleeding time", caused by the RU-1144 substance, showed that the prolongation of bleeding was twice as less pronounced than that caused by ASA and clopidogrel.
Conclusion. The performed studies demonstrated a pronounced antithrombotic activity of compound RU-1144, which exceeded that of acetylsalicylic acid, clopidogrel and EMHPS, while the ability to prolong the bleeding time was reliably lower than that of reference drugs.
Keywords: antithrombotic activity, thrombosis, benzimidazole, ASA, clopidogrel, ethylmethylhydroxypyridine succinate, the *Görög Thrombosis Test*, bleeding time
Abbreviations: EMHPS – ethylmethylhydroxypyridine succinate; ASA – acetylsalicylic acid.

АНТИТРОМБОТИЧЕСКАЯ АКТИВНОСТЬ НОВОГО ПРОИЗВОДНОГО БЕНЗИМИДАЗОЛА, ИМЕЮЩЕГО В СВОЕЙ СТРУКТУРЕ ПРОСТРАНСТВЕННО ЗАТРУДНЕННЫЙ ФЕНОЛЬНЫЙ ЗАМЕСТИТЕЛЬ

А.А. Спасов[1], А.Ф. Кучерявенко[1], К.А. Гайдукова[1], М.В. Черников[2], О.Н. Жуковская[3]

[1] Федеральное государственное бюджетное учреждение высшего образования «Волгоградский государственный медицинский университет» Министерства здравоохранения Российской Федерации
400131, Россия, г. Волгоград, площадь Павших Борцов, д. 1
[2] Пятигорский медико-фармацевтический институт – филиал федерального государственного бюджетного образовательного учреждения высшего образования «Волгоградский государственный медицинский университет» Министерства здравоохранения Российской Федерации
357532, Россия, Ставропольский край, г. Пятигорск, пр. Калинина, 11
[3] НИИ физической и органической химии Южного федерального университета
344090, г. Ростов-на-Дону, пр. Стачки, 194/2

E-mail: aspasov@mail.ru

Получено 10.02.2020 Рецензия (1) 10.04.2020 Рецензия (2) 20.04.2020 Принята к печати 28.04.2020

For citation: Spasov A.A., Kucheryavenko A.F., Gaidukova K.A., Chernikov M.V., Zhukovskaya O.N. Antithrombotic activity of a new benzimidazole derivative with a spatially difficult phenolic substitute in its structure. *Pharmacy & Pharmacology*. 2020;8(2):78-85. **DOI:** 10.19163/2307-9266-2020-8-2-78-85

Для цитирования: Спасов А.А., Кучерявенко А.Ф., Гайдукова К.А., Черников М.В., Жуковская О.Н. Антитромботическая активность нового производного бензимидазола, имеющего в своей структуре пространственно затрудненный фенольный заместитель. *Фармация и фармакология.* 2020;8(2):78-85. **DOI:** 10.19163/2307-9266-2020-8-2-78-85

Научно-практический журнал
ФАРМАЦИЯ И ФАРМАКОЛОГИЯ

ОРИГИНАЛЬНАЯ СТАТЬЯ

DOI: 10.19163/2307-9266-2020-8-2-78-85

Цель – изучение антитромбогенных свойств соединения РУ-1144 с ранее выявленной выраженной антиагрегантной и антиоксидантной активностью, на модели артериального тромбоза сонной артерии крыс, индуцированного хлоридом железа (III), в сравнении с известными антиагрегантными препаратами – ацетилсалициловой кислотой и клопидогрелом, а также антиоксидантным препаратом – этилметилгидроксипиридина сукцинат.
Материалы и методы. Антитромботическая активность соединения РУ-1144 была изучена на модели артериального тромбоза сонной артерии крыс, вызванного аппликацией 50% хлорида железа (III) и модели Global Thrombosis Test (по Горогу). Оценку данного вида активности производили по удлинению времени образования тромба. Исследования влияния соединения РУ-1144 на параметр времени кровотечения проводили на мышах. В качестве препаратов сравнения использовали ацетилсалициловую кислоту, клопидогрел и ЭМГПС.
Результаты. Выявленное на модели артериального тромбоза, индуцированного аппликацией хлорида железа (III), антитромботическое действие субстанции РУ-1144, превосходило таковое как у ацетилсалициловой кислоты, так и у клопидогрела в 3,5 раза, и в 2,9 раза – у ЭМГПС. На модели Global Thrombosis Test (тест Горога) *in vitro* соединение РУ-1144 снижало тромбогенный потенциал крови в равной степени с ацетилсалициловой кислотой и клопидогрелом. При оценивании «времени кровотечения» вещество РУ-1144 пролонгировало кровотечение в среднем в 2 раза менее выражено, чем АСК и клопидогрел.
Заключение. Проведенные исследования продемонстрировали у соединения РУ-1144 выраженную антитромботическую активность, превышающую таковую у ацетилсалициловой кислоты, клопидогрела и ЭМГПС, при этом способность удлинять время кровотечения была достоверно ниже, чем у препаратов сравнения.
Ключевые слова: антитромботическая активность, тромбоз, бензимидазол, АСК, клопидогрел, этилметилгидроксипиридина сукцинат, тромбоз по Горогу, время кровотечения
Сокращения: ЭМГПС – этилметилгидроксипиридина сукцинат; АСК – ацетилсалициловая кислота.

INTRODUCTION

Cardiovascular diseases are currently the leading cause of global disability and mortality worldwide [1–3]. According to the World Health Organization, by 2030, more than 20 million deaths per year from the diseases associated with an increase in blood thrombogenic potential, will have been registered. Among them there is a coronary heart disease, a stroke, impaired peripheral circulation, complications of diabetes mellitus, therefore, antiplatelet therapy is an important component in various areas of clinical practice [4].

It is known that an atherosclerotic plaque causes narrowing of the vessel section and, as a result, when the bloodstream passes through this place, turbulent accelerations occur. In their turn, they affect blood corpuscles primarily erythrocytes and thrombocytes, increasing their aggregation ability. In addition to the formed elements, the vessel wall is exposed, which results in the endothelium damage. There is also the possibility of collagen fibers to come into contact with thrombocytes, followed by their adhesion to the damaged surface, activation, aggregation, and thrombus formation [5].

Thus, the activation of the platelet element of hemostasis, can become a cause of complications on behalf of the cardiovascular system, i. e. the formation of arterial thromboses [1]. Besides, an important role in the pathogenesis of thrombus formation is played by the activation of lipid peroxidation processes, the enhancement of which causes an increase in the thrombocyte aggregation and the coagulation element of hemostasis [6, 10]. This concept is the theoretical justification for the use of antioxidant agents as an additional pathogenetic therapy of arterial thromboses. Thus, timely and correct preventive measures aimed at inhibiting thrombocyte aggregation and lipid peroxidation, can prevent premature deaths, increase life expectancy, improve its quality, and reduce the economic costs of society for patients' treatment and rehabilitation [7–9].

In the course of the previous studies, among heterocyclic compounds, the substances exhibiting antiaggregant and antioxidant properties, had been identified [11–13]. The compound under code RU-1144 (1-(2,6-ditretbutyl-4-(1-hydroxyethyl)-phenyl-pyrimidobenzimidazole hydrochloride) can inhibit thrombocyte aggregation and lipid peroxidation, exceeding the reference drugs – acetylsalicylic acid, clopidogrel and ethylmethylhydroxypyridine succinate [31]. There are various methods for studying these types of activity [15, 16]. However, the most common is the study of antithrombotic properties in the models of arterial thromboses.

Therefore, the aim of the study was a comparative investigation of the antithrombotic activities of compound RU-1144 and antiaggregant drugs with a high evidence base – acetylsalicylic acid and clopidogrel, as well as the antioxidant agent EMGPS in the model of carotid arterial thromboses in rats, as well as in the model of the Global Thrombosis Test (the *Görög Thrombosis Test*) and their effect on "the bleeding time" parameters.

MATERIALS AND METHODS

Animals

The experiments were performed in 108 nonlinear white male rats weighing 250–300 g and 24 white mongrel male mice weighing 20–22 g, kept under vivarium conditions (the temperature of 22–24 °C, the relative humidity of 40–50%) with natural light on a standard diet (GOST R 50258-92). All the animals were obtained from the nursery of the Research Center for Biomedical Technologies, Ltd. The animals were kept under standard conditions in accordance with the Decree of the Chief State Sanitary Doctor of the Russian Federation No. 51 dated 29.08.2014, "On approval of SP 2.2.1.3218-14 "Sanitary and epidemiological requirements for the

design, equipment and maintenance of experimental biological clinics (vivariums)". All the animals (rats and mice) were quarantined for 14 days in separate vivarium boxes of the FSBEI HE of Volgograd State Medical University of the Ministry of Health of Russia. During the quarantine, the body weight of the animals was measured more than twice (on the 1st and the 14th days). The clinical condition was monitored in the groups every day by visual inspection. The animals with deviations found out during the examination, were excluded from the experimental groups. During the study, all the procedures with the animals were carried out in accordance with generally accepted ethical standards for the treatment of animals adopted by the European Convention for the Protection of Vertebrate Animals used for experimental and other scientific purposes (1986) and considering the International Recommendations of the European Convention for the Protection of Vertebrate Animals used in experimental research (1997). All the procedures with the animals were performed in accordance with the standards, outlined in the eighth edition of the Guide for the Care and Use of Laboratory Animals and ARRIVE (Animal Research: Reporting of In Vivo Experiments). The experimental study was approved by the Regional Research Ethics Committee of the Volgograd Region, protocol No. 2083-2016, dated November 18, 2016. This study was carried out in accordance with the requirements of the "Guidelines for the Preclinical Studies of Medicines" [15].

Study design

An experimental study of the antithrombotic activity of the benzimidazole derivative under code RU-1144 (1-(2,6-ditretbutyl-4-(1-hydroxyethyl)-phenyl-pyrimidobenzimidazole hydrochloride) (Scientific Research Institute of Physicochemical Physics of Southern Federal University), which has a spatially hindered phenolic substituent in its structure, has been carried out. Well known antiaggregant agents with a high evidence of activities – acetylsalicylic acid (Sigma, USA) and clopidogrel (Sanofi, France), and an antioxidant agent – ethylmethylhydroxypyridinesuccinate (EMGPS ®, Pharmasoft LLC, Russia) [16] – had been chosen as reference drugs. Compound RU-1144 and reference drugs were administered intragastrically with the help of an intragastric tube.

As a solvent, purified distilled water was used. The antithrombotic activities of compound RU-1144 and reference drugs were studied in the model of rats' carotid arterial thromboses caused by the surface application of a 50% solution of *ferric chloride* ($FeCl_3$). The studied substances were administered to rats intragastrically once, 2 hours before the application of a thrombotic agent to the carotid artery of the animals [17]. 30 minutes before the start of the experimental arterial thromboses, the rats were intraperitoneally anaesthetized with chloral-hydrate (400 mg/kg). After the onset of anaesthesia, the skin and tissues were opened in layers, highlighting the carotid artery. A cotton pad moistened with a 50% solution of *ferric chloride* ($FeCl_3$) (0.025 ml) was placed on a small area of the carotid artery. The surrounding tissues were isolated with the help of a special "Parafilm" film. To record the changes in the blood flow, a Minimax-Doppler-K ultrasound dopplerograph (Minimax, St. Petersburg) was used. The ultrasound probe of the apparatus was installed at a small distance from the cotton pad, placed on the carotid artery. The blood flow was recorded till the complete occlusion of the vessel.

Compound RU-1144 and the reference drugs were studied at the doses of an equimolar dose of 19 mg/kg of acetylsalicylic acid (a pharmacologically active dose obtained in the model of ADP-induced rat aggregation in an *in vivo* test). For the test substance RU-1144, this dose was 48 mg/kg, and for the reference drugs of clopidogrel and EMHPS it was 32 and 28 mg/kg, respectively). In order to determine the ED_{50} depending on the manifested antithrombotic effect, (the dose at which the studied compounds increase the time of the onset of the complete vessel occlusion by a thrombus to the control by 50%), the studied doses of the substances and the reference drugs were either increased or decreased. Compound RU-1144 was also studied at the doses of 24 and 12 mg/kg, ASA – at 100 and 150 mg/kg, clopidogrel – at 60; 120 and 180 mg/kg, and the comparison drug EMGPS was studied at the doses of 150 and 100 mg/kg, respectively.

The antithrombotic activity of compound RU-1144 was investigated using the Global Thrombosis Test *in vitro* with the *ex vivo* study of the biological material after a single intragastric administration at the dose of 18.8 mg/kg (a pharmacologically active dose obtained in the model of ADP-induced thrombocyte aggregation in rats in the *in vivo* test) [18]. The reference drugs – acetylsalicylic acid and clopidogrel – were studied at the doses of 28.5 and 13.8 mg/kg, respectively. 2 hours after the administration of the test compounds, the blood was drawn from the abdominal aorta with a 5 ml syringe containing 20 μM of ADP thrombocyte aggregation inducer. The animals had been pre-anaesthetized with chloral hydrate (400 mg/kg, intraperitoneally). The resulting blood was immediately placed in a special *Görög* tube without any addition of stabilizers and preservatives. The main criteria for evaluating the antithrombotic effect of the test compound and reference drugs were indicators of the occlusion time and the lysis time, the analysis of which was carried out using the GTT Draw 2.3 software.

In order to determine the undesirable effect of the

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2020-8-2-78-85

antiaggregant drugs, "the bleeding time in mice" model was used [19]. To reproduce this model, the animals were preliminarily anaesthetized using chloral hydrate at the dose of 400 mg/kg. After that 5 mm of the tip of the tail was cut off, then placed in a test tube with physiological saline in a water bath (37 °C).

To evaluate the effect, the time expressed in seconds, from the moment of cutting off the tip of the tail to the moment the bleeding stopped completely, was recorded. According to the effect on this parameter, compound RU-1144 was studied at the dose of 18.8 mg/kg and the reference drugs ASA and clopidogrel – at the doses of 28.5 and 13.8 mg/kg, respectively. The administration of the studied compounds was carried out 2 hours before the start of the experiment.

The groups of the control animals were given purified distilled water as a single dose in the equivalent volume intragastrically.

Statistical processing of results

Statistical processing of the experimental data was carried out using the Mann-Whitney criterion, the one-way ANOVA criterion with Bonferroni correction using the GraphPad Prism 5.0 statistical software package ("GraphPad", USA) and Microsoft Excel 2007 (Microsoft, USA).

RESULTS

In the course of the arterial thrombosis study, the data indicating the presence of antithrombotic properties of the test substance and reference drugs, were obtained.

The average time of the carotid artery occlusion of the control group animals, was 19.4±1.5 min. (Table 1), which is consistent with the published data [23, 26].

Compound RU-1144 at the dose of 48 mg/kg, reliably prolonged the time of the carotid artery complete occlusion to 31.4 minutes, which was 61.1% reliably higher than this indicator in the control group animals. With a further decrease of the test compound dose to 24 mg/kg, the time of thrombus formation also reliably decreased and amounted to 27 minutes. A further dose reduction to 12 mg/kg, reliably prolonged the onset of the carotid artery complete occlusion by 14.1% (Table 1).

At the dose of 19 mg/kg, ASA unreliably with respect to the control, prolonged the time of thrombus formation by 6.4%. Therefore, in the further study, the doses of acetylsalicylic acid were increased to 100 and 150 mg/kg. At the same time, at the dose of 100 mg/kg, the reference drug increased the time of the onset of the carotid artery complete occlusion by 29.5%, and at the dose of 150 mg/kg – by 58.5%. Thus, an increase of the dose of acetylsalicylic acid increased the studied parameter by 58.5% (Table 1).

At the dose of 32 mg/kg, clopidogrel reliably extended the thrombosis time by 9.0% compared with the control group animals. A further increase of the dose to 60 mg/kg and then to 120 and 180 mg/kg, led to an increase in time till the complete occlusion of the carotid artery by 21.8, 34.6 and 65.4%, respectively (Table 1).

At the dose of 28 mg/kg, EMHPS increased the onset time of the rats' carotid artery complete occlusion by 8.11%. The increase of the drug doses to 100 and 150 mg/kg, led to the prolongation of this indicator by 41.03 and 75.21%, respectively.

Based on the obtained data, ED_{50} antithrombotic activities of compound RU-1144 and reference drugs were calculated. So, for the tested RU-1144 sample, this value was 37.8 mg/kg, for acetylsalicylic acid – 133.0 mg/kg, and for clopidogrel and EMHPS – 132.0 and 108.4 mg/kg, respectively. Thus, in terms of ED_{50} antithrombotic activities, RU-1144 compound exceeded the antiaggregant drugs – acetylsalicylic acid and clopidogrel – by 3.52 and 3.49 times, respectively, and the antioxidant agent EMHPS – by 2.87 times.

At the next stage, the antithrombotic activities of compound RU-1144 and the reference drugs of ASA and clopidogrel, were studied in the Global Thrombosis Test model (the *Görög Thrombosis Test*). When performing this experiment in the control group of the animals, the onset of the complete occlusion in the test system was 95.2 seconds (Table 2). The study of the biological material of the animals administered intragastrically with compound RU-1144, showed a statistically reliable increase in the time of the complete occlusion onset by 37% compared with the values obtained in the control group, and amounted to 130.5 seconds. At the same time, the test compound unreliably increased the lysis time relative to the control (Table 2).

The reference drug ASA, studied at the dose of 28.5 mg/kg, also reliably led to the prolongation of the complete occlusion time in the test system, while this indicator was 1.2 times higher than that of the control group animals, though not affecting the lysis clot time.

In the group of the animals treated with clopidogrel, the occlusion time was 57.6% longer than in the control group, but the lysis time was comparable to the values obtained in the control group animals (Table 2).

Thus, the results of the study obtained in this model, showed that the greatest antithrombotic effect was demonstrated by the reference drug clopidogrel, which, in the studied dose, increased the occlusion time of the test system by 1.7 times, unreliably exceeding compound RU-1144 and reliably exceeding acetylsalicylic acid by 1, 3 times. The studied compounds have shown no effect on the rate of lysis either.

Table 1 – The effect of compound RU-1144, ASA, clopidogrel and EMHPS on the complete occlusion time of rats' carotid artery in the model of arterial thromboses induced by the application of *ferric chloride* ($FeCl_3$) (M±m, n=6)

No. in sequence	Tested samples	Dose, mg/kg	Thrombus formation time, min	Δ% of prolonging thrombus formation time	ED_{50}, mg/kg
1	Control		19.4±1.5		
2	RU-1144	12	22.3±0.7*	14.1±3.6*	37.8
		24	27.0±0.6*	38.5±2.8*	
		48	31.4±1.0*	61.1±5.4*	
3	ASA	19	20.8±0.3*	6.4±1.6*	133.0
		100	25.3±0.5*	29.5±2.5*	
		150	30.9±0.3*	58.5±1.4*	
4	Clopidogrel	32	21.3±0.3*	9.0±1.3*	132.0
		60	23.8±0.3*	21.8±1.6*	
		120	25.8±0.4*	34.6±1.3*	
		180	32.3±0.4*	65.4±2.2*	
5	EMHPS	28	21.1±0.3*	8.1±1.6*	108.4
		100	27.5±0.6*	41.0±2.9*	
		150	34.2±0.8*	75.2±4.3*	

Notes:
n – the number of the animals in the group;
*– the data relative to the control (the Mann-Whitney test, p≤0.05).

Table 2 – Antithrombotic activities of compound RU-1144 and reference drugs – ASA and clopidogrel – in the model of the *ex vivo* Global Thrombosis Test (the *Görög Thrombosis Test*) (M±m, n=6)

No. in sequence	Tested samples	Dose, mg/kg	Occlusion time, sec	Lysis time, sec
1	Control	–	95.2 ± 1.4	635.2 ± 29.0
2	RU-1144	18.8	130.5 ± 7.8*	711.2 ± 39.4
3	ASA	28.5	117.5 ± 4.1*	629.3 ± 15.7
4	Clopidogrel	13.8	150.0 ± 4.0*	631.5 ± 17.1

Notes:
n – the number of the animals in the group;
* – the data reliable relative to the control (the Mann-Whitney test, p<0.05).

Table 3 – The effects of compound RU-1144 and reference drugs on the time of bleeding from the mice's tail veins, at the doses of ED_{50} antiaggregant activity *in vivo* (M±m, n=6)

No. in sequence	Tested samples	Dose, mg/kg	Bleeding time, sec	Δ% of prolonging thrombus formation time
1	Control		349.3 ± 7.2	
2	RU-1144	18.8	445.5 ± 10.5*#	27.5 ± 3.0*#
3	ASA	28.5	583.9 ± 9.1*	67.2 ± 2.6*
4	Clopidogrel	13.8	566.0 ± 10.0*	62.0 ± 2.9*

Notes:
n – the number of the animals in the group;
the data reliable relative to the control, one-way ANOVA criterion with Bonferroni correction (p<0.05);
– the data reliable relative to the reference drugs, one-way ANOVA criterion with Bonferroni correction (p <0.05).

Table 4. Antiaggregant activities of compound RU-1144 and reference drugs in *in vitro* (IC_{50}) and *in vivo* (ED_{50}) studies

No. in sequence	Tested samples	IC_{50}, μM	ED_{50}, mg/kg
1	RU-1144	5.5	18.8
2	ASA	120.0	28.5
3	Clopidogrel	–*	13.8

Note:
* – in view of being a prodrug, clopidogrel cannot be used in in vitro tests.

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2020-8-2-78-85

Thus, compound RU-1144 led to a reliable increase in the bleeding time by 27.5% relative to the control mouse group, while ASA and clopidogrel prolonged the bleeding time twice as active – by 67.2 and 62.0%, respectively (Table 3). Thus, compound RU-1144 had a weaker effect on this parameter than the reference drugs.

DISCUSSION

Complex mediator interactions between thrombocytes with the involvement of various aggregation factors. play a significant role in initiating the processes of the arterial thrombosis development. Preliminary studies of the antiaggregant activity of compound RU-1144 in comparison with clopidogrel and acetylsalicylic acid, both *in vitro* and *in vivo*, made it possible to determine the inhibitory concentrations (IC_{50}) and effective doses (ED_{50}) of these compounds (Table 4).

As Table 4 shows, in the *in vitro* studies, compound RU-1144 predominates over the ASA activity by 21.8 times, *in vivo – by* 1.5 times, while it is 1.3 times weaker than clopidogrel. According to the published data, by application of 50% *ferric chloride* ($FeCl_3$), in the thrombotic lesion of the vascular wall Fe directly interacts with hydrogen peroxide, causing the formation of hydroxyl anions and a change of thrombocyte membranes in the phospholipid composition. The above-mentioned processes increase the functional activity of thrombocytes [21–23, 29].

Besides, oxidized fibrinogen accumulates in blood, activating the process of thrombus formation. Lipid peroxidation in endotheliocyte membranes leads to systemic endothelial dysfunctions and increases its penetration. This model of thrombosis allows us to study the effect of the compounds on the formation rate of an arterial (white) thrombus, which mainly consists of thrombocytes. That is why this model was chosen to study compound RU-1144, combining two types of activity: antiaggregant and antioxidant.

Based on the data obtained, it can be concluded that the compound RU-1144 exhibits a pronounced antithrombotic activity which exceeds that of the reference drugs due to its ability to inhibit thrombocyte aggregation and lipid peroxidation, and thereby prevent the occurrence of arterial thrombosis in the rats' carotid artery.

The Global Thrombosis Test makes it possible to research not only the antithrombotic but also the thrombolytic activities of the compounds. Under the conditions of increased turbulence of blood flow in the *Görög Thrombosis Test*, compound RU-1144 showed the ability to increase the time of thrombus formation compared to the values in the control group animals and the unreliable predominancy of the activity compared to acetylsalicylic acid, however, it was unreliably weaker than clopidogrel. The lysis time under the action of the test sample did not change, which makes it possible to conclude that RU-1144 compound does not have any fibrinolytic activity.

It is known that during a prolonged therapy with antiaggregant drugs, a side effect such as bleeding, is observed. Bleedings in the gastrointestinal tract are most often, and intracranial hemorrhages resulting in increased risks of ischemic events, are not uncommon either [25, 27, 28].

The study showed that the compound RU-1144 increases the bleeding time from the tail vein of mice, however, in contrast to ASA and clopidogrel, this effect is less pronounced. That makes it possible to suppose a low probability of a side effect in the form of bleeding.

CONCLUSION

In the model of rats' carotid arterial thrombosis induced by a 50% solution of *ferric chloride* ($FeCl_3$), the compound under the laboratory code RU-1144 exerts a pronounced antithrombotic effect, exceeding that of the antiaggregant drugs – acetylsalicylic acid and clopidogrel – by 3.52 and 3.49 times, respectively, and the antioxidant agent EMHPS by 2.87 times. Under the conditions of increased turbulence of blood flow in the *Görög Thrombosis Test*, the tested sample of RU-1144 showed the ability to increase the time of thrombus formation, comparable to acetylsalicylic acid and clopidogrel, without affecting the time of its lysis. When studying compound RU-1144 in the test "bleeding time", the ability of the test sample to prolong this indicator was shown. This factor is typical of the group of antiplatelet agents, but at the same time, in comparison with acetylsalicylic acid and clopidogrel, it can prolong this time to a lesser extent.

FINANCIAL SUPPORT

This study did not have any financial support from outside organizations.

AUTHORS' CONTRIBUTION

All authors equally contributed to the research work.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

REFERENCES

1. Papapanagiotou A, Siasos G, Gargalionis A, Papavassiliou AG. et al. The Role of Platelets in Cardiovascular Disease: Molecular Mechanisms. Curr Pharm Des. 2016;22(29):4493–505. DOI: 10.2174/1381612822666160607064118
2. Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, et al. Heart disease and stroke statistics (a report from the American Heart Association). Circulation. 2016;133:38–60. DOI: 10.1161/CIR.0000000000000485
3. Reed GW, Rossi JE, Cannon CP. Acute myocardial infarction in women. Lancet. 2017;14(389): 197-210. DOI: 10.1016/S0140-6736(16)00267-1
4. Grove EL, Würtz M, Thomas MR, Kristensen SD. Antiplatelet therapy in acute coronary syndromes// Expert. Opin. Pharmacother. 2015;16(14):2133–47. DOI: 10.1517/14656566.2015.1079619
5. Shaturnyĭ VI, Shakhidzhanov SS, Sveshnikova AN, Panteleev MA. Activators, receptors and signal transduction pathways of blood platelets. Biomed Khim. 2014; 60(2):182–200. DOI: 10.18097/pbmc20146002182
6. Ambrosio D, Tritto I, Golino P. Reactive oxygen metabolites and arterial thrombosis. Cardiovascular Research. 1998; 34:4445–524. DOI: 10.1016/s0008-6363(97)00101-6.
7. Aboonabia A, Singh I. The effectiveness of antioxidant therapy in aspirin resistance, diabetes population for the prevention of thrombosis. Biomedicine &Pharmacotherapy. 2016; 83: 277–2. DOI: 10.1016/j.biopha.2016.06.044
8. Chaschewic TV, Zhernosekov DD. Platelet aggregation. Mechanism of participation of adhesive molecules and mitochondria. Bulletin of Polessky State University. 2017: 51–61.
9. Roka-Moya YM, et al. Novel aspects of platelet aggregation. Biopolym. cell. 2014. 30(1): 10. DOI: http: //dx.doi.org/10.7124/bc.000874
10. Gordeev IG, Becchu EA, Lysov VA, Volov NA, Ilyin EE, et al. Evaluation of the effect of myocardial cytoprotectors on the processes of lipid peroxidation in patients with stable angina before and after surgical revascularization of the myocardium. Russian journal of cardiology. 2005; 3(53):41–6.
11. Spasov AA, Kucheryavenko AF, Sirotenko VS, Gaidukova KA, Morkovnik AS, Anisimova VA, Divaeva LN, Kuzmenko TA. Antithrombotic activity of a new derivative of diazepinobenzimidazole compound DAB-15. Bulletin of experimental biology and medicine. 2016; 162(11):585–8.
12. Spasov AA, Kucheryavenko AF, Kosolapov VA, Anisimova VA. Antithrombogenic activity of antioxidant compounds. Bulletin of experimental biology and medicine. 2013. 155(6):740–2.
13. Kosolapov VA, Spasov AA, Anisimova VA, Zhukovskaya ON. Condensed Benzimidazoles Are a Novel Scaffold for Antioxidant Agents' Search and Development. Antioxidants. 2019: 245–53. DOI: 10.5772/intechopen.82817
14. Spasov AA, Nedogoda VV, Konan K, Kucheryavenko AF. Mechanism of reduction of platelet sensitivity to medicines in response to low-energy laser radiation of blood. Hematology and transfusiology. 2001; 46(2):36–9.
15. Makarov VA, Spassov AA, Plotnikov MB, Belozerskaya GG, Vasileva TM, et al. Guidelines for the study of drugs that affect hemostasis. In the book: a Guide to preclinical trials of medicinal products of the fgbi "NCESMP" health Ministry of Russia. Moscow. 2012: 453–79.
16. Zhitnikova LM. ASA in the prevention and treatment of cardiovascular disease: clinical guidelines for practitioners. Regular issues of "BC". Cardiology. 2012;14:708–13
17. Kurz KD, Main BW, Sandusky GE. rat model of arterial thrombosis induced by ferric chloride. Thromb Res. 1990;15:269–80. DOI: 10.1016/0049-3848(90)90106-m
18. Yamamoto J, Inoue N. et al. Global Thrombosis Test (GTT) can detect major determinants of haemostasis including platelet reactivity, endogenous fibrinolytic and thrombin generating potential. Thrombosis Research. 2014;133: 919-26. DOI: 10.1016/j.thromres.2014.02.018
19. Greene TK, Schiviz A. et al. Towards a standardization of the murine tail bleeding model//J. Thromb Haemost. 2010;8(12):2820-2. DOI: 10.1111/j.1538-7836.2010.04084.x
20. Haber F, Weiss J. On the catalysis of hydroperoxide. Naturwissenschaften. 1932;20:948–50.
21. Freedman JE. Oxidative Stress and Platelets. Arteriosclerosis, Thrombosis and Vascular Biology. 2008;28:11-6. DOI: 10.1161/ATVBAHA.107.159178. Epub 2008 Jan 3.
22. Sies H. Oxidative stress: a concept in redox biology and medicine. Redox Biol. 2015;4:180–3. DOI: 10.1016/j.redox.2015.01.002
23. Dogne JM, Hanson J, Leval X, et al. Pharmacological characterization of N-tert-Butyl-N-[2-(4-methylphenylamino)-5-nitrobenzenesulfonyl] urea (BM-5730, a novel Thromboxane A2 receptor antagonist and thromboxane synthase inhibitor in a rat model of arterial thrombosis and its effects on bleeding time. J of Pharmacol and Exp Therap. 2004;309:498–505. DOI: 10.1124/jpet.103.063610
24. Buccheri S, Capodanno D, James S, Angiolillo DJ. Bleeding after antiplatelet therapy for the treatment of acute coronary syndromes: a review of the evidence and evolving paradigms. Expert Opin Drug Saf. 2019;25:1–19. DOI: 10.1080 / 14740338.2019.1680637.
25. Qiu L, Han JX, See AAQ, King NKK. Effects of anticoagulant and antiplatelet agents in severe traumatic brain injury in an Asian population – A matched case-control study. J Clin Neurosci. 2019:61–6. DOI: 10.1016 / j.jocn.2019.08.08.087.
26. Spasov AA, Kucheryavenko AF, Tien M, Anisimova VA. Antithrombotic activity of the compound RU-891. Experimental and Clinical Pharmacology. 2013;76(6):25–6. DOI: https: // doi.org/10.30906/0869-2092-2013-76-6-25-26
27. Spronk HMH., Padro T, Siland JE. Atherothrombosis and Thromboembolism: Position Paper from the Second Maastricht Consensus Conference on Thrombosis. ThrombHaemost. 2018;118(118):229–50. DOI: 10.1160 / TH17-07-0492
28. Dadjou Y, Safavi S, Javad K. Risks and Benefits of Dual Antiplatelet Therapy Beyond 12 Months After Coronary Stenting. Medicine (Baltimore). 2016;95(22):1–7. DOI: 10.1097 / MD.0000000000003663

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2020-8-2-78-85

29. Shakhmardanova SA, Gulevskaya ON, Seletskaya VV, Zelenskaya AV, et al. Antioxidants: classification, pharmacotherapeutic properties, use in practical medicine. Journal of Fundamental Medicine and Biology. 2016;3:4–12.
30. Bath PM, May J, Heptinstall S. Clinical utility of remote platelet function measurement using P-selectin: assessment of aspirin, clopidogrel, and prasugrel and bleeding disorders. Platelets. 2018. 29(5):425–30. DOI: 10.1080 / 09537104.2018.1445839
31. Spasov AA, Kucheryavenko AF, Gaidukova KA, Kosolapov VA, Zhukovskaya ON. Antiplatelet activity of new derivatives of benzimidazole containing sterically hindered phenolic group in their structure. Research Results in Pharmacology. 2020;6(1):1–9. DOI: 10.3897 / rrpharmacology.6.50373

AUTHORS

Alexander A. Spasov – Doctor of Sciences (Medicine), Academician of the Russian Academy of Sciences, Professor, Head of the Department of Pharmacology and Bioinformatics, Volgograd State Medical University. ORCID 0000-0002-7185-4826. E-mail: aspasov@mail.ru

Aida F. Kucheryavenko – Doctor of Sciences (Medicine), Associate Professor, Professor of the Department of Pharmacology and Bioinformatics, Volgograd State Medical University. ORCID 0000-0003-1406-6919. E-mail: aidakuchryavenko@yandex.ru

Ksenia A. Gaidukova – Assistant, the Department of Pharmacology and Bioinformatics, Volgograd State Medical University. ORCID 0000-0003-4376-6332. E-mail: ksenijagajjdukva@rambler.ru

Maxim V. Chernikov – Doctor of Sciences (Medicine), the Head of the Department of Biology and Physiology, Pyatigorsk Medical and Pharmaceutical Institute – a branch of Volgograd State Medical University. ORCID 0000-0001-8340-1296. E-mail: pharmax@list.ru

Olga N. Zhukovskaya – Candidate of Sciences (Chemistry), Researcher at the Laboratory of Organic Synthesis, Research Institute of Physical and Organic Chemistry, Southern Federal University. ORCID 0000-0003-0865-6656. E-mail: zhukowskaia.ol@yandex.ru

RESEARCH ARTICLE

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY

COMPLEX BIOSTIMULATION OF INTRAPLEURAL ADHESIOGENESIS IN THORACAL SURGERY

A.V. Kalashnikov[1], A.A. Vorobiev[2], S.A. Kalashnikova[1], D.Sh. Salimov[3]

[1] Pyatigorsk Medical and Pharmaceutical Institute – a branch of Volgograd State Medical University
11, Kalinin ave., Pyatigorsk, Russia, 357532
[2] Volgograd State Medical University
1, pl. Fallen Fighters, Volgograd, Russia, 400131
[3] Central Military Clinical Hospital named after P.V. Mandryka
8A, Bolshaya Olenya street, Moscow, Russia, 107076

E-mail: kalashnikova-sa@yandex.ru

Received 15 January 2020 Review (1) 30 March 2020 Review (2) 15 April 2020 Accepted 15 May 2020

The aim of the study is to determine the effectiveness of the use of platelet enriched plasma in the complex treatment of chest trauma and chronic pleural empyema.

Materials and methods. The work was performed on 450 male rats, simulated with chest trauma (n=180) and chronic pleural empyema (n=270). In the experimental groups, biostimulation of adhesiogenesis as an intrapleural injection of 1 ml of platelet-enriched plasma was carried out; in the comparison group; the animals with pleural empyema were injected with 1 ml of doxycycline solution; in the negative control groups, the treatment was not carried out at all. Withdrawal from the experiment took place on the 10th, 20th, 30th days. The samples of intrapleural adhesions were fixed in 10% formalin, followed by histological tracing and preparation of micropreparations, staining with hematoxylin and eosin. The morphometric study included determination of the volume fraction (VF) of collagen and reticular fibers; fibrin; inflammatory cells; bloodstream (%).

Results. An intrapleural administration of platelet-rich plasma is an effective way to stabilize the rib cage in chest injuries, and to eliminate residual cavities in chronic pleural empyema. When assessing the severity of the adhesions in chest trauma, it was found out that adhesions are most often visualized at the sites of rib fractures (from 13.3 to 40%). In pleural empyema, during the entire process of observation, the VF of collagen fibers forming adhesions was higher in the group with biological stimulation of adhesiogenesis than in the NCpe group and in the CG. In the PRP group, already at the initial stages of the experiment, this indicator was significantly lower than in the NC and CG ($p<0.05$).

Conclusion. Based on the data obtained, the effectiveness of the use of platelet-enriched plasma in thoracic surgery for the biological potentiation of adhesiogenesis has been proved in experimental chest injuries and chronic pleural empyema.

Keywords: thoracic surgery; biostimulation of adhesiogenesis; platelet-enriched plasma; chest trauma; pleural empyema

Abbreviations: Ch.Inj.T – chest injury trauma; NCt – negative control group with chest trauma (without pharmacological correction); PRPt – administration of platelet-rich plasma for the treatment of chest trauma; PE – pleural empyema; NCpe – negative control group with pleural empyema (without pharmacological correction); PRPpe – administration of platelet-rich plasma for the treatment of pleural empyema; CG – comparison group, CGpe – comparison group without pharmacological correction.

For citation: Kalashnikov A.V., Vorobiev A.A., Kalashnikova S.A., Salimov D.Sh. Complex biostimulation of intrapleural adhesiogenesis in thoracal surgery. *Pharmacy & Pharmacology*. 2020;8(2):86-99. **DOI:** 10.19163/2307-9266-2020-8-2-86-99

Для цитирования: Калашников А.В., Воробьев А.А., Калашникова С.А., Салимов Д.Ш. Комплексная биостимуляция внутриплеврального адгезиогенеза в торакальной хирургии. *Фармация и фармакология.* 2020;8(2): 86-99. **DOI:** 10.19163/2307-9266-2020-8-2-86-99

Научно-практический журнал
ФАРМАЦИЯ И ФАРМАКОЛОГИЯ

ОРИГИНАЛЬНАЯ СТАТЬЯ

DOI: 10.19163/2307-9266-2020-8-2-86-99

КОМПЛЕКСНАЯ БИОСТИМУЛЯЦИЯ ВНУТРИПЛЕВРАЛЬНОГО АДГЕЗИОГЕНЕЗА В ТОРАКАЛЬНОЙ ХИРУРГИИ

А.В. Калашников[1], А.А. Воробьев[2], С.А. Калашникова[1], Д.Ш. Салимов[3]

[1] Пятигорский медико-фармацевтический институт – филиал федерального государственного бюджетного учреждения высшего образования «Волгоградский государственный медицинский университет» Министерства здравоохранения Российской Федерации
357532, Россия, Ставропольский край, г. Пятигорск, пр. Калинина, 11

[2] Федеральное государственное бюджетное учреждение высшего образования «Волгоградский государственный медицинский университет» Министерства здравоохранения Российской Федерации
400131, Россия, г. Волгоград, пл. Павших Борцов, д. 1

[3] Федеральное казенное учреждение «Центральный военный клинический госпиталь имени П.В. Мандрыка» Министерства обороны Российской Федерации
107076, Россия, г. Москва, Большая Оленья улица, владенье 8А

E-mail: kalashnikova-sa@yandex.ru

Получено 15.01.2020 Рецензия (1) 30.03.2020 Рецензия (2) 15.04.2020 Принята к печати 15.05.2020

Цель. Определить эффективность применения плазмы, обогащенной тромбоцитами в комплексном лечении травмы грудной клетки и хронической эмпиемы плевры.

Материалы и методы. Работа выполнена на 450 крысах-самцах, которым моделировали травму грудной клетки (n=180) и хроническую эмпиему плевры (n=270). В опытных группах осуществлялась биостимуляция адгезиогенеза: внутриплевральное введение 1 мл плазмы, обогащенной тромбоцитами, в группе сравнения при эмпиеме плевры вводили 1 мл раствора доксициклина, в группах негативного контроля лечения не проводилось. Выведение из эксперимента на 10-е, 20-е, 30-е сутки. Образцы внутриплевральных сращений фиксировали в 10%-ом формалине с последующей гистологической проводкой и изготовлением микропрепаратов, окраской гематоксилином и эозином. Морфометрическое исследование включало определение объемной доли (ОД) коллагеновых и ретикулярных волокон; фибрина; клеток воспалительного ряда; сосудистого русла (%).

Результаты. Внутриплевральное введение плазмы, обогащенной тромбоцитами, является эффективным способом стабилизации реберного каркаса – при травмах грудной клетки, и ликвидации остаточных полостей – при хронической эмпиеме плевры. При оценке выраженности спаечного процесса при травме грудной клетки установлено, что наиболее часто спайки визуализируются в местах перелома ребер (от 13,3 до 40%). При эмпиеме плевры на протяжении всего наблюдения ОД коллагеновых волокон, формирующих спайки, была выше в группе с биологической стимуляцией адгезиогенеза, чем в группе НКэп и в ГСэп. В PRP-группе данный показатель уже на начальных сроках эксперимента был достоверно ниже, чем в группе НК и ГС ($p<0,05$).

Заключение. На основании полученных данных доказана эффективность применения плазмы, обогащенной тромбоцитами в торакальной хирургии для биологического потенцирования адгезиогенеза: при экспериментальных травмах грудной клетки и хронической эмпиеме плевры.

Ключевые слова: торакальная хирургия; биостимуляция адгезиогенеза; плазма, обогащенная тромбоцитами; травма грудной клетки; эмпиема плевры

Сокращения: ТГК – травма грудной клетки; НКт – группа негативного контроля с травмой грудной клетки (без фармакологической коррекции); PRPт – введение плазмы, обогащенной тромбоцитами для лечения травмы грудной клетки; ЭП – эмпиема плевры; НКэп – группа негативного контроля с эмпиемой плевры (без фармакологической коррекции); PRPэп – введение плазмы, обогащенной тромбоцитами для лечения эмпиемы плевры; ГС – группа сравнения; ГСэп – группа сравнения без фармакологической поддержки.

INTRODUCTION

At present, one of the promising directions of regenerative medicine is biological stimulation of repair processes on the basis of platelet rich plasma (PRP) containing numerous growth factors and cytokines [1–3].

The drugs developed on the basis of autologous components, are increasingly being introduced into cosmetology and traumatology. However, the biotechnology based on PRP technologies, has not yet found application in thoracic surgery which determines the relevance of this work.

The problems of the rib cage stabilization in severe concomitant injuries and the elimination of residual cavities in chronic pleural empyema, are still unresolved. Such problems require search for new solutions on the basis of biological stimulation of adhesiogenesis in the complex treatment of these nosologies [4–7].

THE AIM of the study is to determine the effectiveness of the use of platelet enriched plasma in the complex treatment of chest trauma and chronic pleural empyema.

MATERIALS AND METHODS

Experimental animals

The experiment was carried out on 450 nonlinear sexually mature male rats (confluence), weighing 280–300 g, which were kept under standard vivarium conditions, with a natural change of the daily cycle, free access to extruded feed and water. The maintenance and manipulations were carried out in accordance with the order of the Ministry of Health of the USSR No. 755 dated 08/12/1977 and the European Convention for the Protection of Vertebrate Animals used for Experiments or for Other Scientific Purposes (Strasbourg, March 18, 1986) [8, 9]. A positive conclusion on experimental studies of the Local Independent Ethical Committee of Volgograd State Medical University was received on September 29, 2016, Protocol No. 12 – 2016.

Study Design

The design of the experiment is shown in Figure 1.

The animals were modeled for chest trauma (n=180) and chronic pleural empyema (n=270) using the authors' post-anesthesia techniques (chloral hydrate 350 mg/kg intraperitoneally). When modeling Ch.Inj.T, a negative control group with chest injury trauma (NCt) and an experimental group (PRPt) were isolated, then underwent biological potentiation of adhesiogenesis by introducing platelet-rich plasma (PRP) into the pleural cavity: a set for blood sampling Plasmolifting™, Ltd Plasmolifting, Kazan, Russia; TU 9437-002-27837594-2015, registration certificate No. RZN 2016/3980 dated 04.19.2016. The protocol of the procedure was as follows:

Stage I. Blood sampling in the volume of 5 ml from the tail vein using a syringe, into a specialized Plasmolifting™ tube.

Stage II. Centrifugation 1000 G (3200 revolutions) for 5 min., separation into fractions, obtaining platelet autoplasm.

Stage III. Supernatant sampling (thrombotic autologous plasma), located in the upper part of the tube above the separation gel (Fig. 2Б).

Stage IV. Injection of 1 ml of the drug in fractured ribs: pointwise into the fracture zone, subpleurally into the pleural cavity; directly into the residual pleural cavity – in chronic pleural empyema.

In addition to the above-described groups, in the simulation of chronic PE, a comparison group (CGpe) was added, the animals of which were injected intrapleurally with 1 ml of a doxycycline solution. Doxycycline (Doxycyclinum) is a semisynthetic bacteriostatic antibiotic from the group of tetracyclines with a broad spectrum of activity. Its chemical name is (4S, 4aR, 5S, 5aR, 6R, 12aS)-4-dimethylamine-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxy-1,4,4a,5,5a,6,11,12a-octahydronaphthacene-2-carboxyamide monohydrochloride, mixed with ethanol (2: 1), hemihydrate $C_{22}H_{24}N_2O_8$*HCl*½C_2H_5OH*½H_2O. Its invented name is Doxycycline, BINERGIYA Ltd (Russia), lyophilisate for preparation of solution for infusions 100 mg, packed in vials (5) of contoured plastic (1), in cardboard packs. The code is ATX J01AA02. Its composition is: doxycycline (in the form of hydrochloride) 100 mg; the excipients are: sodium disulfite – 6 mg, disodium edetate – 0.02 mg. In addition to the antibacterial effect of Doxycycline, this substance, according to the National Clinical Guidelines for Thoracic Surgery, is the drug of choice for chemical pleurodesis [10-12]. Withdrawal from the experiment was carried out on the 10th, 20th and 30th days of the experiment. The description of the macroscopic picture of the pleural cavity and the collection of intrapleural adhesions was carried out with a maximum preservation of the intrinsical points [13].

Histological study

1350 samples of intrapleural adhesions were taken with a maximum preservation of the intrinsical points of adhesions between the parietal and visceral pleura and fixation of the material in a 10% solution of neutral formalin (the exposure for 24 h); a standard histological processing was carried out, passing the fixed material through ethyl alcohol in the ascending concentrations (from 70° up to 100°) and chloroform. A comprehensive morphological study included the production of serial sections of intrapleural adhesions at different periods of the experiment with staining with hematoxylin and eosin. Micrographs were made using a LeicaDM 100 microscope with a digital camera, magnification ×100, ×200, ×400.

Morphometric study

A morphometric study was carried out in accordance with the established principles of quantitative morphological investigations, according to which the measurements are carried out on microphotograms obtained by photographing serial sections (and determining the number of objects on at least 10 glasses in 10 fields of view) [avtandilov]. The Videotest Morpho software was used to determine the volume fraction (VF) of collagen and elastic fibers, fibrin, cellular composition of adhesions (lymphocytes, leukocytes, fibroblasts) and VF of the blood-stream [14].

Statistical processing of results

The results were processed using the STATISTICA 7.0 software package (StatSoft, USA), (M±SEM were determined), Wilcoxon's nonparametric test, Student's test and confidence index (p). The results were considered reliable at $p<0.05$.

RESULTS

Efficiency of platelet-rich plasma in intrapleural administration for potentiating adhesiogenesis in chest trauma according to the results of macroscopic examination

As a result of a comparative analysis of the experimental group and the negative control group, it was found out that on the 10th day of the experiment with chest injury trauma, there were no cases of consolidation of rib fractures. In the negative control group with chest trauma

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2020-8-2-86-99

(without pharmacological correction), the adhesion process was absent in 16.7% of cases; in 36.7% the adhesions were formed locally, exclusively in the area of the surgical trauma, and were represented by whitish translucent cords (arachnoid adhesions), Figure 2A.

When PRP was injected intrapleurally, adhesions were not formed only in 6.7%, which was significantly less than in the negative control group with chest trauma (without pharmacological correction) ($p<0.05$). The fact that intrapleural adhesions were represented by denser (filmy) adhesions, localized mainly between the thoracotomy scar and the parietal and visceral pleura, is of great importance (Fig. 2B).

On the 20th day of the experiment, consolidation of rib fragments was recorded in 66.7% of the animals in the negative control group with chest trauma (without pharmacological correction) and in 77.8% in the experimental group. Adhesions in the negative control group with chest trauma (without pharmacological correction), as in the previous period of the experiment, were predominantly formed in the area of surgical trauma (in 43.3%) and were represented by arachnoid and membranous adhesions (Fig. 3A).

When platelet-rich plasma (PRPt) was injected into the pleural cavity on day 20, adhesions were absent only in 1 case (3.3%), in other cases adhesion was recorded in 13.3% (4 cases) with total pleural cavity filling. The most frequently detected adhesions were in the fracture zone (40.0%), there were also single local adhesions (20.0%) and single adhesions outside the fracture zone (23.3%). The adhesions were represented by dense, planar adhesions (Fig. 3B).

On the 30th day of the experiment, the negative control group with chest trauma (without pharmacological correction) showed consolidation of fracture sites with the formation of a pronounced callus. Narrowing and deformation of the intercostal spaces, formed due to the adhesive process, was noted. No total obliteration of the pleural cavity was detected (Fig. 4A).

By the end of the experiment (day 30) in the PRPt group, the total pleural cavity obliteration was observed in 13.3% (4 cases). Figure 4B shows the most typical situation characterized by the formation of a planar intimate adhesion at the fracture site during biostimulation of adhesiogenesis with platelet-rich plasma. The adhesion length was 10 mm, the width – 4 mm, thickness – up to 1 mm, the total area of the organ adhesion was 40 mm^2; there was a depletion of vascularization compared with the previous period.

Thus, a comparative analysis of the adhesion process in the pleural cavity with chest trauma revealed significant differences in NCt rats and the PRPt experimental group. In the NCt group, arachnoid and filmy adhesions prevailed, while biostimulation of adhesiogenesis with platelet-rich plasma revealed ribbon-like and planar adhesions, the density of which increased in the dynamics of the experiment.

Efficiency of platelet-rich plasma when administrated intrapleurally, for potentiating adhesiogenesis in chest trauma according to the of histological study results

On the 10th day of the experimental chest trauma, histological examination of pleural adhesions showed the predominance of chaotically located connective tissue fibers with a pronounced leukocyte infiltration in both – the NCt group and the PRPt group. At the same time, in the studied samples of the NCt group, diffusely located serous-hemorrhagic exudate with fibrin concretions was found (Fig. 5A).

On the 10th day of the experiment, thin fibers of connective tissue, located unevenly, mainly in the zone of fracture and drug administration, were determined in the experimental group. The amount of exudate was insignificant, its organization was notified against the background of a persisting inflammatory reaction (Fig.5B).

On the 20th day of the experiment, the volume of serous-hemorrhagic exudate in the NCt group was insignificant; the edema of the connective tissue, represented by chaotically localized fibers, by the type of myxomatous transformation, was revealed. Single vessels and diffuse, predominantly neutrophilic, inflammatory infiltration were identified. There was a discrepancy between immature pevral adhesions and the time of the experiment (Fig. 6A).

When PRP was adminisrated on the 20th day, young pleural adhesions containing fibers oriented parallel to the surface of the walls of the pleural cavity, were determined. In loosely located connective tissue fibers, single hemosiderin granules were visualized. It was associated with a change in the permeability of the endothelium and basement membrane and indicated continuing adhesiogenesis (Fig. 6B).

On the 30th day of the experiment, adhesions were still immature in the NCt group. Vascular filling and the presence of hemosiderin granules with siderophages indicated ongoing angiogenesis and the formation of pleural adhesions. The vessels were thin-walled, full-blooded and located unevenly among the fibers of the connective tissue. The presence of single lymphocytes, plasma cells and neutrophils was detected, as well as an abundance of macrophages (siderophages) (Fig. 7A).

By the end of the experiment, mature adhesions in the area of the rib fracture had been revealed. It took place in histological examination of morphological changes in the pleural cavity during biostimulation of adhesiogenesis by the introduction of platelet-rich plasma. The adhesions were formed by strictly oriented collagen fibers and included fibroblasts, fibrocytes, with single vessels in the field of view (Fig. 7B).

Morphometric assessment of the effectiveness of platelet-enriched plasma for potentiating adhesiogenesis in dynamics of chest trauma

A morphometric analysis was completely consistent with the results of histological examination (Table 1).

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY

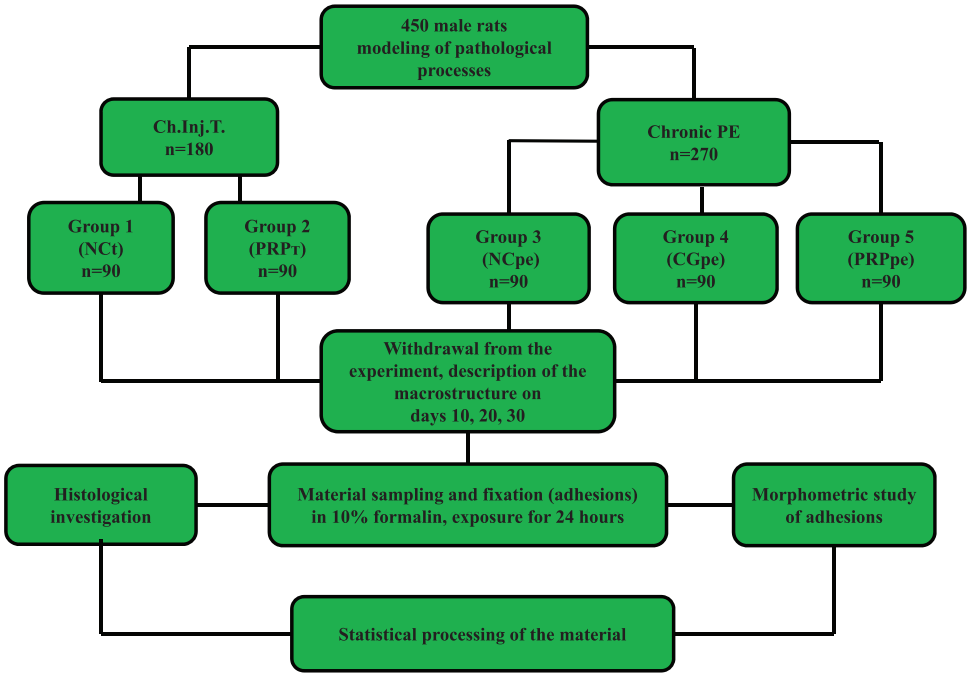
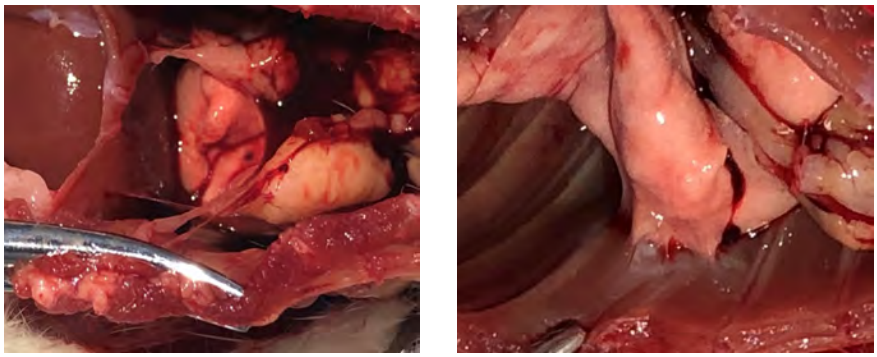


Figure 1 – The design of the experiment

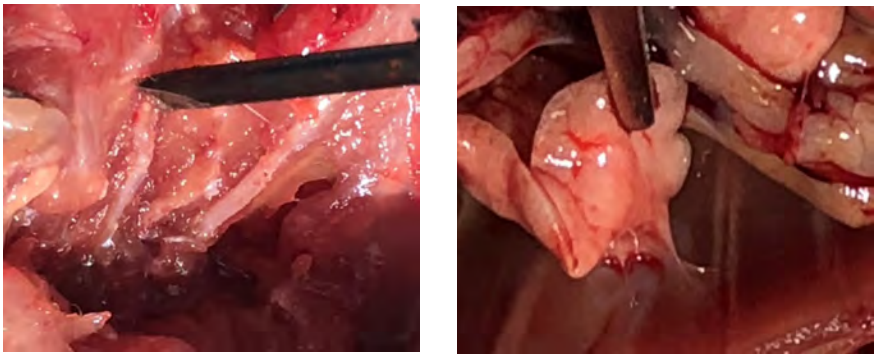
Notes: Ch.Inj.T – chest injury trauma; NCt – negative control group with chest trauma (without pharmacological correction); PRPt – administration of platelet-rich plasma for the treatment of chest trauma; PE – pleural empyema; NCpe – negative control group with pleural empyema (without pharmacological correction); PRPpe – administration of platelet-rich plasma for the treatment of pleural empyema.



A **B**

Figure 2 – Pleural cavity on the 10th day of experimental chest injury

Note: A. Negative control group. B. Experimental group. Administration of platelet-rich plasma (PRPt). Lack of consolidation of rib fragments.

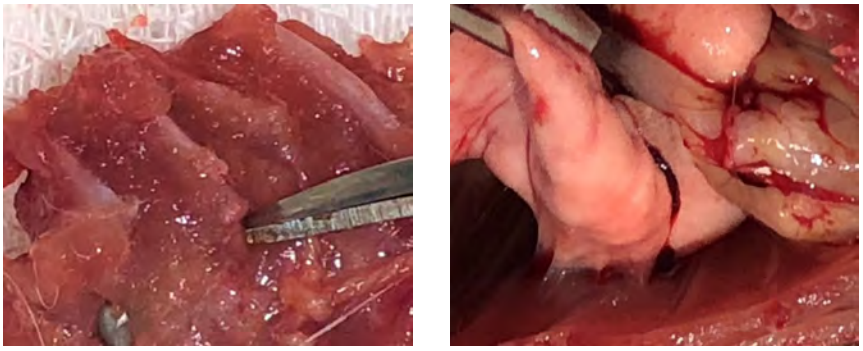


A **B**

Figure 3 – Pleural cavity on day 20 of experimental chest injury

Note: A. Negative control group. The presence of consolidation of rib fractures, the absence of intrapleural adhesions. B. Experimental group. Administration of platelet-rich plasma (PRPt), formation of pleural adhesions.

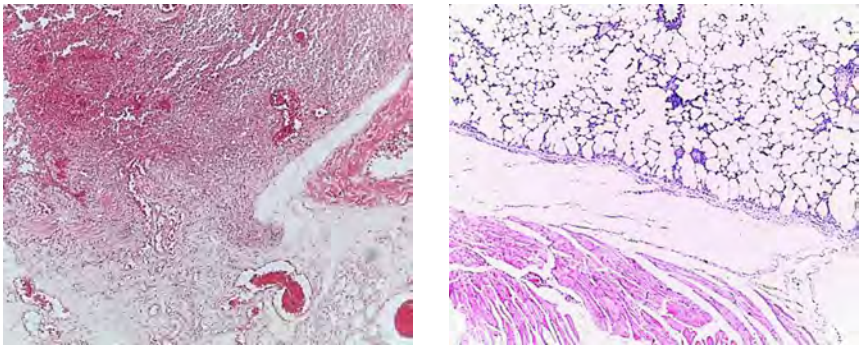
ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2020-8-2-86-99



A B

Figure 4 – Pleural cavity on day 30 of experimental chest injury

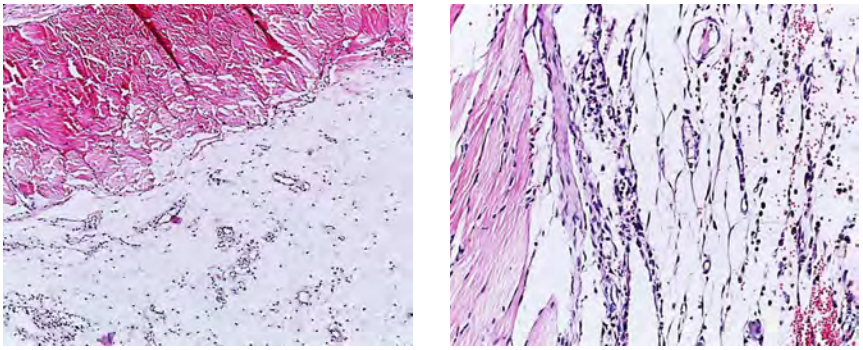
Note: A. Negative control group. The presence of consolidation of rib fractures, the absence of intrapleural adhesions. B. Experimental group. Administration of platelet-rich plasma (PRPt). Formation of plane adhesions.



A B

Figure 5 – Experimental chest injury on day 10

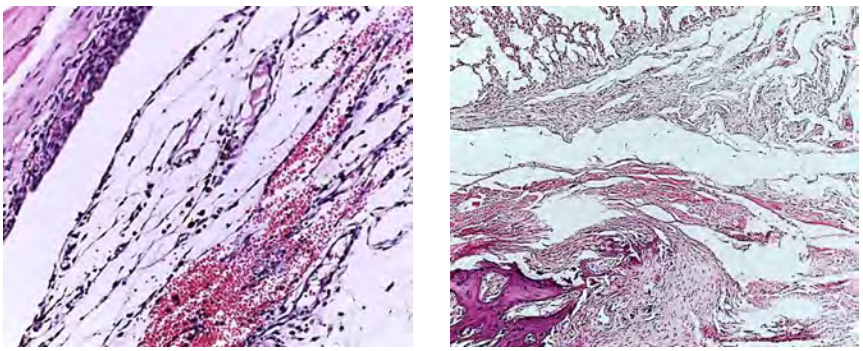
Note: A. NCt group. B. PRPt group. Staining with hematoxylin and eosin. Magnification ×400.



A B

Figure 6 – Experimental chest injury on day 20

Note: A. NCt group. Staining with hematoxylin and eosin. Magnification ×100. B. Experimental group PRPt. Staining with hematoxylin and eosin. Magnification ×200.



A B

Figure 7 – Experimental chest injury on day 30

Note: A. Group NCt. Staining with hematoxylin and eosin. Magnification ×200. B. Experimental PRPt group. Staining with hematoxylin and eosin. Magnification ×400.

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY

Table 1 – Morphometric indices of the composition of adhesions in rats with chest trauma against the background of platelet-rich plasma, for potentiation of adhesiogenesis (M±m, %)

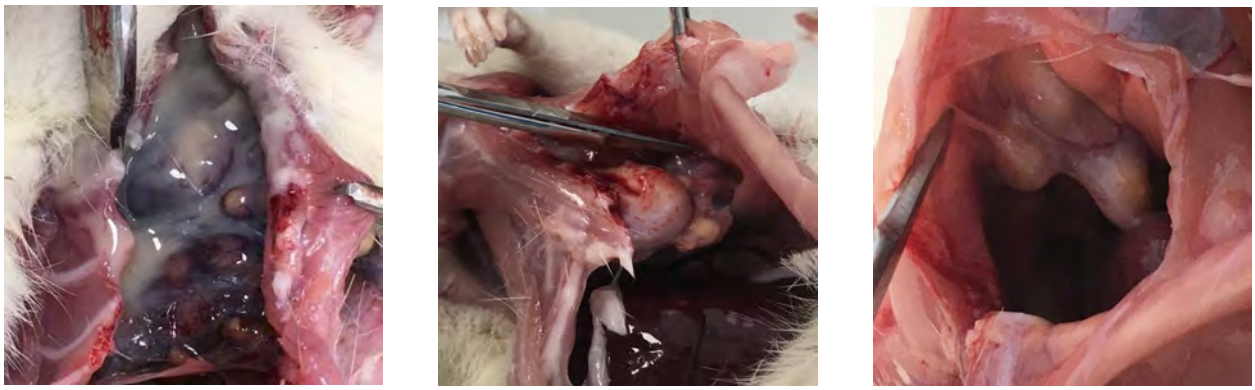
Indices, VF (%)	Experimental groups					
	NCt			PRPt		
	Day 10	Day 20	Day 30	Day 10	Day 20	Day 30
Collagen fibers	3.04±0.8	15.11±2.3#	33.72±9.7#	8.23±0.9*	29.15±3.1*#	37.23±8.3*#
Reticular fiber	31.56±3.5	25.05±0.9#	2.95±0.7#	30.19±3.7	15.03±1.1*#	4.15±0.3#
Fibrin	8.11±0.9	6.09±0.3	5.21±0.3#	9.15±0.9	4.25±0.8*#	4.08±0.5
Leukocytes	22.17±1.7	18.75±3.5	9.73±0.5#	21.95±3.9	17.21±2.5	8.92±0.8#
Lymphocytes	11.01±0.9	14.15±0.8	20.12±7.3#	10.27±0.9	15.95±1.9	19.75±1.3
Fibroblasts	6.88±0.5	10.05±1.3	18.36±3.5#	7.07±0.8	10.21±0.7	21.03±1.5#
Vessels	17.23±2.5	10.80±2.5#	9.91±0.8#	13.14±1.5	8.2±0.9*#	4.84±0.3*#

Note: * – reliability of differences in comparison with the NCt group ($p < 0.05$);
– reliability of differences in comparison with the previous period ($p < 0.05$).

Table 2 – Morphometric indices of the composition of adhesions in rats with chronic pleural empyema against the background of the administration of platelet-rich plasma to potentiate adhesiogenesis (M±m, %)

Indices, VF (%)	Experimental groups					
	NCpe			PRPpe		
	Day 10	Day 20	Day 30	Day 10	Day 20	Day 30
Collagen fibers	1.09±0.7	11.13±1.7#	30.08±3.1#	8.19±0.8*	23.07±1.5*#	32.64±4.1#
Reticular fiber	33.72±2.3	29.16±3.6#	7.26±0.8#	30.75±2.9	15.28±3.5*#	5.11±0.5*#
Fibrin	9.23±0.8	7.95±0.9	5.83±0.3#	10.67±0.7	8.17±0.9	4.97±0.1#
Leukocytes	21.16±1.3	19.31±2.1	11.12±1.1#	23.13±4.1	16.81±3.3	8.13±0.9
Lymphocytes	13.11±1.9	14.29±0.7	19.19±5.7	12.87±0.8	16.03±1.1#	21.40±1.1
Fibroblasts	8.02±0.3	9.83±1.1	17.23±2.9	6.92±0.8	12.91±0.8	19.73±1.1
Vessels	13.67±1.9	8.33±0.3	9.29±0.9	7.47±1.1#	7.73±0.9*#	8.02±0.5

Note: * – reliability of differences in comparison with the NCpe group ($p<0.05$);
– reliability of differences in comparison with the previous period ($p<0.05$).



A **B** **C**

Figure 8 – Pleural cavity of experimental pleural empyema in the comparison group on day 10

Note: A. Negative control group (NCpe). The cavity is filled with liquid pus, multiple abscesses. B. Comparison group (CGpe). C. Experimental group (PRPpe).

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2020-8-2-86-99

When determining the VF of collagen and reticular fibers forming adhesions, significant differences were found in the NCt group and in the PRPt experimental group. Thus, on the 10th day of the experiment in the NCt group, the VF of collagen fibers was significantly less than in the experimental group with stimulation of adhesiogenesis with platelet-rich plasma (3.04±0.8% and 8.23±0.9%, respectively) p<0.05. Against the background of an increase in the proportion of collagen fibers, there was a significant decrease in the VF of reticular fibers, which sharply decreased on the 30th day of the experiment (p<0.05).

Besides, at all stages of the experiment, significant differences were detected in the NC group and experimental groups in determining the VF of the cellular composition of adhesions: leukocytes, lymphocytes, fibroblasts (p<0.05).

Thus, as a result of a complex morphological study, the following was found out: biological potentiation of adhesiogenesis by plasma enriched with platelets is an effective measure for stabilizing the rib cage in chest injuries with multiple rib fractures.

Efficieny of plasma, platelet-enriched through intrapleural administration for potentiating adhesiogenesis in chronic pleural empyema, according to the results of macroscopic examination

As a result of the study, significant differences were established in the morphogenesis of residual cavities in animals of the comparison groups and experimental groups. When conducting a comparative characteristic of experimental chronic pleural empyema and adhesions in the pleural cavity without treatment and with various methods of biological stimulation of adhesion formation, the following results took place: on the 10th day of the experiment, in all the study groups including the negative control group, the comparison group and the three experimental groups, the residual cavity was preserved macroscopically. The severity of the adhesive process depended on the tactics of managing the residual pleural cavity, which arose during experimental modeling of pleural empyema.

In the NCpe group, the volume of the residual pleural cavity was maximum and averaged 25.1±3.1 mm^3. Basically, single adhesions were determined in the animals of this group (50.0%); adhesions were absent in 36.7% of cases, multiple adhesions were found only in 13.3%, while spider adhesions were determined predominantly morphologically. The cavity was filled with liquid pus with no signs of organization. The visceral pleura was thickened to 1-1.5 mm, abscesses with a diameter of 1 to 3 mm were determined in its thickness (Fig. 8A).

In the CGpe, as well as in the NCpe group, the total obliteration of the residual cavity was not observed. However, intrapleural adhesions, both single (in 56.7%) and multiple (in 23.3%), were determined. The volume of the residual pleural cavity was less than in the NC group – 23.2±2.5 mm^3 (p>0.05).

In the CG, the residual empyema cavity was formed by the parietal pleura, visceral pleura of the upper lobe, an interlobar groove, a lower lobe and a diaphragm. In the thickness of the pleura, multiple encapsulated abscesses up to 3 mm in diameter were determined. There were focal fibrin deposits on the pleural surface (Fig. 8B).

In the experimental group, on the 10th day of experimental PE, against the background of the administration of platelet-enriched plasma, residual cavities of a small volume were determined, fragmented into sectors by single organ adhesions. Intrapleural adhesions were most often located in the costophrenic sinus; they were penetrated by newly formed vessels, which indicated the active formation of adhesions (Fig. 8C).

On the 20th day of experimental EP in the NC group, a residual cavity containing purulent exudate, was determined. The pyogenic membrane was thickened due to the deposition of fibrin up to 2 mm, infiltrated by numerous microabscesses. The dome of the diaphragm was smoothed, the sinus deformity was detected, while there were no intrapleural adhesions (Fig. 9A).

In the antero-inferior parts of the pleural cavity in CGpe rats, a residual empyema cavity with thickened walls, containing organized exudate, was determined. Numerous adhesions, mainly membranous and planar, were localized in the pleurophrenic sinuses and the interlobar sulcus. A pronounced intra-adhesion inflammatory process and rich neovascularization indicated the active formation of adhesions (Fig. 9B).

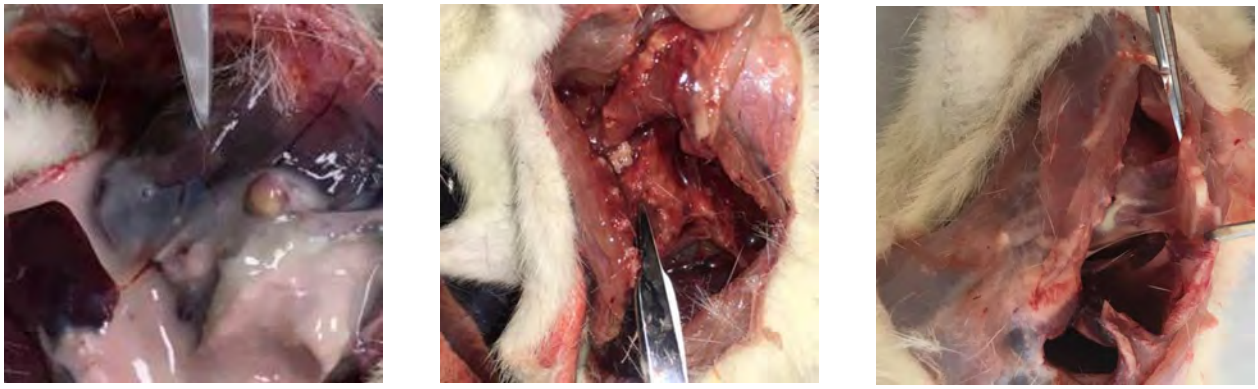
Residual cavities without purulent contents, with focal fibrin deposits on the walls, up to 2 mm thick, were determined deformed (due to the adhesion process) during biological potentiation of adhesiogenesis as a result of the administration of platelet-rich plasma. Morphologically, adhesions were represented by a wide spectrum: single organ adhesions were combined with multiple filmy and ribbon-like adhesions, which were randomly located inside the residual cavity, significantly reducing its volume (Fig. 9C).

By the end of the experiment (day 30), the cases of elimination of residual cavities had been recorded in all the groups. However, in the NCpe group, the empyema cavity was determined significantly more often than in the comparison group and in the experimental PRPpe group (p<0.05). At the same time, in the NCpe group, the volume of the residual cavity was the largest (19.3±1.7 mm^3). There was thickening of the parietal and visceral pleura, with subpleural abscesses. In the animals of this group, single adhesions prevailed (50.0%), in 30.0% of cases adhesions were absent, multiple adhesions were found only in 13.3%, the total obliteration was recorded only in 6.6% (Fig. 10A).

When doxycycline was administrated (CGpe), the volume of the residual empyema cavity averaged 16.5±1.5 mm3, the residual cavity was found to be obliterated in 13.3%; in 16.7% there were single adhesions, in 46.7% – multiple ones. Adhesions were represented by mature massive moorings located in the lower sections (Fig. 10B).

ISSN 2307-9266 e-ISSN 2413-2241

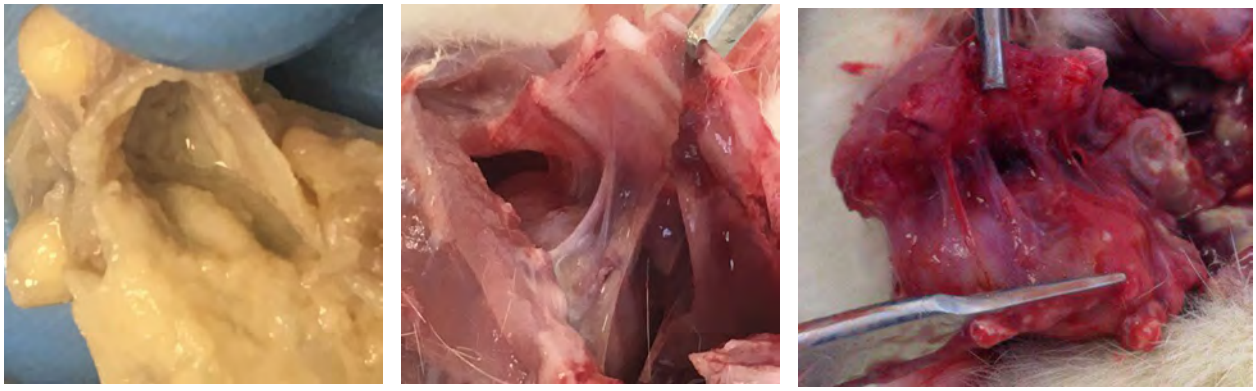
Scientific and Practical Journal
PHARMACY & PHARMACOLOGY



A **B** **C**

Figure 9 – Pleural cavity of experimental pleural empyema on day 20

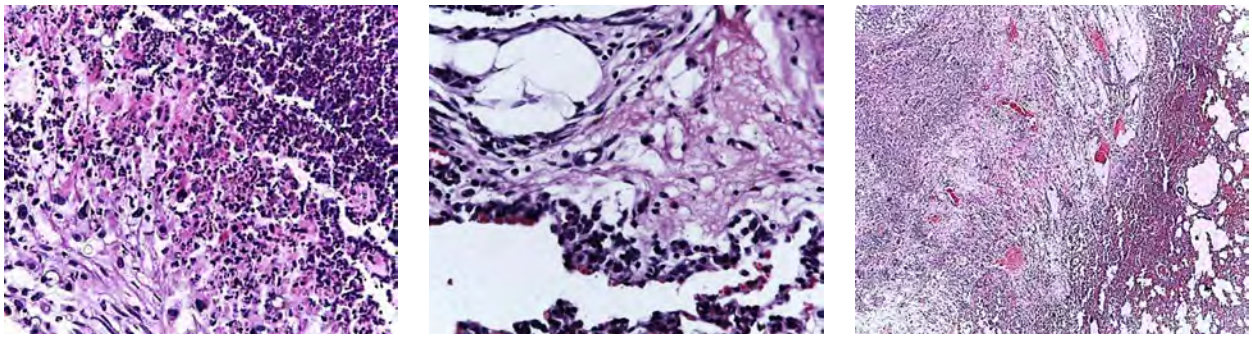
Note: A. Negative control group (NCpe). The cavity is filled with liquid pus, multiple abscesses. B. Comparison group (CGpe). C. Experimental group (PRPpe).



A **B** **C**

Figure 10 – Pleural cavity on the 30th day of experimental pleural empyema

Note: A. Negative control group (NCpe). B. Comparison group (CGpe). C. Experimental group (PRPpe).

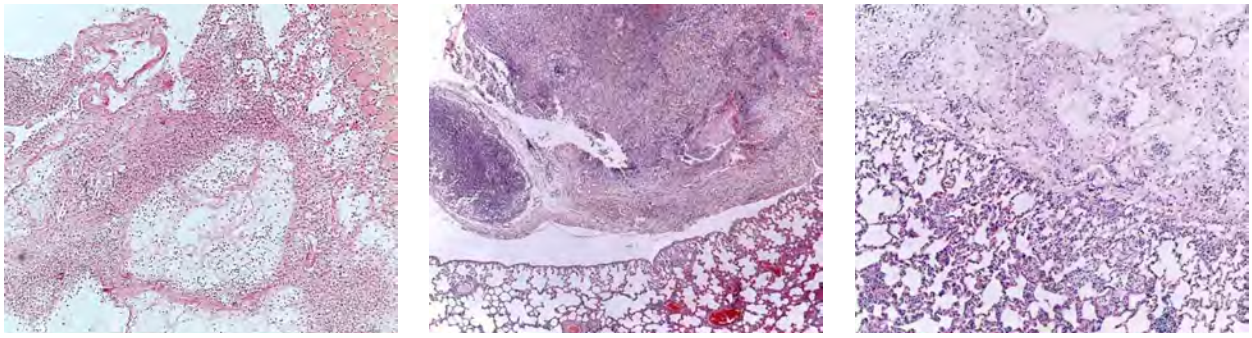


A **B** **C**

Figure 11 – Formation of immature connective tissue in pleural empyema on day 10 of the experiment against the background of chronic pleural empyema in rats

Note: A. Negative control group (NCpe). B. Comparison group (CGpe). C. Experimental group (PRPpe) with the administration of platelet-rich plasma. Staining with hematoxylin and eosin. Magnification ×400.

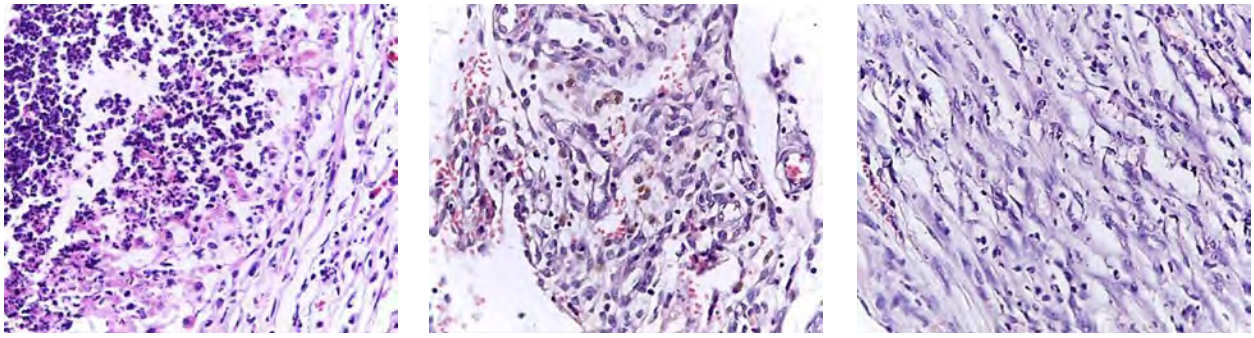
ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2020-8-2-86-99



A B C

Figure 12 – Experimental pleural empyema on day 20 of the experiment

Note: A. NCpe group. Staining with hematoxylin and eosin. Magnification x100. B. Comparison group. Staining with hematoxylin and eosin. Magnification ×100. C. Experimental group (PRPpe) with the administration of platelet-rich plasma. The phase of young adhesions with the presence of immature connective tissue against the background of chronic pleural empyema in rats on day 20 with the administration of platelet-rich plasma. Staining with hematoxylin and eosin. Magnification ×100.



A B C

Figure 13 – Experimental pleural empyema on day 30 of the experiment

Note: A. NCpe group. B. Comparison group. C. Experimental group (PRPpe) with the administration of platelet-rich plasma. Mature adhesion in the pleural cavity of rats with the presence of oriented connective tissue fibers, a small number of lymphocytes against the background of chronic pleural empyema. Staining with hematoxylin and eosin. Magnification ×400.

In the experimental group (PRPpe) on the 30th day, the total obliteration of the empyema cavity was registered in 13.3%. The volume of the residual pleural cavity averaged 12.1±0.8 mm^3. The absence of adhesions was registered in 20.0%. Ribbon-like and planar adhesions were morphologically identified (Figure 10C).

Efficiency of plasma, platelet-enriched through intrapleural administration for potentiating adhesiogenesis in chest trauma, according to the results of histological study

On the 10th day of the experiment in the pleural cavity in the NCpe group, the impregnation of the pleural layers with purulent fibrinous exudate was determined; the involvement of adjacent tissues in the pathological process was detected. Deposits of fibrin filaments were determined in the inflammatory detritus of the exudate. In the parietal pleura, reactive changes, characterized by the phenomena of nuclear hyperchromia and violations of the nuclear-cytoplasmic relationship, were found out. The inflammatory infiltrate was represented by an abundance of polymorphonuclear neutrophils with single lymphocytes and macrophages (Fig. 11A).

In the comparison group, purulent-fibrinous exudate was also found in the residual PE cavity. The signs of inflammation were revealed, consisting in visualization of mesothelial cells with inflammatory changes. In pleural adhesions, chaotically located connective tissue fibers, mainly reticular, were determined, in the thickness of which there were single fibroblasts and multiple neutrophilic leukocytes (Fig. 11B).

In the experimental group, the residual pleural cavity was delimited from the lung tissue due to the formation of granulation tissue represented by connective tissue fibers with an abundance of blood vessels (Fig. 11C).

In the NCpe group, on the 20th day of experimental EP, a partial organization of exudate with a precipitate of fibrin and single connective tissue fibers was revealed. A few microabscesses with a tissue dendrite located in the center, were found. The pathological process was delimited by immature connective tissue fibers, inside which there was a massive inflammatory infiltration, represented by neutrophils with single lymphocytes and macrophages. There was a discrepancy between the degree of maturity of pleural adhesions and the timing of the experiment (Fig. 12A).

When doxycycline was administrated on the 20th day of the experiment, adhesiogenesis had a number of features associated with fragmentation of the residual cavity by connective tissue adhesions into microcavities containing an insignificant amount of serous-purulent exudate. The detected adhesions were infiltrated with neutrophilic leukocytes and richly vascularized, which indicated active neoangiogenesis (Fig. 12B).

The administration of platelet-rich plasma at this time of the experiment led to the active formation of connective tissue, represented by a network of loosely located thin fibers with edema phenomena. At the same time, the severity of the inflammatory response was significantly lower than in the NCpe and CGpe groups. Morphologically, moderate diffuse lymphocytic infiltration of pleural adhesions with single neutrophilic leukocytes was determined. On the part of the microvasculature, the signs of active neoangiogenesis were revealed, the vessels were evenly distributed among the fibers of the connective tissue (Fig. 12C).

Histological examination of pleural tissues in the negative control group on the 30th day showed a pronounced edema of the connective tissue, abundantly infiltrated by neutrophils, single plasmocytes and macrophages. The mesothelium of the visceral and parietal sheets was not visualized, between the thin bands of the connective tissue exudate was determined; its composition was mainly represented by neutrophils. Diffuse impregnation and thickening of the pleural sheets due to the pronounced edema and abundant neutrophilic infiltration was revealed. The vessels of the microvasculature had moderate blood filling, with symptoms of perivascular edema, which was histologically manifested by the fragmentation of the vascular wall and the presence of optically empty perivascular spaces (Fig. 13A).

Against the background of doxycycline administration, in the animals of the comparison group the presence of single immature adhesions in the pleural cavity was detected. The adhesions were formed by loose fibrous connective tissue, while thin connective tissue fibers had a different direction, between which an abundance of moderately plethorical vessels was revealed. Between the fibers of the connective tissue, an accumulation of fibroblasts was clearly visible; they had rounded nuclei and a small amount of cytoplasm. In addition, lymphocytic infiltration with the presence of single plasma cells was determined. In pleural adhesions, hemosiderin granules as well as large cells with the presence of brown pigment in the cytoplasm (siderophages) were determined. Attention was drawn to the fact that the vessels of the microvasculature were lined with endothelial cells with a rounded nucleus, which indicated "irritation" of the endothelium and was a morphological sign of endothelial dysfunction (Fig. 13B).

In the experimental group with biological potentiation of adhesiogenesis in the pleural cavity with plasma enriched with platelets, the formation of multiple adhesions with the presence of strictly oriented connective tissue fibers was registered; fibroblasts and fibrocytes with an elongated nucleus and a small amount of cytoplasm were detected there. Single vessels with typical endothelium were visualized. Morphological signs of inflammation were minimal and characterized by the presence of single lymphocytes and plasma cells (Fig. 13C).

Morphometric essessment of the efficiency of platelet-rich plasma for potentiation of adhesion in chronic pleural empyema in dynamics

The summary data on the results of the morphometric study in the animals with the experimental chest trauma of the NCpe group and with various methods of biological stimulation of adhesiogenesis, are presented in Table 2.

As a result of a complex morphological study, it was found out that the composition of the adhesions within the experimental groups varied significantly. Thus, during the entire observation, the VF of collagen fibers forming adhesions was higher in the group with biological stimulation of adhesiogenesis than in the NCpe group and in the CGpe group. On the 10th day, in the group with the use of PRP technologies, this indicator was 7.5 times higher than in the group of NC ($p<0.01$). As the duration of the experiment increased, the VF of collagen fibers in the adhesions formed in the NC and CG group steadily increased. On the 30th day it did not have significant differences from the adhesions softened during potentiation of adhesiogenesis.

Alongside this with an increase in the VF of collagen fibers, a decrease in VF of reticular fibers occurred, which indicated the maturation of the adhesion. However, in the PRP group, this indicator, already at the initial stages of the experiment, was significantly lower than in the NC and CG groups ($p<0.05$). The changes in the cellular composition of adhesions were less pronounced, but leukocyte infiltration significantly decreased in the PRPpe group compared to the NC and CG groups. So, on the 20th day, in the group with combined stimulation of adhesions with the plasma enriched with platelets, the VF of leukocytes was 2.1 times lower than in the NC group. Alongside with this, there was an increase in VF of lymphocytes and VF of fibroblasts, which was recorded significantly earlier (10-20 days) than in the NC group.

Thus, the results obtained indicate the earlier formation and maturation of adhesions during the biological stimulation of platelet-rich plasma, as well as of their stability in this group.

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2020-8-2-86-99

DISCUSSION OF RESULTS

Adhesiogenesis is a compensatory reaction that occurs in response to surgical (or any other) trauma. Platelet-rich plasma (PRP technologies) was chosen as a biological substance that provides potentiation of adhesiogenesis.

The choice of a chest injury with multiple floating fractures of ribs and residual cavity in chronic pleural empyema as nosological units for the use of biotechnology in stimulation of adhesion is not accidental. In chest trauma, stimulation of the adhesions should have a dual role: stabilization of the rib cage – on the one hand and protection of the lung parenchyma from damage – on the other. In pleural empyema, the residual cavity is invaded with connective tissue, which leads to its obliteration and elimination of the chronic focus of purulent infection.

According to the literature data, the use of platelet-rich plasma is pathogenetically justified, because platelets contain growth factors (PDGF, VEGF, EGF, FGF, etc.) that increase the activity of fibroblasts. Fibroblasts produce elastin, collagen, hyaluronic acid, promoting the formation of connective tissue and its neovascularization. In addition, the growth factors inhibit the decrease in bone tissue volume by stimulating the proliferation of osteoblasts and blocking osteoclasts. There is some information about the immunostimulating effect of PRP, its participation in the normalization of metabolic processes, tissue respiration, optimization of microcirculation [15, 16].

For chest trauma, a model for stabilizing the rib cage by stimulating adhesiogenesis using platelet-rich plasma has been developed. This model is pathogenetically substantiated, has validity and makes it possible to assess the morphological structure of adhesions formed under the influence of platelet growth factors. When modeling and treating experimental chest trauma with multiple rib fractures, significant differences were found out in the negative control group and in the experimental group. When assessing the severity of the adhesion process, it was found out that adhesions are most often visualized at the sites of rib fractures (from 13.3 to 40%). At the same time, in the NC group, single adhesions prevailed (23.3%-63.3%), while in the experimental group on the 20th and 30th days, single adhesions were absent ($p<0.01$). Similar results were obtained when analyzing the cases of the absence of adhesions: the animals without adhesions in the pleural cavity were determined in the NC group at all periods of the experiment (16.7% on the 10th and 20th days; 13.3% on the 30th day). While in the group with PRP technology, the percentage of rats without adhesions in the pleural cavity was significantly lower: from 6.7% on day 10, 3.3% on day 20, to 0% on day 30 ($p<0.05$).

The macroscopic differences in the structure of adhesions are noteworthy; arachnoid and membranous adhesions predominated in the NC group, while ribbon-like and planar adhesions prevailed in the experimental groups. The thickness and density of adhesions was increasing in the duration of the experiment in all the studied groups.

So, this study makes it possible to conclude that the biological potentiation of adhesion is a logical measure of stabilization in chest injuries with multiple rib fractures. The morphological substrate of this method is the formation of mature adhesions without manifestations of activity and further development, thereby confirming the maturity and formation of adhesions. The results obtained are consistent with the literature data, according to which the use of PRP technology has a stimulating effect on the development and neovascularization of adhesions due to the contained growth factors; in addition, there is information about the stimulating effect of PRP technology on the formation of callus [17].

When modeling and treating experimental pleural empyema, the authors' methods of biological stimulation of adhesiogenesis by injecting platelet-rich plasma was used. The elimination of the residual pleural cavity is based on the stimulation of adhesion formation by the growth factors contained in PRP, which is consistent with the literature data [18, 19].

At the same time, significant differences were established in the morphogenesis of residual cavities in the animals of the NC group, comparison groups and the experimental group. It is noteworthy that in the comparison group (treatment with doxycycline) the adhesions consisted mainly of loose fibrous connective tissue infiltrated with cellular elements, which confirmed the fact of the inflammatory process in the adhesion and is prognostically unfavorable in terms of the occurrence of complete obliteration of the pleural cavity adhesions. At the same time, in the experimental group, intrapleural adhesions were formed mainly by connective tissue fibers containing lymphocytes, histiocytes, fibroblasts, a small number of desolate vessels, and no signs of inflammation were detected. The described histological picture is typical of mature adhesions. It means that with targeted administration of PRP into the pleural cavity, adhesiogenesis can be considered controlled.

So, in the experiment it was established that the applied biotechnologies make it possible to potentiate intrapleural adhesions in residual cavities in chronic pleural empyema, which leads up to the complete obliteration of the empyema cavity – a chronic source of infection and a risk factor for disease recurrence.

CONCLUSION

As a result of the experimental study, the efficiency of the use of platelet-rich plasma for the biological potentiation of adhesiogenesis in experimental chest injuries and chronic pleural empyema has been proved. The results obtained may be a sufficient basis for recommending clinical trials.

FINANCIAL SUPPORT AND SPONSORSHIP

This study did not have any financial support from outside organizations.

AUTHORS' CONTRIBUTION

All authors equally contributed to the research work.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

1. Akhmerov RR, Zarudiy RPh, Aminova ZM, Emelin AL, Ovechkina MV. Primenenie trombocitarnoj autoplazmy pri lechenii gonartrozov i koksartrozov [Use of thrombocytic autoplasma in treatment of gonarthrosis and coxarthrosis]. Prakticheskaja medicina. 2013;1(2):17–20. Russian.
2. Jhang JF, Wu SY, Lin TY, Kuo HC. Repeated intravesical injections of platelet-rich plasma are effective in the treatment of interstitial cystitis: a case control pilot. Low Urinary Tract Symptoms. 2017;11(2):42–47. DOI: 10.1111/luts.12212.
3. Cieslik-Bielecka A, Skowroński R, Jędrusik-Pawłowska M, Pierchała M. The application of L-PRP in AIDS patients with crural chronic ulcers: A pilot study. Adv Med Sci. 2017;63(1):140–146. DOI: 10.1016/j.advms.2017.10.002.
4. Sakakura N, Mizuno T, Kuroda H, Sakao Y, Uchida T. Surgical treatment of empyema after pulmonary resection using pedicle skeletal muscle plombage, thoracoplasty, and continuous cavity ablution procedures: a report on three. Journal of Thoracic Disease. 2016; 8(6):1333–9. DOI: 10.21037/jtd.2016.04.04.
5. Botianu PV, Botianu AM, Bacarea VC. Muscle flaps and thoracomyoplasty as a re-redo procedure for postoperative empyema. The Journal of Thoracic and Cardiovascular Surgery. 2016;64(3)252–257. DOI: 10.1055/s-0034-1387820.
6. Zhestkov KG, Barskij BG, Atjukov MA, Pichurov AA. Nacional'nye klinicheskie rekomendacii po lecheniju spontannogo pnevmotoraksa. Moskow, RF. 2014:23.
7. MacDuff A, Arnold A, Harvey J Management of spontaneous pneumothorax: British Thoracic Society pleural disease guideline 2010. Thorax. 2010;65(2):18–31. DOI: 10.1136/thx.2010.136986.
8. Rybakova AV, Makarova MN. Metody jevtanazii laboratornyh zhivotnyh v sootvetstvii s evropejskoj direktivoj 2010/63 [Methods of euthanasia of laboratory animals, in accordance with european directive 2010/63]. Mezhdunarodnyj vestnik veterinarii. 2015;2:96–107.
9. Rybakova AV, Makarova MN. Sanitarnyj kontrol' jeksperimental'nyh klinik (vivariev) v sootvetstvii s lokal'nymi i mezhdunarodnymi trebovanijamiMezhdunarodnyj vestnik veterinarii [Sanitary inspection of experimental clinic (vivarium) with using local and internation requirement]. 2015;4:81–89.
10. Song KS, Keum D, Kim JB. Chemical pleurodesis using doxycycline and viscum album extract. Korean Journal of Thoracic and Cardiovascular Surgery. 2017;50(4):281–286. DOI: 10.5090/kjtcs.2017.50.4.281.
11. Thomas R, Piccolo F, Miller FD, MacEachern Chee PR, Huseini AC. Intrapleural fibrinolysis for the treatment of indwelling pleural catheter-related symptomatic loculations: a multicenter observational study. Chest. 2015;148(3):746–751. DOI: 10.1378/chest.14-2401.
12. Boshuizen RC, Noort VVd, Burgers JA, Herder GJM, Hashemi SMS, Hiltermann TJN. A randomized controlled trial comparing indwelling pleural catheters with talc pleurodesis (NVALT-14). Lung Cancer. 2017;108:9–14. DOI: 10.1016/j.lungcan.2017.01.019.
13. Vorob`jov AA, Beburishvili AG. Hirurgicheskaja anatomija operirovannogo zhivota i laparoskospicheskaja hirurgija speak. Volgograd, RF: Izdatel`. 2001:230.
14. Avtandilov GG. Osnovy kolichestvennoj patologicheskoj anatomii. M.: Medicina. 2002:240s.
15. Bohlen HL, Schwartz ZE, Wu VJ, Thon SG, Finley ZJ, O'Brien MJ, Savoie FH. Platelet-Rich Plasma Is an Equal Alternative to Surgery in the Treatment of Type 1 Medial Epicondylitis. Sports Med. 2020;8(3):2325967120908952. DOI: 10.1177/2325967120908952.
16. Picard F, Hersant B, Padula SLa, Meningaud JP. Platelet-rich plasma-enriched autologous fat graft in regenerative and aesthetic facial surgery: Technical note. Journal of Stomatology Oral Maxillofac Surgery. 2017;118(4):228–231. DOI: 10.1016/j.jormas.2017.05.005.
17. Blazhenko AN, Muhanov ML, Lysyh EG, Samojlova AS. Primenenie obogashhjonnoj trombocitami plazmy dlja stimuljacii reparativnogo osteogeneza na rannej stadii formirovanija kostnoj mozoli Sovremennye problemy nauki, tehnologij, innovacionnoj dejatel'nosti: sbornik. trudov po materialam. mezhdunarodnoj. nauchno-prakticheskoj. konferencii. Belgorod: Agentstvo perspektivnyh nauchnyh issledovanij. 2017:9–12.
18. Korymasov EA, Jablonskij PK, Sokolovich EG, Lishenko VV, Motus IJa, Skrjabin SA. Nacional'nye klinicheskie rekomendacii «Jempiema plevry». 2015:33.
19. Achkasov EE, Bezuglov EN, Ul`yanov EN, Ul`yanov AA, Kurshev VV, Repetyuk AD, Egorova ON, Primenenie autoplazmy, obogashhennoj trombocitami, v klinicheskoj praktike [Application platelet-rich plasma in clinical practice]. Biomedicina. 2013;4:46:59.

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2020-8-2-86-99

AUTHORS

Anton V. Kalashnikov – Candidate of Sciences (Medicine), the Head of the Department of Surgical Disciplines of Pyatigorsk Medical and Pharmaceutical Institute – a branch of Volgograd State Medical University. ORCID 0000-0002-7688-9366. E-mail: cos@pmedpharm.ru

Alexander A. Vorobyev – Honored Scientist of the Russian Federation, Doctor of Sciences (Medicine), Professor, the Head of the Department of Operative Surgery and Topographic Anatomy of Volgograd State Medical University. ORCID 0000-0001-8378-0505. E-mail: cos@volgmed.ru

Svetlana A. Kalashnikova – Doctor of Sciences (Medicine), Associate Professor, the Head of the Department of Morphology of Pyatigorsk Medical and Pharmaceutical Institute – a branch of Volgograd State Medical University. ORCID 0000-0002-7688-9366. E-mail: kalashnikova-sa@yandex.ru

Dmitry S. Salimov – Candidate of Sciences (Medicine), the Head of the 2-nd Surgical Department of Central Military Clinical Hospital n. a. P.V. Mandryk. ORCID 0000-0001-8647-1505. E-mail: salimow.dmitry@yandex.ru

RESEARCH ARTICLE

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY

STUDY OF ANTIATHEROSCLEROTIC AND ENDOTHELIOPROTECTIVE ACTIVITY OF PEPTIDE AGONISTS OF EPOR/CD131 HETERORECEPTOR

Puchenkova O.A.[1], Nadezhdin S.V.[1], Soldatov V.O.[2], Zhuchenko M.A.[3], Korshunova D.S.[2], Kubekina M.V.[2], Korshunov E.N.[2], Korokina L.V.[1], Golubinskaya P.A.[4], Kulikov A.L.[1], Gureev V.V.[1], Pokrovskiy V.M.[1], Patrakhanov E.A.[1], Lebedev P.R.[1], Denisyuk T.A.[5], Belyaeva V.S.[1], Movchan E.A.[1], Lepetukha E.I.[1], Pokrovskiy M.V.[1]

[1] Belgorod State National Research University, 85, Pobeda Str., Belgorod, 30801
[2] FInstitute of Gene Biology of the Russian Academy of Sciences, 34/5, Vavilov Str., Moscow, Russia, 11933
[3] Russian Research Center "Kurchatov Institute" – State Science Research Institute of Genetics, 1, Academician Kurchatov Square, Moscow, Russia, 123098
[4] Clinical diagnostic laboratory Voronezh Regional Clinical Ophthalmological Hospital, 22, Revolution of 1905 Str., Voronezh, Russia, 394030
[5] Kursk State Medical University, 3, Karl Marx Str., Kursk, Russia, 305041

E-mail: zinkfingers@gmail.com

Received 10 April 2020 Review (1) 20 May 2020 Review (2) 28 May 2020 Accepted 30 May 2020

Introduction. The drugs affecting a mitochondrial dysfunction, oxidative stresses, apoptosis and inflammation of the vascular wall, have a high potential for the prevention and treatment of atherosclerotic lesions. In this regard, the use of EPOR/CD131 heteroreceptor agonists which have a similar spectrum of pharmacological effects, is one of the promising strategies in the treatment of cardiovascular diseases.

Materials and Methods. The study was carried out on 68 C57Bl/6J male mice. Atherosclerosis was simulated in transgenic animals with an endotheliospecific knockdown of the *Polg* gene by simulating a balloon injury and keeping on a Western diet. Then, the studied drugs were injected once every 3 days at the dose of 20 μg/kg for 27 days. On the 28th day, the animals were euthanized and the area of atherosclerotic plaques was assessed. The gene expression associated with the processes of inflammation, antioxidant protection, apoptosis, and angiogenesis was also determined in the aortic tissues. In addition, the endothelium protective effect of peptides on primary cultures of endothelial cells of wild and transgenic *Polg-D257A* mice was studied.

Results. No statistically significant effect of drugs on the area of lipid infiltration have been found. However, the studied peptides have significantly reduced the expression of proinflammatory genes (*iNos, Icam1, Vcam1, Sele, Il6, Tnfa),* the genes associated with angiogenesis (*Vegfa, Kdr, and Hif1a),* the expression of proapoptic factors; they decreased the Bax/Bcl-2 ratio by more than 1.5 times. In addition, when supplemented with H_2O_2 *in vitro*, peptides dose-dependently increased endothelial cell survival.

Conclusion. The erythropoietin-based peptides can be used to improve the functional state of the vascular wall against the background of atherosclerotic lesions and have a depressing effect on pathobiological processes associated with a mitochondrial dysfunction. In addition, the studied peptides have a significant endothelial protective effect in the induction of oxidative stress *in vitro.*

Keywords: atherosclerosis, erythropoietin derivatives, mitochondrial dysfunction, oxidative stress

For citation: Puchenkova O.A., Nadezhdin S.V., Soldatov V.O., Zhuchenko M.A., Korshunova D.S., Kubekina M.V., Korshunov E.N., Korokina L.V., Golubinskaya P.A., Kulikov A.L., Gureev V.V., Pokrovskiy V.M., Patrakhanov E.A., Lebedev P.R., Denisyuk T.A., Belyaeva V.S., Movchan E.A., Lepetukha E.I., Pokrovskiy M.V. Study of antiatherosclerotic and endothelioprotective activity of peptide agonists of EPOR/CD131 heteroreceptor. *Pharmacy & Pharmacology*. 2020;8(2):86-97. **DOI:** 10.19163/2307-9266-2020-8-2-100-111

Для цитирования: Пученкова О.А., Надеждин С.В., Жученко М.А., Коршунова Д.С., Кубекина М.В., Коршунов Е.Н., Корокина Л.В., Голубинская П.А., Куликов А.Л., Гуреев В.В., Покровский В.М., Патраханов Е.А., Лебедев П.Р., Денисюк Т.А., Беляева В.С., Мовчан Е.А., Лепетюха Е.И., Покровский М.В. Изучение антиатеросклеротической и эндотелиопротективной активности пептидных агонистов гетерорецептора EPOR/CD131. *Фармация и фармакология.* 2020;8(2):100-111. **DOI:** 10.19163/2307-9266-2020-8-2-100-111

Научно-практический журнал
ФАРМАЦИЯ И ФАРМАКОЛОГИЯ

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2020-8-2-100-111

ИЗУЧЕНИЕ АНТИАТЕРОСКЛЕРОТИЧЕСКОЙ И ЭНДОТЕЛИОПРОТЕКТИВНОЙ АКТИВНОСТИ ПЕПТИДНЫХ АГОНИСТОВ ГЕТЕРОРЕЦЕПТОРА EPOR/CD131

О.А. Пученкова[1], С.В. Надеждин[1], В.О. Солдатов[2], М.А. Жученко[3], Д.С. Коршунова[2], М.В. Кубекина[2], Е.Н. Коршунов[2], Л.В. Корокина[1], П.А.Голубинская[4], А.Л. Куликов[1], В.В. Гуреев[1], Покровский В.М.[1], Патраханов Е.А.[1], Лебедев П.Р.[1], Т.А. Денисюк[5], В.С. Беляева[1], Мовчан Е.А.[1], Лепетюха Е.И.[1], Покровский М.В.[1]

[1] ФГАОУ ВО «Белгородский государственный национальный исследовательский университет», 308015, Россия, г. Белгород, ул. Победы, 85
[2] ФГБУН «Институт биологии гена РАН», 119334, Россия, г. Москва, ул. Вавилова, 34/5
[3] НИЦ «Курчатовский институт» – ГосНИИгенетика, 123098, Россия, г. Москва, пл. Академика Курчатова, 1
[4] Клинико-диагностическая лаборатория, Бюджетное учреждение здравоохранения Воронежской области «Воронежская областная клиническая офтальмологическая больница» (БУЗ ВО «ВОКОБ»), 394030, Россия, г. Воронеж, ул. Революции 1905 года, д. 22
[5] ФГБОУ ВО «Курский государственный медицинский университет», 305041, Россия, г. Курск, ул. Карла Маркса, 3

E-mail: zinkfingers@gmail.com

Получено 10.04.2020 Рецензия (1) 20.05.2020 Рецензия (2) 28.05.2019 Принята к печати 30.05.2020

Введение. Препараты, воздействующие на митохондриальную дисфункцию, оксидативный стресс, апоптоз и воспаление сосудистой стенки, обладают высоким потенциалом при профилактике и лечении атеросклеротических поражений. В этой связи применение агонистов гетерорецептора EPOR/CD131, которые обладают подобным спектром фармакологических эффектов, является одной из перспективных стратегий в лечении кардиоваскулярных заболеваний.

Материалы и методы. Исследование было проведено на 68 самцах мышей C57Bl/6J. Атеросклероз моделировали на трансгенных животных с эндотелиоспецифичным нокдауном гена *Polg* путем моделирования баллонной травмы и содержания на западной диете. Затем в течение 27 дней вводили изучаемые препараты 1 раз в 3 дня в дозе 20 мкг/кг. На 28-й день животных эвтаназировали и оценивали площадь атеросклеротических бляшек. Также в тканях аорты определяли экспрессию генов, связанных с процессами воспаления, антиоксидантной защиты, апоптоза, ангиогенеза. Кроме того, было изучено эндотелиопротективное действие пептидов на первичных культурах эндотелиоцитов диких и трансгенных мышей *Polg-D257A*.

Результаты. Мы не обнаружили статистически значимого влияния препаратов на площадь липидной инфильтрации. Однако исследуемые пептиды значимо уменьшили экспрессию провоспалительных генов *iNos, Icam1, Vcam1, Sele, Il6, Tnfa*, генов, связанных с ангиогенезом *Vegfa, Flt-1* и *Hif1a*, экспрессию проапоптических факторов и более чем в 1,5 раза снизили соотношение *Bax/Bcl-2*. Кроме того, пептиды дозозависимо увеличили выживаемость эндотелиоцитов при добавлении H_2O_2 *in vitro*.

Заключение. Используемые пептиды на основе эритропоэтина способны улучшать функциональное состояние сосудистой стенки на фоне атеросклеротического поражения и оказывают угнетающее влияние на патобиологические процессы, связанные с митохондриальной дисфункцией. Кроме того, исследуемые пептиды оказывают значимый эндотелиопротективный эффект при индукции оксидативного стресса *in vitro*.

Ключевые слова: атеросклероз, производные эритропоэтина, митохондриальная дисфункция, оксидативный стресс.

INTRODUCTION

Atherosclerosis is a chronic disease of the walls of vascular walls. It is characterized by aseptic inflammation, impaired perfusion of organs and tissues, a tendency to thrombosis and a progressive dysfunction of vascular walls in the process of aging. In 1912, at the meeting of the Society of Russian Physicians in St. Petersburg, a prominent Russian scientist N.N. Anitschkow together with S.S. Khalatov presented the first results of his revolutionary research concerning the identification of the relationships between alimentary factors, blood cholesterol levels and atherosclerosis. The prize for the most outstanding research in the field of atherosclerosis is currently named after N.N. Anitschkow. Since the time of these works, atherosclerosis has been considered primarily as a disease caused by the accumulation of cholesterol in the vascular walls [1]. This concept is still the key to reducing a cardiovascular risk. However, it is now generally accepted that dyslipidemia is the only cause of atherosclerosis in familial hypercholesterolemia only. In other cases, atherosclerosis is the result of the combined effect of a number of pathogenetic factors; among them there is an endothelial dysfunction [2], hemodynamic overload [3–5], migration of smooth

muscle cells [6], chronic sterile vascular inflammation and a number of other processes.

In the pathobiology of atherosclerosis, a special place is given to the disruption of a mitochondrial function [7]. Mitochondria are the main generator of reactive oxygen species (ROS) in the cell. For example, it is known that passing along the redox gradient of the electron transport chain, 1–3% of electrons prematurely react with oxygen in complexes I and III to form superoxide and other types of ROS [8]. Under various pathological conditions, including hypoxia and inflammation, their number may increase. In addition, mitochondria play not only a metabolic role in vascular cells, but also an important regulatory and signaling one [8].

It is also important that a mitochondrial dysfunction in other cells, including neurons and cardiomyocytes, leads to a decrease in their resistance to ischemia, which is expressed in an increase in deaths against the background of cerebral strokes, coronary artery occlusions, infarctions of kidneys and other organs. In connection with the indicated information, the pathogenetic cascade combining a mitochondrial dysfunction and atherosclerosis, is becoming a relevant target for pharmacological effects.

Agonists of the heterodimeric erythropoietin receptor EPOR/CD131 can be considered as a promising therapeutic approach for influencing the mitochondrial link in case of damage to the vascular wall. The first drug from this group was the 11-amino acid peptide pHBSP (pyroglutamate helix B surface peptide), discovered by a research group guided by Michael Brines, in 2008 [9]. Previously, this peptide was demonstrated to have a pronounced endothelium protective effect in modeling an L-NAME-induced endothelial dysfunction in rats [10, 11]. However, in this study, a side effect in the form of a prothrombotic action has also been identified. In this regard, an attempt to modernize this molecule by adding tripeptide motifs with an antiplatelet effect was made. As a result, two fundamentally new compounds combining cytoprotective [12] and antiplatelet effects (the unpublished data of the authers' own), were obtained. Here, the reports on the results of studying the antiatherosclerotic activity of these compounds are presented.

THE AIM of the study was to evaluate the anti-atherosclerotic and endothelium protective properties of short peptide derivatives of erythropoietin.

MATERIALS AND METHODS

Animals and diet

C57Bl/6J mice were used as the main test system. The animals were donated to the Center for Collective Use of the Institute of Gene Biology, the Russian Academy of Sciences, and were kept in the preclinical research center of the Research Institute of Pharmacology of Living Systems. After passing a 14-day quarantine regime, the mice were stratified by weight and placed in separate conventional cages in accordance with their belonging to the experimental group. Before and during the study, the animals were kept in the rooms with artificial lighting (12h/12h mode) at the temperature of 21–23° C, the humidity of 38–50%; they had a free access to food and water. The number of the conclusion of the independent ethical committee is 06-09/02-1 dated 12/16/2019.

The study included 16 wild-type males and 52 males (25–30 g) with the Polg-D257A/Cdh5-CRE genotype against the background of C57Bl/6J. The genotype is associated with an endothelial-specific knockdown of the Polg gene encoding the polymerase gamma enzyme. Disturbances in the work of this enzyme lead to the development of a mitochondrial dysfunction [13, 14]. This line was created at the Genome editing center of the IGB RAS to study the effects of the oxidative stress and mitochondrial dysfunction. The line is characterized by the presence of a mutant form of the Polg gene under the control of the CAG promoter and a stop cassette flanked by LoxP sites (the unpublished data); the basic structure is described in the article [15]. After cross-breeding with Cdh5-CRE mice, endothelial-specific removal of the stop cassette and overexpression of the mutant Polg form in the endothelium occur. 2 weeks before the operation, the animals were put on a Western diet with 2% cholesterol.

The carried out work met the requirements of the Law of the Russian Federation "On the Protection of Animals from Cruelty" dated June 24, 1998, the rules of laboratory practice when conducting preclinical studies in the Russian Federation (GOST 3 51000.3-96 and GOST R 53434-2009), European Community directives (86/609 EU), the rules of the International Recommendations of the European Convention for the Protection of Vertebrate Animals used in experimental research (1997) and the Rules of laboratory practice adopted in the Russian Federation (order of the Ministry of Health of the Russian Federation No. 708 dated 29.08.2010).

Surgical procedures

The operation was performed on a heated table under a preparative microscope with anesthesia zolazepam (Virbac, Russia) 2.5 mg/100 g + xylazine (Biogel, Belarus) 2 mg/100 g intraperitoneally 2 mg/100 g intraperitoneally); after performing a median laparotomy, the animals were isolated at the level between the bifurcation and the discharge of the renal arteries.

Two clips were placed on the exposed vessel, the arterial wall was incised between them, and the initial section of the balloon catheter was inserted (Fig. 1). Then the proximal clip was removed and the catheter was advanced cranially by 10 mm.

After that, water was pumped into the catheter balloon for 40 seconds, inflating it to a diameter of 1.5 mm at the pressure of 10 bar. Finally, the catheter was removed, three stitches were applied to the incision in the aorta, and the wound was sutured. After the operation, the animals were placed in individual cages with sterilized paper bedding and watched until their awakening.

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2020-8-2-100-111

To alleviate the postoperative pain syndrome, within 3 days from the moment of the operation, the animals received Metamizole sodium (Pharmstandard-Leksredstva (Russia) with drinking water ad libitum at the concentration of 50 mg of the substance per 100 ml of water [16, 17].

Experiment design and drug administration

The animals with the *Polg-D257ACdh5-CRE* genotype were divided into 5 equal groups:

1) Intact – the animals without pathology modeling and without drug administration (n=12);

2) Control – the animals with pathology modeling (balloon injury + Western diet with a high cholesterol content), which, starting from the 1st day, were injected with water subcutaneously in the volume of 0.1 ml/10 g (n=12);

3) P-αB – the animals with pathology modeling, which, starting from the 1st day, were injected with the P-αB peptide (Pharmapark LLC) subcutaneously at the dose of 20 mcg /kg one time per 3 days for 27 days (total dose 180 mcg /kg, n =12);

4) P-αB1 – the animals with pathology modeling, which, starting from the 1st day, were injected with the P-aB1 peptide (Farmapark LLC) subcutaneously at the dose of 20 mcg/kg once every 3 days for 27 days (total dose 180 mcg /kg, n=12);

5) P-αB3 – the animals with pathology modeling, which, starting from the 1st day, were injected with the P-αB3 peptide (Farmapark LLC) subcutaneously at the dose of 20 mcg/kg once every 3 days for 27 days (total dose 180 mcg/kg, n=12) (Table 1).

Measuring the atherosclerotic plaque area

Macroscopic examination of atherosclerotic aortic plaques was performed in four animals from each group. Briefly, on day 28 after the balloon trauma simulation, the animals were euthanized with an anesthesia overdose Zolazepam (Virbac, Russia) 10 mg/100 g intraperitoneally and the abdominal aorta was carefully removed from the bifurcation to the diaphragm level.

Then the preparations were longitudinally dissected, spread out on a foam substrate, washed with a 50% ethanol solution and immersed in an Oil Red O solution for 15 minutes. After that, the preparations were washed with distilled water, and digital photographs were taken. The ratio of the area of the atherosclerotic plaque (colored red) to the intact tissue was calculated using the imageJ program.

Quantitative polymerase chain reaction

After euthanasia, the aortic tissue in the area of the balloon injury was sampled from the other animals, homogenized and incubated for 10 minutes at 37°C in the "Extract RNA" solution. After lysing the sample in the reagent, it was subjected to chloroform cleansing, the supernatant sample was collected and washed with isopropyl alcohol and 70% ethyl alcohol. The concentration of the obtained RNA was measured using an IMPLEN NanoPhotometer spectrophotometer and adjusted to the concentration of 300 ng/µl.

A reverse transcription was performed using the MMLVRTSK021 set according to the manufacturer's protocol (Evrogen). The mixture was carefully mixed and heated for 2 minutes at 70 °C to melt the secondary structures of RNA and then anneal the OligoDT primer. Then the samples were transferred to ice. The entire reaction mixture was incubated for 60 min at 40 °C in a T100™ ThermalCycler (Bio-Rad).

To stop the reaction, the mixture was heated at 70°C for 10 minutes. The resulting DNA was diluted to the concentration of 1 ng/µl. The level of the gene expression was assessed relative to the values of the reference Gapdh gene. The calculation of the expression at the specific point was made according to the following formula: Gene expression = [(Ct (Gapdh)/Ct (Gene of interest)] (Table 2).

In vitro study of cytoprotective activity

In 8 intact mice (4 animals with the *Polg-D257A/Cdh5-CRE* genotype and 4 wild-type animals) after euthanasia under sterile conditions, the inferior vena cava was isolated and washed with a DPBS solution (Thermo FS) until the blood was completely removed. Then the vein was dissected to expose the inner surface and placed in a 0.2% collagenase solution in DPBS with the addition of 0.9 mM $CaCl_2$, 0.493 mM $MgCl_2$, 5.56 mM glucose, 0.327 mM sodium pyruvate, penicillin and streptomycin (Lonza), exposing the intima to enzymatic dissociation. To increase the efficiency of the endothelial cell separation, the inner layer was scraped off with sterile forceps. The collagenase solution containing the cells was collected in a 5 ml tube, the resulting endothelial cells were cultured in DMEM-F12 medium (Lonza) supplemented with 20 mM HEPES buffer (Lonza), 5 U/ml heparin, 200 µg/ml ECGF (Sigma-Aldrich), 10% fetal calf serum (Thermoscientific) at 37 °C in a humid atmosphere containing 5% CO_2 [18].

Cell viability was measured by a quantitative colorimetric MTT analysis, which provides sensitive measurements of cellular metabolic statuses, in particular a mitochondrial status, which can reflect early redox changes. Briefly, exponentially growing cells were seeded in a 96-well plate at the density of 4×104 cells per well. Then the cells were treated with the studied peptides (P-aB, P-aB1, P-aB3 (LLC "Pharmapark") in 3 concentrations – 5, 30, 50 µg/ml for 2 hours. After pretreatment, 200 µM H_20_2 was added for 24 hours to the culture medium. The negative control cells were treated with H_2O_2 only, and the positive control cells were not treated with anything at all. After the incubation for 24 hours, 10 µl of MTT assay set reagent was added to each well and the cells were incubated for an additional hour. The absorption of each reaction product was measured using a microplate reader at the wavelength of 450 nm. The results are expressed as a percentage of MTT uptake in control cells, which was assumed to be 100% (Fig. 2).

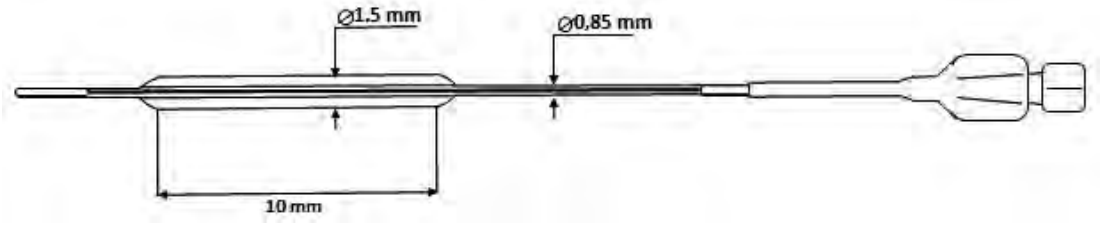


Figure 1 – Schematic image and dimensions of the balloon catheter

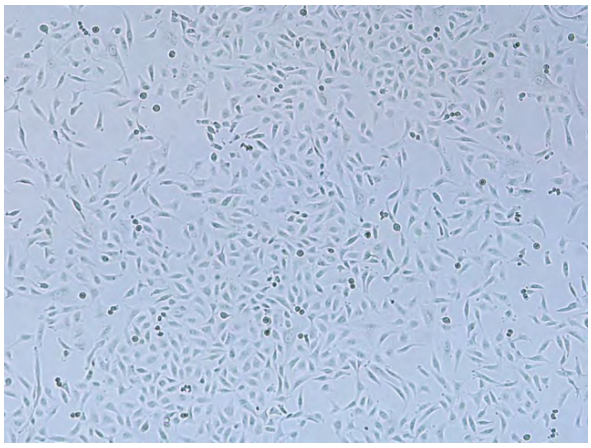


Figure 2 – Primary monolayer of mouse endothelial cells (a 40× magnification)

Table 1 – Amino acid sequence of the tested compounds

Laboratory cipher	Amino acid sequence
P-αB	____QEQLERALNSS
P-αB1	RGDQEQLERALNSS
P-αB3	KGDQEQLERALNSS

Table 2 – Primers used for quantitative PCR

Gene	F-primer	R- primer	Product length (b.p.)	GenBank
Trp53 (p53)	CGACTACAGTTAGGGGGCAC	CCATGGCAGTCATCCAGTCT	95	NM_001127233.1
Bcl2	TCACCCCTGGTGGACAACAT	TTCCACAAAGGCATCCCAGC	102	NM_009741.5
Bax	CCCGAGCTGATCAGAACCAT	GAGGCCTTCCCAGCCAC	96	NM_007527.3
Pon2	CTTCCACACTGCCACCTGAT	TCCTGGGAATTTTAGACCCACA	105	NM_000305.3
Sod2	GGCTGGCTTGGCTTCAATAAG	AGCGGAATAAGGCCTGTTGTT	95	NM_013671.3
Vegfa (VEGF-A)	GGGCCTCCGAAACCATGAA	TGCAGCCTGGGACCACTTG	95	NM_001025250.3
Kdr(VEGFR)	TGCAGGAAACTACACGGTCA	CGATCTGGGGTGGGACATTC	95	NM_001363216.1
Nos2 (iNOS)	GCTCTAGTGAAGCAAAGCCCA	GGGATTCTGGAACATTCTGTGC	103	NM_001313921.1
Icam1	CTCCGGACTTTCGATCTTCCA	CCTTCCAGGGAGCAAAACAAC	98	NM_010493.3
Vcam1	TACTGTTTGCAGTCTCTCAAGC	CGTAGTGCTGCAAGTGAGGG	101	NM_011693.3
Sele (E-selectin)	GGGAAGAAGACTGTCCTAGCC	AGGGGAGCTGGCTTCCTAAG	96	XM_006496715.3
Hif1a	AGAACAACTTGAGCTGGCGT	TGGAGGTGAACTAGGCTCTGT	103	NM_001092957.1
Casp1 (Caspase-1)	TGTATTCACGCCCTGTTGGA	CCCTCAGGATCTTGTCAGCC	100	NM_009807.2
Casp3 (Caspase-3)	GCTTGGAACGGTACGCTAAG	CTTGCTCCCATGTATGGTCTT	105	NM_001284409.1
Il6	GACTGGGGATGTCTGTAGCTC	TGGATGGAAGTCTCTTGCAG	103	NM_001314054.1
Tnfa (TNFa)	ACTGAACTTCGGGGTGATCG	ACTTGGTGGTTTGTGAGTGTG	105	NM_001278601.1
Gapdh	GGGTCCCAGCTTAGGTTCATC	CCCAATACGGCCAAATCCGT	100	NM_001289726.1

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2020-8-2-100-111

Statistical processing

The data obtained were checked for a normal distribution using the Shapiro-Wilk test. The data with the normal distribution were compared with each other using One-way ANOVA with Tukey's HSD post hoc test. The data with the abnormal distribution were compared using the Kruskal-Wallis test and the post-hoc analysis according to Dunn's method.

RESULTS

Macroscopic assessment of plaque

Macroscopic analysis revealed that by the 28th day after the balloon injury modeling, the lipid deposits characteristic of atherosclerosis were visualized in all aortic preparations stained with Oil Red O.

At the same time, the degree of damage varied greatly within the groups, which made it difficult to interpret the results obtained. As a result, no significant differences between the control group and the groups using the tested drugs have been found, although a certain tendency to reduce the area was observed in the group using P-αB1 (Fig.3).

Quantitative PCR

Using a molecular biological analysis of plaque tissues, it was found out that the studied peptides significantly reduce the expression of pro-apoptotic factors Bax, caspase-1 and caspase-3, and also slightly increase the expression of antiapoptic factor Bcl-2. As the heat map in Fig. 2 shows, the greatest effect was demonstrated by preparation P-***α***B1 (Fig.4A).

For an integral assessment of the pro-apoptotic orientation of tissues, the ratio of Bax expression to Bcl-2 was calculated. The average calculated Bax/Bcl-2 ratio was 0.67 in the group of the intact animals, 1.81 in the control group, 1.19 in the group treated with P-aB, 0.96 in the group treated with P-αB1 and 1.09 in the group treated with P-αB3 (Fig. 4B).

Along with the anti-apoptotic effect, the studied drugs decreased the expression of the genes for inflammatory marker Nos2, and molecules of intercellular adhesion Icam1, Vcam1, and E-selectin, which had been increased against the background of trauma. The most pronounced effect was obtained in the group treated with the P-αB1 compound (Fig. 5).

In addition, the studied drugs reduced the expression of factors VEGF-A, VEGFR, and HIF-1a associated with angiogenesis (Fig. 6).

Finally, against the background of modeling atherosclerosis in *Polg-D257A/Cdh5-CRE* mice, it was found out that an increase in the expression of the genes of the antioxidant enzymes Pon2, Sod2 occurs. At the same time, in comparison with the control, the expression of the antioxidant system genes was reduced against the background of the use of the tested peptides. As well as in assessing the effect of peptides on pro-inflammatory and pro-angiogenic genes, the most pronounced effect was obtained in the group treated with the P-αB1 compound (Fig. 7).

In vitro study of cytoprotective activity

When carrying out the MTT test on primary cultures of endothelial cells, it was found out that even without the addition of H_2O_2, endothelial cells expressing Polg-D257A are characterized by a lower signal intensity in comparison with the wild type. During the incubation with H_2O_2, most of Polg-D257A endothelial cells lost their signal intensity almost five times from 80.60 (95% CI 77.29-84.94) to 15.79 (95% CI 11.97-25.42) (Fig. 8).

The studied drugs, dose-dependently increased the cell survival under the conditions of the oxidative stress induced by the addition of H_2O_2. Moreover, the figure shows that modified peptides (P-***α***B1 and P-***α***B3) had a more pronounced effect compared to the base compound (P-***α***B) when added in equivalent doses.

DISCUSSION

Peptide agonists of the EPOR/CD131 heteroreceptor are activators of erythropoietin-associated cytoprotection cascades. Compounds P-αB1 and P-αB3, along with primary anti-apoptotic properties, also have an antiplatelet activity, which is achieved through the introduction of the KGD and RGD tripeptide motifs. In this study, as an experimental model for studying the antiatherosclerotic activity of P-αB1 and P-αB3, transgenic mice were used; they had a mitochondrial dysfunction against the background of the tissue-specific knockdown of the Polg gene encoding gamma polymerase.

Polymerase gamma is an enzyme that plays a key role in mitochondrial DNA replication. This enzyme demonstrates a high accuracy of work and, at the same time, has its own 3'->5' exonuclease activity, due to which it is possible to correct polymerization errors. The inclusion of "wrong" nucleotides without subsequent correction leads to the accumulation of mitochondrial mutations and a mitochondrial dysfunction [19]. As a result, there is an increase in the production of active radicals and damage to the cell. Homozygous animals with a systemic *Polg* knockout do not survive, therefore, in this experiment an endotheliospecific gene knockdown was used [20]. This model reflects one of the key links in the pathogenesis of atherosclerosis – an oxidative stress against the background of a mitochondrial dysfunction. Nevertheless, in any animal model of atherosclerosis, individual differences in timing and a degree of plaque formation are so great that very large groups of animals are needed to test drugs [21–23]. In this regard, a decision to standardize the process of atherogenesis through the induction of atherosclerosis by a balloon trauma and a Western diet was made.

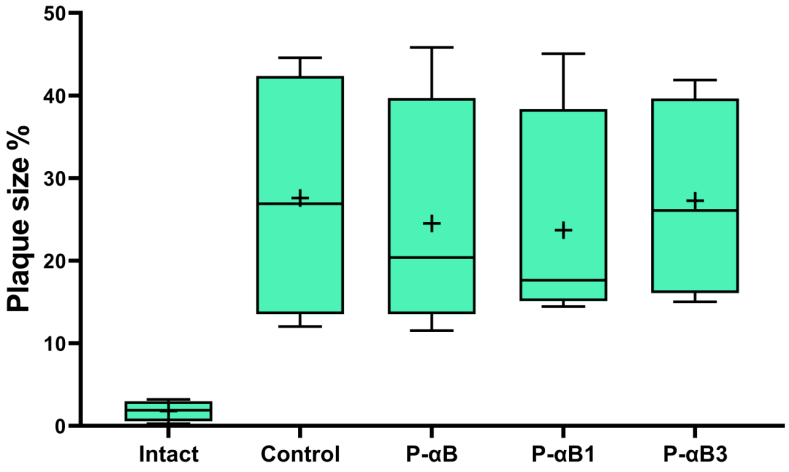


Figure 3 – Area of lipid deposits

Note: + is arithmetic mean

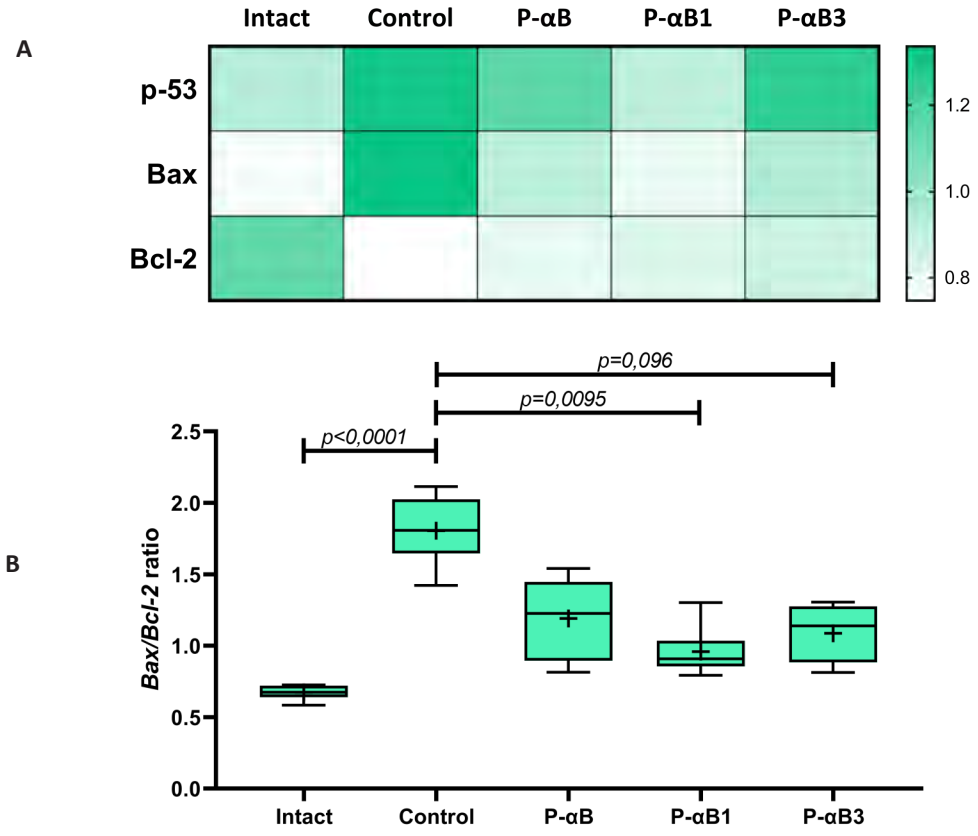


Figure 4 – Influence of the studied drugs on the relative expression of apoptosis markers

Note: A) Fig. A shows that against the background of the balloon damage modeling, the expression of the programmed cell death markers p53 and Bax increases significantly, and the expression of the anti-apoptotic marker Bcl-2 decreases. In almost all cases, the studied drugs return the expression of p53, Bax and Bcl to the level of the values in the intact group; B) Fig. B reflects the Bax/Bcl-2 ratio. The ratio characterizes the Pro-apoptic orientation of the cell: the higher it is, the more pronounced the activation of cascades of the programmed cell death. Fig. B shows that the P-αB1 peptide significantly reduces the Bax/Bcl-2 ratio.

+ is arithmetic mean; the statistical significance of the intergroup differences was detected using the Kruskal-Wallis test and the post-hoc analysis according to Dunne's method.

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2020-8-2-100-111

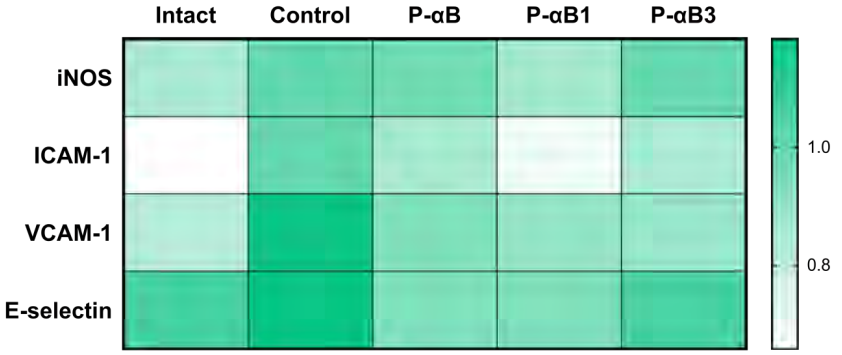


Figure 5 – The expression level of genes *Nos2* (*iNOS), Icam1, Vcam1* and *Sele* (*E-selectin)*

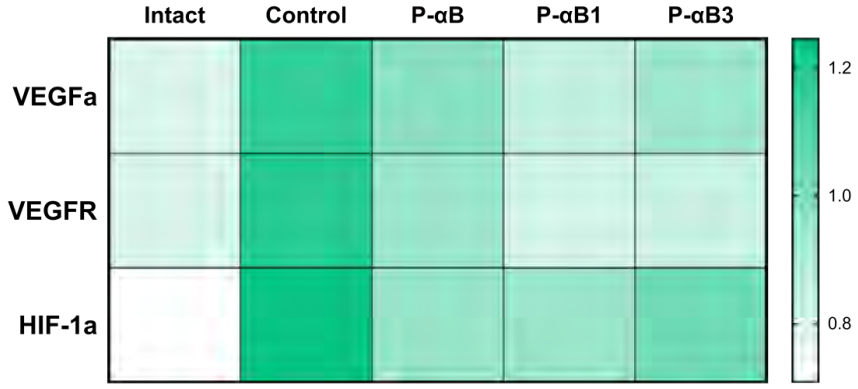


Figure 6 – Expression level of *Vegfa* (*Vegf-A), Kdr (VEGFR-1), and HIF1alpha (HIF-1a)*

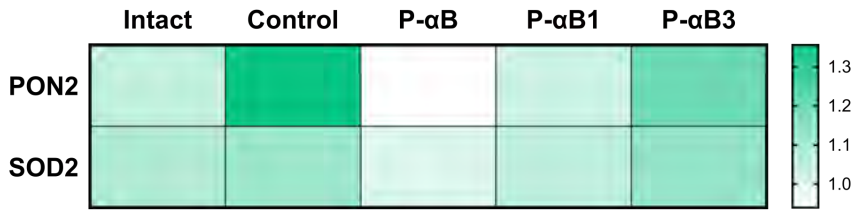
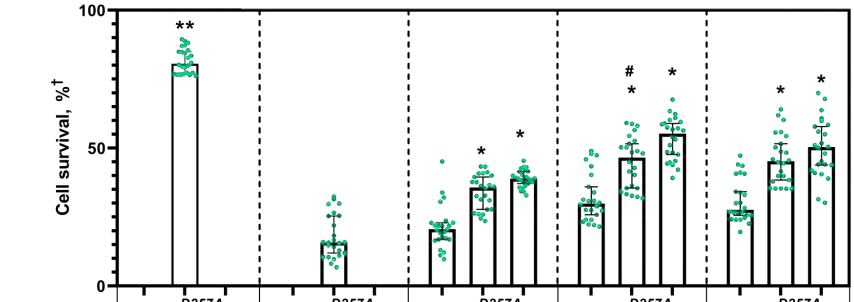


Figure 7 – Expression level of genes PON2, SOD2



Genotype:	$Polg^{D257A}$	$Polg^{D257A}$	$Polg^{D257A}$	$Polg^{D257A}$	$Polg^{D257A}$
H_20_2	-	+	+	+	+
P-αB (μg/ml)	-	-	5 30 50	-	-
P-αB1 (μg/ml)	-	-	-	5 30 50	-
P-αB3 (μg/ml)	-	-	-	-	5 30 50

Figure 8 – Influence of H_2O_2 and tested peptides on the survival of endothelial cells with the *Polg-D257A* genotype

Note: † – relative to intact wild-type endothelial cells; * – p<0.0001 when compared with the group treated only with H_20_2; # – p=0.0783 when compared with the group treated with supplemented P-αB 30 μg/ml

In this model, atherosclerosis is associated with a traumatic effect on the vessel against the background of damage to endothelial cells due to a mitochondrial dysfunction. To confirm the in vivo effects, an in vitro study of the effectiveness of the selected peptides on a primary culture of endothelial cells *Polg*-^{D257A} was also carried out. To enhance the oxidative stress, the cells in the presence of 200 μM H_2O_2 were incubated.

The studied peptides have demonstrated a pronounced endothelioprotective effect in the oxidative stimulus the in vitro model. It has also been found out that the drugs have a pronounced reducing effect on the expression of pro-apoptotic markers. These results are consistent with the concept of the basic mechanism of the action of the erythropoietin derivatives. When the cytoprotective heteroreceptor EPOR/CD131 is activated, Jak/STAT-mediated signal transmission to the nucleus occurs, leading to "survival" signaling by reducing the expression of Pro-apoptotic genes [24, 25]. Similar effects of erythropoietin receptors stimulation have previously been shown in modeling of atherosclerosis [26].

In addition, it has also been found out that the expression of the genes of the antioxidant system PON2 and SOD2 decreased in the treated animals compared to the control. The observed effect is associated with the fact that a strong oxidative and toxic stress develops in the vessels of the control group animals, stimulating an increase in the expression of genes of the antioxidant system. At the same time, against the background of the treatment, pathological phenomena in the cells were reduced, and the stimulating activity against the PON2 and SOD2 genes also decreased.

It has also been found out the peptide agonists EPOR/CD131 have a pronounced anti-inflammatory activity, reducing the expression of pro-inflammatory cytokines and intercellular adhesion molecules. Inflammation is an active factor in the development of atherosclerosis and it contributes to the destabilization of atherosclerotic plaques [27]. VCAM-1, ICAM-1, IL-6, TNF-a molecules play a special role in regulating inflammatory cascades and vascular infiltration by immune cells [28-30]. In general, the anti-inflammatory effect of erythropoietin and its derivatives is a widely studied phenomenon [31, 32]. Therefore, our data fit into the general idea of the pharmacodynamics of EPOR/CD131 agonists.

Further on, a decision to evaluate the effect of the studied peptides on the expression of genes encoding angiogenic factors was made. Angiogenic factors play an important role in the atherosclerosis progression, and erythropoietin is known to be able to stimulate angiogenesis [33]. In atherosclerosis areas, specific local conditions (relative anoxia, inflammation, oxidative stresses) increase the expression of classical and nonclassical angiogenic factors that promote the proliferation of pre-existing vasa vasorum [34]. Neovascularization increases the local flow of nutrients and O_2 and thus may contribute to the progression and remodeling of plaques [35]. The obtained results demonstrated that, in contrast to erythropoietin, peptide agonists EPOR/CD131 exhibit antiangiogenic effects, at least against atherosclerotic plaques.

CONCLUSION

Previously, the following hypothesis had been formulated and confirmed: by adding the tripeptide motifs KGD and RGD, cytoprotective peptide derivatives of erythropoietin can acquire antiplatelet properties. In the course of this study two innovative peptides and a base compound were demonstrated. In this study, it has been demonstrated that two innovative peptides and a basic compound P-αB (pHBSP) protect endothelial cells in vitro; they also reduce pro-apoptotic, pro-inflammatory, and angiogenic activation of vascular wall cells in the model of atherosclerosis combined with a mitochondrial dysfunction. Such a pharmacological activity of the studied drugs seems to be very promising in combination with the information on the presence of an antiplatelet activity in them. Thus, the observed effects complement the information on the cardiovascular activity of innovative peptides P-αB1 and P-αB3, as well as new prospects in the development of peptides combining atheroprotective and antiplatelet properties.

FINANCIAL SUPPORT

This work is carried out with the support of the Ministry of Education and Science of Russia. Subsidy Agreement No. 05.605.21.0191 (unique agreement identifier RFMEFI60519X0191).

AUTHORS' CONTRIBUTION

O.A. Puchenkova – administration of drugs to animals, collection of organs for molecular biological and macroscopic studies, article writing; **S.V. Nadezhdin** – isolation of the primary culture of endothelial cells, in vitro study of the cytoprotective activity of erythropoietin derivatives, article writing; **V.O. Soldatov** – article writing, development of the research design; **M.A. Zhuchenko** – peptide synthesis and literature analysis, article writing; **D.S. Korshunova** – isolation of RNA, conversion of RNA to cDNA, analysis of the expression of targeted genes; **M.V. Kubekina** – isolation of RNA, conversion of RNA into DNA, the analysis of the target genes; **E.N. Korshunov** – handling and caring for animals, preparation of the experimental group of animals; **L.V. Korokina** – article writing, developing a research design; **A.L. Kulikov** – pharmaceutical service, statistical processing and work with graphic materials; **P.A. Golubinskaya** – article writing, formalizing the bibliography; **V.M. Pokrovsky** – observation and care of animals, animal handling, administration of drugs; **E.A. Patrakhanov** – observation and care of animals, animal handling, administration of

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2020-8-2-100-111

drugs; **P.R. Lebedev** – observation and care of animals, administration of drugs. Animal necropsy; **V.V. Gureev** – article writing, consulting on planning, methodology and implementation of the experiment, modeling of balloon injury; **T.A. Denisyuk** – statistical processing, article writing; **V.S. Belyaeva** – analysis of the graphic images and measurement of the area of atherosclerotic plaque, isolation of the primary culture of endothelial cells, study of the cytoprotective activity of erythropoietin derivatives *in vitro*; **E.A. Movchan** – isolation of the primary culture of endothelial cells, in vitro study of the cytoprotective activity of erythropoietin derivatives, preparation of aorta samples for graphic analysis; **E.I. Lepetyukha** – isolation of RNA, quantitative PCR; **M.V. Pokrovskiy** – creation of the idea, planning of research, consultation on the implementation of individual stages of experimental work, quality assurance.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Zárate A, Manuel-Apolinar L, Basurto L, De la Chesnaye E, Saldívar I. Cholesterol and atherosclerosis. Historical considerations and treatment. Arch Cardiol Mex. 2016; 86(2):163–9. DOI: 10.1016/j.acmx.2015.12.002.
2. Davignon J, Ganz P. Role of endothelial dysfunction in atherosclerosis. Circulation; 2004; 109(23):27–32. DOI: 10.1161/01.CIR.0000131515.03336.f8.
3. Davies PF. Hemodynamic shear stress and the endothelium in cardiovascular pathophysiology. Nat ClinPract-Cardiovasc Med. 2009; 6(1):16–26. DOI: 10.1038/ncpcardio1397.
4. Sorokin A, Kotani K, Bushueva O, Taniguchi N, Lazarenko V. The cardio-ankle vascular index and ankle-brachial index in young Russians. Journal of atherosclerosis and thrombosis. 2015; 22(2):211–8. DOI: 10.5551/jat.26104.
5. Polonikov A, Bykanova M, Ponomarenko I, Sirotina S, Bocharova A, Vagaytseva K, Shvetsov Y. The contribution of CYP2C gene subfamily involved in epoxygenase pathway of arachidonic acids metabolism to hypertension susceptibility in Russian population. Clinical and Experimental Hypertension. 2017; 39(4):306–311. DOI: 10.1080/10641963.2016.1246562.
6. Bennett M.R, Sinha S, Owens G.K. Vascular Smooth Muscle Cells in Atherosclerosis. Circ Res. 2016; 118(4):692–702. DOI: 10.1161/CIRCRESAHA.115.306361.
7. Kattoor AJ, Pothineni NVK, Palagiri D, Mehta JL. Oxidative Stress in Atherosclerosis. CurrAtheroscler Rep. 2017; 19(11):42s DOI: 10.1007/s11883-017-0678-6.
8. Quintero M, Colombo SL, Godfrey A, Moncada S. Mitochondria as signaling organelles in the vascular endothelium. Proc. Natl. Acad. Sci. U.S.A. 2006; 103:5379–5384. DOI: 10.1073/pnas.0601026103.
9. Brines M, Patel NS, Villa P, et al. Nonerythropoietic, tissue-protective peptides derived from the tertiary structure of erythropoietin. Proc Natl AcadSci U S A. 2008; 105(31):10925–10930. DOI: 10.1073/pnas.0805594105.
10. Korokin MV, Soldatov VO, Tietze AA, Golubev MV, Belykh AE, Kubekina MV, Puchenkova OA, Denisyuk TA, Gureyev VV, Pokrovskaya TG, Gudyrev OS, Zhuchenko MA, Zatolokina MA, Pokrovskiy MV. 11-amino acid peptide imitating the structure of erythropoietin α-helix b improves endothelial function, but stimulates thrombosis in rats. Pharmacy & Pharmacology. 2019; 7(6):312–320. Russian. DOI: 10.19163/2307-9266-2019-7-6-312-320.
11. Korokin M, Gureev V, Gudyrev O, Golubev I, Korokina L, Peresypkina A, Pokrovskaia T, Lazareva G, Soldatov V, Zatolokina M, Pobeda A, Avdeeva E, Beskhmelnitsyna E, Denisyuk T, Avdeeva N, Bushueva O, Pokrovskii M. Erythropoietin Mimetic Peptide (pHBSP) Corrects Endothelial Dysfunction in a Rat Model of Preeclampsia. Int. J. Mol. Sci. 2020; 21:6759. DOI: 10.3390/ijms21186759.
12. Golubev IV, Gureev VV, Korokin MV, Zatolokina MA, Avdeeva EV, Gureeva AV, Rozhkov IS, Serdyuk EA, Soldatova VA. Preclinical study of innovative peptides mimicking the tertiary structure of the α-helix B of erythropoietin. Research Results in Pharmacology. 2020; 6(2):85–96. DOI: 10.3897/rrpharmacology.6.55385.
13. Trifunovic A, Wredenberg A, Falkenberg M. et al. Premature ageing in mice expressing defective mitochondrial DNA polymerase. Nature. 2004; 429:417–423. DOI: 10.1038/nature02517.
14. Kujoth GC, Hiona A, Pugh TD, et al. Mitochondrial DNA mutations, oxidative stress, and apoptosis in mammalian aging. Science. 2005. 309(5733):481–484. DOI: 10.1126/science.1112125.
15. Zvartsev RV, Korshunova DS, Gorshkova EA., et al. Neonatal Lethality and Inflammatory Phenotype of the New Transgenic Mice with Overexpression of Human Interleukin-6 in Myeloid Cells. DoklBiochemBiophys. 2018; 483(1):344–347. DOI: 10.1134/S1607672918060157.
16. Stubbendorff M, Hua X, Deuse T, et al. Inducing myointimal hyperplasia versus atherosclerosis in mice: an introduction of two valid models. J Vis Exp. 2014; 87:51459. DOI: 10.3791/51459.
17. Tediashvili G, Wang D, Reichenspurner H, Deuse T, Schrepfer S. Balloon-based Injury to Induce Myointimal Hyperplasia in the Mouse Abdominal Aorta. J Vis Exp. 2018; 132:56477. DOI: 10.3791/56477.
18. Molina-Sánchez P, Andrés V. Isolation of Mouse Primary Aortic Endothelial Cells by Selection with Specific Antibodies. Methods in Mouse Atherosclerosis. Methods in Molecular Biology. Humana Press, New York, NY. 2015; 1339. DOI: 10.1007/978-1-4939-2929-0_7.
19. Stumpf JD, Saneto RP, Copeland WC. Clinical and molecular features of POLG-related mitochondrial
20. Kusov P, Deikin A. Developing Novel Transgenic Mice Model Of Atherogenesis With Conditional Oxidative Stress By Introduction Of Epithelium-Specific Inducible Mitochondrial Polg With Mutagenic Activity. Atherosclerosis. 2019; 287:99 s. DOI: 10.1016/j.atherosclerosis.2019.06.287.
21. Poznyak AV, Silaeva YY, Orekhov AN, Deykin AV. Animal models of human atherosclerosis: current progress. Braz J Med Biol Res. 2020. 53(6):9557 s. DOI: 10.1590/1414-431x20209557.
22. Mushenkova NV, Summerhill VI, Silaeva YY, Deykin AV, Orekhov AN. Modelling of atherosclerosis in genetically modified animals. Am J Transl Res. 2019.11(8):4614–4633.
23. Volobueva AS, Orekhov AN, Deykin AV. An update on the tools for creating transgenic animal models

of human diseases – focus on atherosclerosis. Braz J Med Biol Res. 2019; 52(5):8108. DOI: 10.1590/1414-431X20198108.
24. Bittorf T, Jaster R, Lüdtke B, Kamper B, Brock J. Requirement for JAK2 in erythropoietin-induced signalling pathways . Cell Signal. 1997; 9(1):85–89. DOI: 10.1016/s0898-6568(96)00121-0.
25. Peng B, Kong G, Yang C. et al. Erythropoietin and its derivatives: from tissue protection to immune regulation. Cell Death Dis. 2020; 11(79). DOI: 10.1038/s41419-020-2276-8.
26. Warren JS, Zhao Y, Yung R, Desai A. Recombinant human erythropoietin suppresses endothelial cell apoptosis and reduces the ratio of Bax to Bcl-2 proteins in the aortas of apolipoprotein E-deficient mice. Journal of Cardiovascular Pharmacology. 2011; 57(4):424–433. DOI: 10.1097/fjc.0b013e31820d92fd.
27. Bäck M, Yurdagul A, Tabas I. et al. Inflammation and its resolution in atherosclerosis: mediators and therapeutic opportunities. Nat Rev Cardiol. 2019; 16:389–406. DOI: 10.1038/s41569-019-0169-2.
28. Ley K, Huo Y. VCAM-1 is critical in atherosclerosis. J Clin Invest. 2001; 107(10):1209–1210. DOI: 10.1172/JCI13005.
29. Fatkhullina AR, Peshkova IO, Koltsova EK. The Role of Cytokines in the Development of Atherosclerosis. Biochemistry (Mosc). 2016; 81(11):1358–1370. DOI: 10.1134/S0006297916110134.
30. Fotis L, Agrogiannis G, Vlachos IS, Pantopoulou A, Margoni A, Kostaki M, Verikokos C, Tzivras D, Mikhailidis DP, Perrea D. Intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1 at the early stages of atherosclerosis in a rat model. In Vivo. 2012; 26:243–250.
31. Nairz M, Sonnweber T, Schroll A, Theurl I, Weiss G. The pleiotropic effects of erythropoietin in infection and inflammation. Microbes Infect. 2012; 14(3):238–246. DOI: 10.1016/j.micinf.2011.10.005.
32. Liu Y, Luo B, Shi R, et al. Nonerythropoietic Erythropoietin-Derived Peptide Suppresses Adipogenesis, Inflammation, Obesity and Insulin Resistance. Sci Rep. 2015:515134. DOI: 10.1038/srep15134.
33. Kimáková P, Solár P, Solárová Z, Komel R, Debeljak N. Erythropoietin and Its Angiogenic Activity Int J Mol Sci. 2017; 18(7):1519. DOI: 10.3390/ijms18071519.
34. Michel JB, Martin-Ventura JL, Nicoletti A, Ho-Tin-Noe B. Pathology of human plaque vulnerability: mechanisms and consequences of intraplaquehaemorrhages. Atherosclerosis. 2014; 234(2) 311–319.
35. Camaré C, Pucelle M, Nègre-Salvayre A, Salvayre R. Angiogenesis in the atherosclerotic plaque. Redox Biol. 2017; 12:18–34. DOI: 10.1016/j.redox.2017.01.007.

AUTHORS

Olesya A. Puchenkova – 6th year student of the Medical Institute, Belgorod State National Research University (NRU BelSU). ORCID 0000-0002-7657-0937. E-mail: lesya759@yandex.ru

Sergey V. Nadezhdin – Candidate of Sciences (Biology), Researcher, the Research Institute of Pharmacology of Living Systems, Belgorod State National Research University (NRU BelSU). ORCID 0000-0002-6249-2464. E-mail: sergey_nadezhdin@yahoo.com

Vladislav O. Soldatov – Junior Researcher, Center for Collective Use of IBG RAS. ORCID 0000-0001-9706-0699. E-mail: pharmsoldatov@gmail.com

Maxim A. Zhuchenko – Candidate of Sciences (Biology), the Head of the Sector, National Research Center "Kurchatov Institute" – State National Research Institute of Genetics. E-mail: maksim.zhuchenko@pharmapark.ru

Diana S. Korshunova – Junior Researcher, Institute of Gene Biology, Russian Academy of Sciences. ORCID 0000-0002-0259-7045. E-mail: korshunova@genebiology.ru

Marina V. Kubekina – postgraduate student, junior researcher at the Center for High-Precision Editing and Genetic Technologies for Biomedicine at the Institute of Gene Biology of the Russian Academy of Sciences. ORCID 0000-0002-8834-1111. E-mail: marykumy@gmail.com

Evgeny N. Korshunov – The head of the vivarium, junior researcher at the Institute of Gene Biology of the Russian Academy of Sciences. ORCID 0000-0001-8170-4656. E-mail: korshunov@genebiology.ru

Liliya V. Korokina – Candidate of Sciences (Medicine), Assistant Professor, Researcher, the Research Institute of Pharmacology of Living Systems, Belgorod State National Research University (NRU BelSU). ORCID: 0000-0001-5402-0697. E-mail: mkorokin@mail.ru

Aleksandr L. Kulikov – Researcher, Research Institute of Pharmacology of Living Systems, Belgorod State National Research University (NRU BelSU). ORCID ID: E-mail: alex-3031@yandex.ru

Polina A. Golubinskaya – The Head of the Clinical Diagnostic Laboratory, Voronezh Regional Clinical Ophthalmological Hospital". ORCID 0000-0002-1765-9042. E-mail: polinapigeon@gmail.com

Vladimir M. Pokrovsky – 5th year student of the Medical Institute, "Belgorod State National Research University" (NRU "BelSU"). ORCID 0000-0003-3138-2075. E-mail: vmpokrovsky@yandex.ru

Evgeniy A. Patrakhanov – 5th year student of the Medical Institute, "Belgorod State National Research University" (NRU "BelSU"). ORCID 0000-0002-8415-4562. E-mail: pateval7@gmail.com

Petr R. Lebedev – 5th year student of the Medical Institute, "Belgorod State National Research University" (NRU "BelSU"). ORCID 0000-0001-9102-3360. E-mail: Artkeit@yandex.ru

Vladimir V. Gureev – Doctor of Sciences (Medicine), Associate Professor, Professor of the Department of Pharmacology and Clinical Pharmacology, Belgorod State National Research University (NRU BelSU). ORCID 0000-0003-1433-1225. E-mail: produmen@yandex.ru

Tatyana A. Denisyuk – Doctor of Sciences (Medicine), Associate Professor of the Department of Pharmacology, Kursk State Medical University. ORCID 0000-0003-0974-4818. E-mail: denitatyana@yandex.ru

Veronika S. Belyaeva – post-graduate student of the

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2020-8-2-100-111

Department of Pharmacology and Clinical Pharmacology, Belgorod State National Research University (NRU BelSU). ORCID 0000-0003-2941-0241. E-mail: nika.beliaeva@yandex.ru

Evgeniya A. Movchan – postgraduate student of the Department of Pharmacology and Clinical Pharmacology, Belgorod State National Research University (NRU BelSU). ORCID 0000-0002-6244-2563. E-mail: ms.movchan@mail.ru

Elizaveta I. Lepetyukha – postgraduate student of the Department of Pharmacology and Clinical Pharmacology, Belgorod State National Research University (NRU BelSU). E-mail: lisitsa007@bk.ru

Mikhail V. Pokrovskiy – Doctor of Sciences (Medicine), Professor of the Department of Pharmacology and Clinical Pharmacology, the Head of the Research Institute of Pharmacology of Living Systems, Belgorod State National Research University (NRU BelSU). ORCID 0000-0002-2761-6249. E-mail: mpokrovsky@yandex.ru

RESEARCH ARTICLE

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY

USING QUANTUM-CHEMICAL PARAMETERS FOR PREDICTING ANTI-RADICAL (HO·) ACTIVITY OF RELATED STRUCTURES CONTAINING A CINNAMOYL FRAGMENT II. DERIVATIVES OF 2',4'-DIHYDROXYCHALCONE, FLAVANONE AND FLAVONE, CONTAINING A HYDROXY GROUP IN POSITION 7

E.T. Oganesyan, S.S. Shatokhin

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

E-mail: edwardov@mail.ru

Received 30 November 2019 Review (1) 12 February 2020 Review (2) 15 April 2020 Accepted 12 May 2020

42 derivatives of 2',4'-dihydroxychalcone, flavanone and flavone, containing the hydroxy group in position 7 (ring "A"), as well as substituents in the ring "B", have been studied.

The aim is to study the quantum-chemical parameters of 2',4'-dihydroxychalcone, flavanone and flavone derivatives containing a hydroxy group in position 7, in order to identify the effect of substituents on Mulliken charges (a.e) in the aromatic core "A", bond numbers (Nμ), the unsaturation index (IUA) and the electron density of the carbon atoms of the cinnamoyl fragment.

Materials and methods. The listed above parameters have been calculated by the semi-empirical method PM7 (WinMopac 2016 program) on the workstation with an Intel Xeon E5-1620 3.5 GHz processor, 20 GB of RAM.

Results and discussion. The analysis of the values of quantum-chemical parameters, as well as their comparison with the corresponding indicators presented in Report I, revealed a number of important features associated with the influence of the hydroxy group in position 7 (ring "A") on the studied quantum-chemical parameters of molecules. It has been established that the hydroxy group in the ring "A" does not significantly affect the Mulliken charge and the electron density of the carbon atoms of the propenone unit C-7→C-8→C-9. On atom C-9 (carbonyl carbon), the Mulliken charge always has a positive value, and the electron density is about 3.4670-3.4840 for all three groups of compounds. The transition from 2',4'-dihydroxychalcone to flavanone and flavone by the formation of the pyrone heterocycle, is accompanied by an increase in the negative charge on C-8, which can be explained by the involvement of the oxygen heteroatom in the transmission of electronic effects. The hydroxy group in the ring "A", has practically no effect on the charge and electron density of atoms. An analysis of the values of bond numbers and unsaturation indices suggests that atoms C-1 of 2',4'-dihydroxychalcone and 7-hydroxyflavanone derivatives, are characterized by the lowest Nμ value; the lowest bond numbers are characteristic for atom C-8 derivatives of 7-hydroxyflavone. Consequently, the primary attack of the HO·radical will be directed at C-1 (in chalcones and flavanones) and at C-8 in flavones.

Conclusion. The performed quantum-chemical calculations make it possible to analyze the effect on the main quantum-chemical parameters of the molecule, which can be useful in predicting the biological activity of flavanoid compounds due to their antiradical effect on reactive oxygen intermediate species (ROIs).

Keywords: hydroxyl radical, chalcones, flavanones, flavones, Mulliken charges, bond numbers, unsaturation index, electron density

For citation: E.T. Oganesyan, S.S. Shatokhin. Using quantum-chemical parameters for predicting anti-radical (но·) activity of related structures containing a cinnamoyl fragment. II. Derivatives of 2',4'-dihydroxychalcone, flavanone and flavone, containing a hydroxy group in position 7. *Pharmacy & Pharmacology*. 2020;8(2):112-123. **DOI:** 10.19163/2307-9266-2020-8-2-112-123

Для цитирования: Э.Т. Оганесян, С.С. Шатохин. Использование квантово-химических параметров для прогнозирования антирадикальной (но·) активности родственных структур, содержащих циннамоильный фрагмент. II. Производные 2',4'-дигидроксихалкона, а также флаванона и флавона, содержащие гидроксигруппу в положении 7. *Фармация и фармакология.* 2020;8(2):112-123 **DOI:** 10.19163/2307-9266-2020-8-2-112-123

Научно-практический журнал
ФАРМАЦИЯ И ФАРМАКОЛОГИЯ

ОРИГИНАЛЬНАЯ СТАТЬЯ

DOI: 10.19163/2307-9266-2020-8-2-112-123

ИСПОЛЬЗОВАНИЕ КВАНТОВО-ХИМИЧЕСКИХ ПАРАМЕТРОВ ДЛЯ ПРОГНОЗИРОВАНИЯ АНТИРАДИКАЛЬНОЙ (HO·) АКТИВНОСТИ РОДСТВЕННЫХ СТРУКТУР, СОДЕРЖАЩИХ ЦИННАМОИЛЬНЫЙ ФРАГМЕНТ. II. ПРОИЗВОДНЫЕ 2',4'-ДИГИДРОКСИХАЛКОНА, А ТАКЖЕ ФЛАВАНОНА И ФЛАВОНА, СОДЕРЖАЩИЕ ГИДРОКСИГРУППУ В ПОЛОЖЕНИИ 7

Э.Т. Оганесян, С.С. Шатохин

Пятигорский медико-фармацевтический институт – филиал федерального государственного бюджетного образовательного учреждения высшего образования «Волгоградский государственный медицинский университет» Министерства здравоохранения Российской Федерации
357532, Россия, Ставропольский край, Пятигорск, пр. Калинина, 11

E-mail: edwardov@mail.ru

Получено 30.11.2019 Рецензия (1) 12.02.2020 Рецензия (2) 15.04.2020 Принята к печати 12.05.2020

Изучено 42 производных 2',4-дигидроксихалкона, флаванона и флавона, содержащих гидроксигруппу в положении 7 (кольцо «А»), а также заместители в кольце «В».

Цель работы: изучение квантово-химических параметров производных 2',4'-дигидроксихалкона, флаванона и флавона, содержащих гидроксигруппу в положении 7, с целью выявления влияния заместителей в ароматическом ядре «А» на малликеновские заряды (a.e), связевые числа (Nμ), индекс ненасыщенности (IUA) и электронную плотность атомов углерода циннамоильного фрагмента.

Материалы и методы. Полуэмпирическим методом PM7 (программа WinMopac 2016) на рабочей станции с процессором IntelXeonE5-1620 3,5 ГГц, 20 Гб оперативной памяти рассчитаны перечисленные выше квантово-химические параметры анализируемых соединений.

Результаты и их обсуждение. Анализ величин квантово-химических параметров, а также их сравнение с соответствующими показателями, представленными в нашем сообщении I, позволил выявить ряд важных особенностей, связанных с влиянием гидроксигруппы в положении 7 (кольцо «А») на изучаемые параметры молекул. Установлено, что гидроксигруппа в кольце «А» не оказывает существенного влияния на малликеновский заряд и электронную плотность атомов углерода пропенонового звена С-7→С-8→С-9. На атоме С-9 (карбонильный углерод) малликеновский заряд всегда имеет положительное значение, а электронная плотность равна примерно 3,4670–3,4840 у всех трех групп соединений. Переход от 2',4'-дигидроксихалкона к флаванону и флавону путем формирования пиронового гетероцикла сопровождается повышением отрицательного заряда на С-8, что можно объяснить вовлечением гетероатома кислорода в процесс передачи электронных эффектов. Гидроксигруппа в кольце «А» практически не влияет на заряд и электронную плотность атомов. Анализ значений связевых чисел и индексов ненасыщенности свидетельствует о том, что наименьшим значением Nμ характеризуются атомы С-1 производных 2',4'-дигидроксихалкона и 7-гидроксифлаванона; наименьшие связевые числа характерны для атома С-8 производных 7-гидроксифлавона. Из этого следует, что первичная атака электрофильного по характеру радикала НО будет направлена на С-1 (у халконов и флаванонов) и С-8 у флавонов.

Заключение. Проведенные расчеты позволяют проанализировать влияние гидроксигруппы в кольце «А» на важнейшие квантово-химические параметры молекул, что может быть полезно при прогнозировании биологической активности флавоноидных соединений за счет их антирадикального влияния на активные формы кислорода (АФК).

Ключевые слова: гидроксильный радикал, халконы, флаваноны, флавоны, малликеновские заряды, связевые числа, индекс ненасыщенности, электронная плотность

INTRODUCTION

The known facts about the correlation of free radical oxidation with the formation of pathochemical processes and the resulting diseases, actualizes the problem of finding new biologically active compounds exhibiting antiradical properties [1-3]. In this regard, the structures containing three substituents in positions 2,3,4 of the aromatic nucleus of the main conjugation chain [4] (cinnamoyl fragment) – 2,3,4-trihydroxy-, 2,4-dimethoxy-3-hydroxy- and 2,4-di-*tert*-butyl-3-hydroxy derivatives – are of significant interest [5]. In this type of substitution, phenolic hydroxyl in C-3 is surrounded by two ortho substituents, due to the screening effect of which the spatially hindered phenoxy radical is characterized by a sufficient stability. It is important to notify that the Gibbs free energy of the homolytic cleavage of the H–O bond, is quite low and averages -163.61 kJ/mol. Modeling the molecular dynamics of changes in the potential H-O bond energy made it possible to determine the activation energy equal to 34.918 kJ/mol, and this indicates that this reaction is easy at the temperatures of ~ 36–37 °C (~ 310 K).

Further on, 42 compounds – derivatives of chalcone, flavanone and flavone containing substituents in the ring "B" and a hydroxyl group in position 7 of the ring "A", have been studied.

THE AIM is to study quantum-chemical parameters of the derivatives of 2',4'-dihydroxychalcone, flavanone and flavone containing a hydroxy group in position 7 in order to identify the effect of substituents in the aromatic nucleus "A" on Mulliken charges (a.e), bond numbers (Nμ), unsaturation index (IUA) and the electron density of carbon atoms of the cinnamoyl fragment.

MATERIALS AND METHODS

The objects of the study were hydroxy and methoxy substituted, chalcone, flavone and flavanone derivatives in the aryl residue of the cinnamoyl fragment, 42 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.

RESULTS AND DISCUSSION

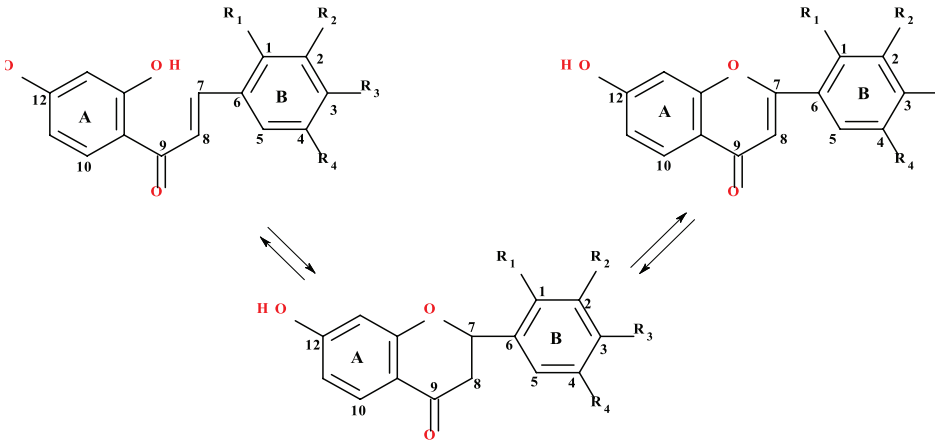
In this paper, the analyzed quantum-chemical parameters of compounds, their structures and symbols are presented in Table 1.

The Mulliken charges (a.e.)

In our previous report [5], in addition to cinnamic acid, 2'-hydroxychalcone derivatives that do not contain substituents in the ring "A" and their corresponding flavanones, were analyzed.

In 2'-hydroxychalcone and the corresponding flavanone [4], atoms C-9 have a positive Mulliken charge of approximately +0.4657 and +0.5222, respectively, and in all the analyzed compounds, without exception, atom C-8 has a negative value equal to approximately -0.300. This gave us grounds to conclude that the primary attack of the HO·radical occurs precisely at position C-8 of both cinnamic acid and 2'-hydroxy chalcone, as well as the corresponding flavanone [5, 6].

Table 1 – Derivatives of chalcone, flavanone and flavone, containing a hydroxy group in position 12* of the ring "A"**

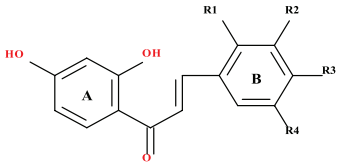
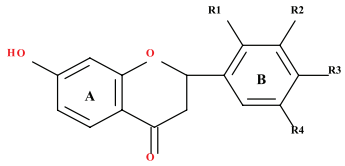
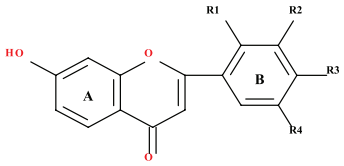


Compounds, No.			Positions of substituents in the ring "B"			
Chalcone	Flavanone	Flavone	1	2	3	4
1x	1anone	1one	H	H	H	H
2x	2anone	2one	OH	H	H	H
3x	3anone	3one	CH_3O	H	H	H
4x	4anone	4one	H	OH	H	H
5x	5anone	5one	H	CH_3O	H	H
6x	6anone	6one	H	H	OH	H
7x	7anone	7one	H	H	CH_3O	H
8x	8anone	8one	H	OH	OH	OH
9x	9anone	9one	H	CH_3O	OH	H
10x	1anone	10one	H	OH	CH_3O	H
11x	11anone	11one	H	CH_3O	CH_3O	H
12x	12anone	12one	H	OH	OH	OH
13x	13anone	13one	H	CH_3O	CH_3O	CH_3O
14x	14anone	14one	H	$(CH_3)_3C$	CH_3O	$(CH_3)_3C$

* Note: The numbering of carbon atoms is given in accordance with the programs generated by the calculation.
** Note: The scheme is presented in accordance with [6]

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2020-8-2-112-123

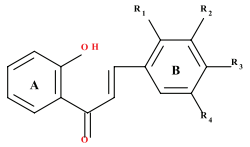
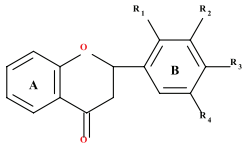
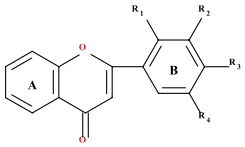
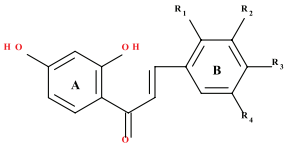
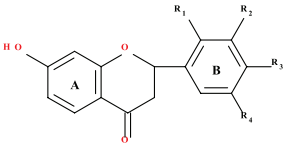
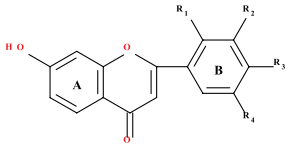
Table 2 – Values of Mulliken charges on atom C-8 of the cinnamoyl fragment of the2',4'-dihydroxychalcone (I), 7-hydroxyflavanone (II), 7-hydroxyflavone (III) derivatives containing the same substituents in the ring "B"

N1*	The position of the substituents in the ring «B»	Structures of the analyzed compounds		
		 I	 II	 III
		a.e.	a.e.	a.e.
1	$R_1=R_2=R_3=R_4=H$	–0.3194	–0.3190	–0.4406	–0.4248
2	$R_1=OH$. $R_2=R_3=R_4=H$	–0.3647	–0.3640	–0.4486	–0.4265
3	$R_1=OCH_3$. $R_2=R_3=R_4=H$	–0.3680	–0.3674	–0.4415	–0.4342
4	$R_2=OH$. $R_1=R_3=R_4=H$	–0.2996	–0.2996	–0.4359	–0.4113
5	$R_2=OCH_3$. $R_1=R_3=R_4=H$	–0.3037	–0.3032	–0.4355	–0.4141
6	$R_3=OH$. $R_1=R_2=R_4=H$	–0.3373	–0.3370	–0.4424	–0.4423
7	$R_3=OCH_3$. $R_1=R_2=R_4=H$	–0.3427	–0.3425	–0.4414	–0.4466
8	$R_2=R_3=OH$. $R_1=R_4=H$	–0.3243	–0.3239	–0.4396	–0.4282
9	$R_2=OCH_3$. $R_3=OH$. $R_1=R_4=H$	–0.3288	–0.3284	–0.4370	–0.4304
10	$R_2=OH$. $R_3=OCH_3$. $R_1=R_4=H$	–0.3276	–0.3274	–0.4387	–0.4315
11	$R_2=R_3=OCH_3$. $R_1=R_4=H$	–0.3329	–0.3327	–0.4380	–0.4341
12	$R_2=R_3=R_4=OH$. $R_1=H$	–0.3016	–0.3012	–0.4342	–0.4158
13	$R_2=R_3=R_4=OCH_3$. $R_1=H$	–0.3190	–0.3130	–0.4378	-0.4495
14	$R_2=R_4=(CH_3)_3C$. $R_3=OH$. $R_4=H$	–0.3467	–0.3454	–0.4379	–0.4179

* According to the nomenclature generally accepted for flavonoids, the structures in question should be designated as 7-hydroxy flavanones and 7 hydroxy flavones. Number 12 in brackets indicates the position generated by the calculation program.

RESEARCH ARTICLE
ISSN 2307-9266 e-ISSN 2413-2241
Scientific and Practical Journal
PHARMACY & PHARMACOLOGY

Table 3 – Electronic density on atom C-8 of the cinnamoyl fragment of 2'-hydroxychalcone (I), flavanone (II), flavone (III), 2',4'-dihydroxychalcone (IV), 7-hydroxy flavanone (V), 7-hydroxyflavone (VI) derivatives containing the identical substituents in the ring "B"

N	Structures of the analyzed compounds						
	 I		 II	 III	 IV	 V	 VI
1	R_1=R_2=R_3=R_4=H	4.3195	4.4400	4.4320	4.3190	4.4406	4.4249
2	R_1=OH. R_2=R_3=R_4=H	4.3648	4.4468	4.4326	4.3640	4.4487	4.4266
3	R_1=OCH_3. R_2=R_3=R_4=H	4.3681	4.4394	4.4412	4.3675	4.4416	4.4343
4	R_2=OH. R_1=R_3=R_4=H	4.2997	4.4374	4.4186	4.2994	4.4359	4.4113
5	R_2=OCH_3. R_1=R_3=R_4=H	4.3037	4.4365	4.4214	4.3033	4.4355	4.4142
6	R_3=OH. R_1=R_2=R_4=H	4.3373	4.4413	4.4491	4.3370	4.4425	4.4423
7	R_3=OCH_3. R_1=R_2=R_4=H	4.3428	4.4406	4.4532	4.3425	4.4414	4.4466
8	R_2=R_3=OH. R_1=R_4=H	4.3244	4.4373	4.4347	4.3239	4.4397	4.4282
9	R_2=OCH_3. R_3=OH. R_1=R_4=H	4.3288	4.4357	4.4373	4.3284	4.4371	4.4304
10	R_2=OH. R_3=OCH_3. R_1=R_4=H	4.3277	4.4383	4.4386	4.3275	4.4387	4.4316
11	R_2=R_3=OCH_3. R_1=R_4=H	4.3329	4.4376	4.4412	4.3327	4.4382	4.4342
12	R_2=R_3=R_4=OH. R_1=H	4.3017	4.4337	4.4233	4.3013	4.4343	4.4158
13	R_2=R_3=R_4=OCH_3. R_1=H	4.3136	4.4370	4.4355	4.3130	4.4378	4.4495
14	R_2=R_4=$(CH_3)_3$C. R_3=OH. R_4=H	4.3468	4.4401	4.4558	4.3455	4.4380	4.4179

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2020-8-2-112-123

In this report, derivatives of 2',4'-dihydroxychalcone are considered. During heterocyclization, they turn into 7 (12)-hydroxyflavanones*, respectively.

Heterocyclization of 2',4'-dihydroxychalcone to the corresponding 7-hydroxyflavanone and then to 7-hydroxyflavone causes changes in the values of Mulliken charges on atoms C-1 – C-8. In the absence of substituents in the ring "B", all three types of structures on atoms C-1 – C-6 and C-8 have a Mulliken charge of a negative value. If there is a substituent (OH or OCH_3) in positions C-1 and/or C-3 of the "B" ring (compounds 2x–7x; 2anone – 7anone; 2one – 7one) in atom C-8, the negative Mulliken charge increases substantially, reaching a maximum of –0.4420 (on average) in flavanone (compounds 2anone, 3anone, 6anone, 7anone) and –0.4374 (on average) in flavone (compounds 2one, 3one, 6one, 7one). In corresponding chalcones, the Mulliken charge on atoms C-8 averages –0.3527 (compounds 2x, 3x, 6x, 7x). On atoms C-1 (compounds 2x, 3x, 2anone, 3anone, 2one, 3one) the charge is positive and equals to +0.3244 on average (compounds 2x and 3x), +0.2700 (compounds 2anone and 3anone) and +0.3191 (compounds 2one, 3one). A similar picture is observed for compounds 4x, 5x, 4anone, 5anone, 8anone – 13anone, 5one – 12one and 14one. On atoms C-3 (compounds 6x – 14x, 6anone – 13 anone, 8one– 12one) and practically on all atoms C-7 (except compounds 4x, 8x – 10x, 12x, 13x), the charge has also a positive value.

The changes in the values of Mulliken charges on atoms C-8 for chalcone derivatives (compounds 1x – 14x), flavanone (compounds 1anone– 14anone) and flavone (compounds 1one – 14one), follow the same pattern: for the compounds containing hydroxy- and methoxy groups in position C-1 of the "B" ring (*ortho*-position to the main conjugation chain); the average charge is equal to –0.3657 (compounds 2x, 3x), –0.4450 (compounds 2anone, 3anone) and –0.4304 (compounds 2one, 3one) (Table 2).

On carbon atoms which the electron-donating OH and OCH_3-groups are bound with, the Mulliken charge has a positive value of +0.3406 (compound 2x), +0.2845 (compound 6anone) and +0.3476 (compound 2one).

There is a similar picture in case if -OH and -OCH_3 groups are located in position C-3 of the ring "B" (*para*-position to the main conjugation chain): in chalcones 6x and 7x, the Mulliken charge on C-8 is equal to -0,3370 and -0.3425, respectively; in flavanones 6anone and 7anone – -0.4424 and –0.4414, in flavones 6one and 7one –0.4423 and -0.4466, respectively. Chalcone derivatives 8x – 11x, flavanones 8anone – 11anone and flavanones 8one– 11one in positions 2 and 3 of the ring "B" simultaneously contain two substituents: 2,3-dihydroxy-, 2-methoxy-3-hydroxy-, 2-hydroxy-3-methoxy- and 2,3-dimethoxy-. In these compounds, the Mulliken charge on C-8 varies slightly: on chalcones 8x – 11x, it is on average equal to –0.3281, for flavanones 8anone– 11anone, it averages -0.4385, for flavones 8one– 11one a.e. -0,4310.

The Mulliken charge on C-8 in compounds 6x, 7x (chalcones), 6anone, 7anone (flavanones), 6one, 7one (flavones) containing one substituent (OH or OCH_3) in position 3 of the ring "B", significantly higher than for compounds 8x – 11x (chalcones), 8anone– 11anone (flavanones) and 8one– 11one (flavones), which contain OH and OCH_3 groups in positions 2,3. It is clear that when there is one substituent in C-3, the polar conjugation with the propenone fragment is much higher than with two substituents in positions C-2 and C-3 simultaneously. This fact is explained by the competing contribution of the substituent in C-2 with that in C-3, which can be seen when comparing the contribution of Taft σ-constants: OH meta +0.127; OH para -0.370; OCH_3 meta +0.115; OCH_3 para -0.268 [7, 8].

Each of the compounds 13x, 14x, 13anone, 14anone, 13one, 14one contains three substituents in positions 2, 3, 4 and, despite the competitive contribution of Taft σ-constants, are characterized by approximately the same values of Mulliken charge on C-8.

It should be notified that on carbonyl carbon C-9, the Mulliken charge is characterized by a high positive value for all the analyzed compounds, and it is in the range from 0.5200 to 0.5335. Based on the data in Table 2, the following can be postulated:

1. the value of Mulliken charges on cinnamoyl fragment C-8 in chalcones in pairs (compounds I and IV), is almost the same (there are differences in the fourth decimal place after the comma). It means that the -OH group in position 4' (compound IV) has almost no effects on the charge of atom C-8;
2. derivatives of 2',4'-dihydroxychalcons (compounds 1x, 4x, 5x, 10x, 12x, 13x) have a very slight negative charge on atom C-7 (-0.0180 on average); here, the electron-donor substituents OH and OCH_3 are in positions C-1 or C-3 of the ring "B";
3. heterocyclization of the chalcones to the corresponding flavanones and flavones contributes to an increase in the negative charge on C-8, which is associated with the involvement of the oxygen heteroatom in the process of transferring electronic effects;
4. the negative charge on C-8 in flavanones (structures II and V) is significantly higher than that of the corre-

* According to the nomenclature generally accepted for flavonoids, the structures in question should be designated as 7-hydroxy flavanones and 7 hydroxy flavones. Number 12 in brackets indicates the position generated by the calculation program.

sponding flavones (structures III and VI): there is no vinyl group C-7 – C-8 in flavanones;

5. if we compare the positive charge on atoms C-7 of the analyzed compounds, it is easy to see that this charge is almost twice lower in flavanones than in flavones;
6. the above listed features of the distribution of the Mulliken charges indicate that the electrophilic hydroxyl radical is primarily attached to C-8 position.

Electrondensity

When discussing the chemical properties of organic compounds containing fragments with conjugated bonds, not only the charge distribution on the reaction sites, but also the electron density on carbon atoms are helpful.

Analyzing our data on the electron density on the carbon atoms of the cinnamoyl fragment, it can be concluded that using the relationship between the Mulliken charge (q_μ) of the given atom, its electron density (Pμμ), and the number of electrons introduced by this atom into the total π-system (η_μ), any of these parameters can be determined using this expression [9–11]:

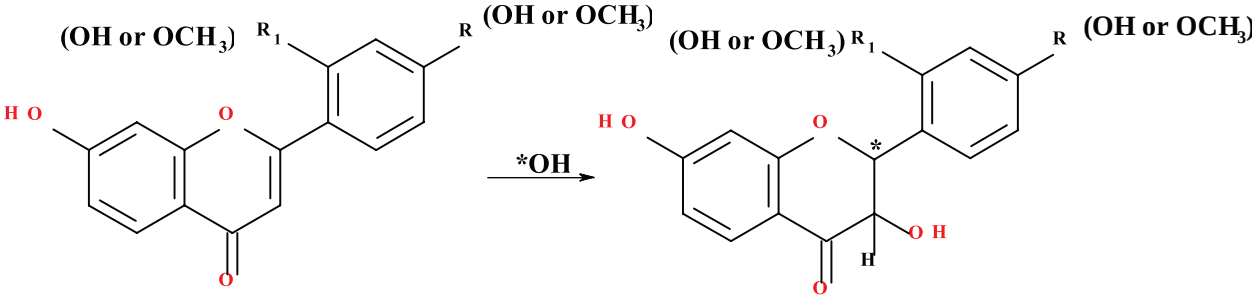
$$q_\mu = \eta_\mu - P\mu\mu$$

Comparing the data presented in Tables 2, 3 and 4, as well as those given in Report I, we found that all atoms of C-7 – C-8 – C-9 propenone unit contribute 4 electrons to form the π-system. Note that the electron density for all compounds is 4 – (a.e.), as can be easily seen from the data in Table 3. For example, if a.e. = –0.26842, then the electron density = 4 – (–0.26842) = 4.2684; if a.e. = +0.34766, then the electron density= 4 – 0.34766 = 3.6523.

In all the analyzed groups of compounds (including those presented in Report I), the highest electron density in the ring "B" bond system → C-7 → C-8 → C-9 is focused on C-8. Analyzing the values of electron densities on C-8 compounds considered in this article, and those previously given in Report 1, it can be noticed that the presence of hydroxy groups in the ring "A" (position 4' in chalcones and 7 in flavanones and flavones) practically does not affect the value of this parameter. For comparison, Table 4 presents the values of electron densities for C-8 compounds containing electron-donating substituents in position 1–4 of the ring "B".*

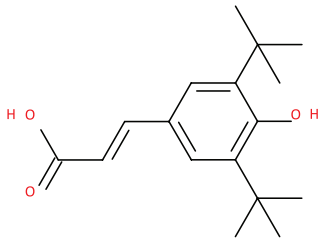
The data of Table 3 show that the electron density values of C-8 in 2'-hydroxy- and 2',4'-dihydroxychalcones are almost the same, identical to Mulliken charges (Table 2). The same dependence is observed in both flavanone derivatives that do not contain a hydroxy group in the "A" ring, and 7-hydroxy flavanones; these compounds lack the C-7→C-8 vinyl group. The absence of the latter, formally excludes the influence of the +M effect from the electron-donating substituents of the ring "B" on the C-6→C-7→C-8 chain of carbon atoms; however, the presence of an oxygen heteroatom can be the cause for the assumption of its participation in the transmission of electronic effects in the following chain order; C-6→C-7→ → ring "A". This assumption is true, since the electron densities in C-8 of flavanones and flavones are very close (Table 3).

Thus, taking into account the electrophilic properties of the hydroxyl radical, as well as the magnitude of Mulliken charges and electron densities, the addition of radical ·OH primarily from the C-8 position, is highly likely. It can be represented as the following diagram:



This scheme is consistent with the conclusions in [13, 14], obtained on the basis of calculations of the enthalpies of radical adducts formation.

Earlier, in accordance with the forecast, we obtained a cinnamic acid derivative, which contained tert-butyl radicals in positions C-2 and C-4 and a hydroxy group in C-3, in the aryl residue of the main conjugation chain (the ring "B") [12]. It showed high cerebroprotective, antioxidant, endothelioprotective and actoprotective activities [15–18]:



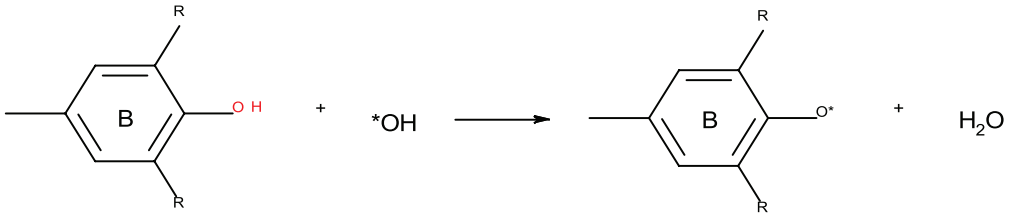
4-hydrohy-3,5-di-tert-butyl cinnamic acid

* To compare the data, Table 3 includes electron densities for a 2'-OH-chalcone and the unsubstituted flavanone on the "A" ring.

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2020-8-2-112-123

Such a high activity can be explained both by the presence of a sterically hindered hydroxy group in C-3, and by a high electron density (-4.3252) in C-8. According to the updated data, the Gibbs energy of homolytic cleavage of the O-H bond is 181.29 kJ/mol.

By the method of molecular dynamics, a simulated reaction according to which a process leading to the formation of a highly active and spatially hindered phenoxyl radical, has been carried out:



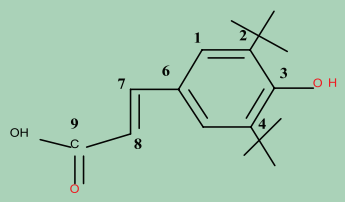
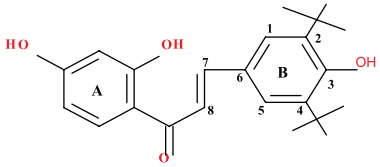
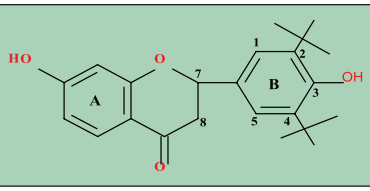
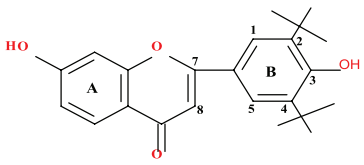
The fact about the cinnamoyl fragment as the main conjugation makes the greatest contribution to the reactivity with respect to reactive oxygen intermediate species (ROIs) and, therefore, to pharmacological activity. It is obvious and undeniable. This conclusion confirms the previously stated results [13, 14].

The above listed conclusions were the basis for predicting derivatives of chalcone, flavanone and flavone containing two *tert*-butyl radicals in positions 2,4 and a hydroxy group in C-3 in the aryl residue "B" (Table 1). These compounds are still virtual and will be synthesized in due course time, however, their quantum-chemical characteristics were found necessary to analyze, and the data are shown in Tables 2–4.

In the previous report it was pointed out that the Gibbs energy of the homolytic bond break H-O depends on the value of the unsaturation index (IUA) of the carbon atom which the hydroxy group is connected with: the higher the IUA value, the lower the bond break energy is.

Considering the closeness of the quantum-chemical characteristics of the predicted structures –14 chalcone, 14flavanone and 14flavone (Table 1) with those of the previously obtained cinnamic acid derivative – it is possible to discuss their high pharmacological activity, which can be proved by the Gibbs energies of the cleavage of the H-O bond (Table 4).

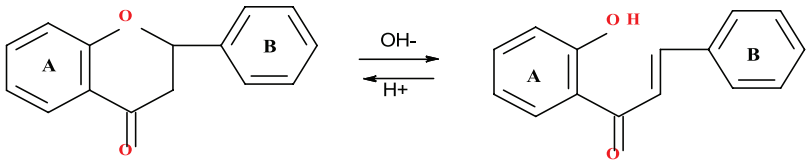
Table 4 – Gibbs free energy of homolytic bond cleavage OH

Structure	IUA unsaturation index (C-3)	Energy of homolytic bond cleavage OH
	0.167	–181.29
	0.166	–178.31
	0.160	–163.08
	0.253	250

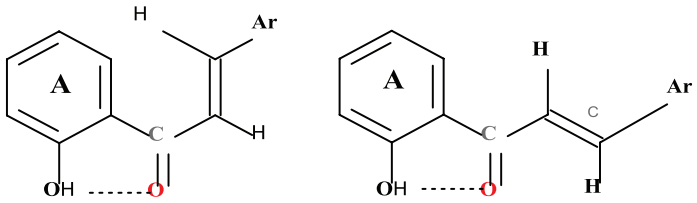
ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY

It should be notified that flavanone derivatives belong to systems with a closed conjugation chain. Although flavanones also belong to the closed system, they lack Ar-C=C-C=O conjugation, and for this reason they are able to transform into the corresponding chalcones with a slight PH>7 deviation:



For chalcones as compounds with an open conjugation chain, various geometric isomers are possible due to the cross-conjugated system, the most stable of which are the derivatives with the trans configuration of the vinylene fragment [19, 20]:



trans-s-trans **trans-s-cis**

Such structural features of chalcones, in contrast to flavones, indicate additional centers of complementarity to the biological substrate, which can explain the presence of a wider spectrum of pharmacological activity, as well as the absence of prooxidant properties [21].

Bond numbers (Nμ) and unsaturation indices (IUA)

In the method of Hückel molecular orbitals (HMO), the values of bond orders [9] are used to characterize molecules, which characterize a relative strength of the covalent bond, its length and reactivity. The sum of all the bond orders belonging to the given atom, determines the bond number (Nμ), which characterizes the degree of saturation of a particular atom. The larger the Nμ value, the higher the degree of saturation, and, conversely, the lower the Nμ value, the greater is the ability of a given atom to form new bonds. This property is closely related to the unsaturation index (IUA) and theoretical valency (Vμ), between which there is a relationship:

$$IUA = V\mu - N\mu$$

Table 5 below examplifies the matrix of quantum chemical parameters obtained by analyzing the compounds considered in this report.

The comparison of the Nμ values on the C-1→C-6→C-7→C-8 site of the cinnamoyl fragment of the analyzed compounds (Table 6), makes it possible to draw a number of conclusions:

1. in the derivatives of 2',4'-dihydroxychalcone, an increase in Nμ from C-1 to C-7 is observed, which then decreases slightly in C-8;
2. a similar pattern is observed in the derivatives of 7-hydroxyflavanones;
3. in 7-hydroxyflavone, the Nμ values in atoms C-1 and C-6 are almost the same and amount to an average of 3.769, but in C-7 there is an increase in this parameter, which reaches an average of 3.803;
4. in C-8 of 7-hydroxyflavone derivatives, Nμ decreases very sharply and averages 3.696; this indicates an increase in the unsaturation of this atom;
5. based on the data obtained, it can be reliably concluded that reactive oxygen intermediate species (ROIs) will primarily be bound by flavonoids in position C-8 of the propenone fragment.

CONCLUSION

In this report, representatives of the extensive group of natural compounds – flavonoids: chalcones, flavanones and flavones containing electron-donating substituents in the ring "B" (i.e. they are in the aromatic core of the cinnamoyl fragment) – have been considered. All the analyzed structures contain a hydroxy group in the position of 4'-chalcones and in the position of 7-flavanones and flavones *.

The analysis and comparison of such parameters as the bond number (Nμ), unsaturation index (IUA), and electron density indicate that they differ insignificantly, but a high electron density in C-8 is common to them.

It is characteristic for C-7 atoms in the cinnamoyl fragment C-1→C-6 →C-7→C-8→C-9 of all three types of structures, to have the highest Nμ value and a positive Mulliken charge, and C-8 atoms are characterized by the smallest bond number and the highest electron density. This indicates the fact that the primary attack of the electrophilic radical HO• is most likely to the position of C-8.

* Compounds 14x, 14anon and 14on are not found in nature and so far are virtual. In one of the messages we give methods for their synthesis.

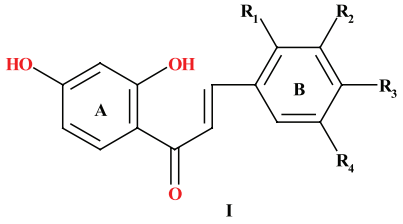
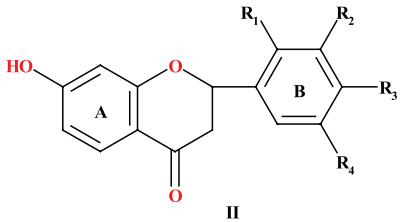
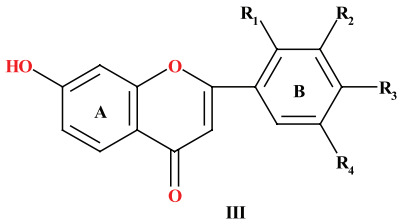
ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2020-8-2-112-123

Table 5 – Quantum-chemical parameters of compounds 2x, 2anone and 2one

Substance 2x									
Atom (CV)	1	2	3	4	5	6	7	8	9
Charges, a.e.	0.34062	–0.26842	–0.04754	–0.23754	–0.04850	–0.23270	0.00332	–0.36401	0.54435
Bond numbers (Nμ)	3.747	3.787	3.831	3.841	3.808	3.768	3.841	3.764	3.737
TheoreticalValency (Vμ)	3.900	3.957	3.966	3.967	3.965	3.961	3.930	3.947	3.811
Unsaturation index(IUA)	0.153	0.170	0.135	0.126	0.157	0.193	0.089	0.183	0.074
Electron density	36594	42684	40475	42375	40485	42327	39667	43640	34556
Substance 2anone									
Atom (C_v)	1	2	3	4	5	6	7	8	9
Charges, a.e.	0.28456	–0.23074	–0.91736	0.19793	–0.08918	–0.18985	0.15385	–0.44670	0.52210
Bond numbers (Nμ)	3.777	3.803	3.842	3.844	3.823	3.788	3.829	3.811	3.761
Theoretical valency (Vμ)	3.922	3.966	3.973	3.973	3.968	3.979	3.895	3.928	3.820
Unsaturation index(IUA)	0.145	0.163	0.131	0.129	0.145	0.191	0.066	0.117	0.059
Electron density	3.7154	4.2307	4.0917	4.1979	4.0892	4.1899	3.8461	4.4487	3.4779
Substance 2one									
Atom (CV)	1	2	3	4	5	6	7	8	9
Charges, a.e.	0.34766	–0.26636	–0.04467	–0.23832	–0.03339	–0.25089	0.31826	–0.42655	0.51493
Bond numbers (Nμ)	3.759	3.784	3.833	3.838	3.808	3.727	3.792	3.709	3.792
Theoretical valency (Vμ)	3.904	3.956	3.966	3.967	3.962	3.947	3.901	3.922	3.851
Unsaturation index(IUA)	0.145	0.172	0.133	0.129	0.154	0.220	0.109	0.213	0.059
Electron density	3.6523	4.2664	4.0447	4.2383	4.0334	4.2509	3.6817	4.4266	3.4851

RESEARCH ARTICLE
ISSN 2307-9266 e-ISSN 2413-2241
Scientific and Practical Journal
PHARMACY & PHARMACOLOGY

Table 6. Values of bond numbers on carbon atoms *C*-1→*C*-6→*C*-7→*C*-8*

Compound, No.	 I 2', 4'-dihydroxychalcone				 II 7-OH-flavanone				 III 7-OH-flavone			
	Nμ (C-1)	Nμ (C-6)	Nμ (C-7)	Nμ (C-8)	Nμ (C-1)	Nμ (C-6)	Nμ (C-7)	Nμ (C-8)	Nμ (C-1)	Nμ (C-6)	Nμ (C-7)	Nμ (C-8)
1	3.813	3.859	3.858	3.815	3.829	3.830	3.838	3.818	3.810	3.830	3.802	3.697
2	3.747	3.768	3.841	3.764	3.777	3.788	3.829	3.811	3.759	3.759	3.792	3.709
3	3.740	3.765	3.839	3.787	3.780	3.789	3.843	3.810	3.770	3.742	3.804	3.708
4	3.750	3.763	3.862	3.820	3.763	3.837	3.838	3.815	3.747	3.747	3.807	3.701
5	3.743	3.757	3.860	3.819	3.757	3.838	3.838	3.816	3.740	3.740	3.805	3.701
6	3.812	3.826	3.852	3.807	3.829	3.821	3.838	3.819	3.805	3.805	3.797	3.689
7	3.130	3.819	3.849	3.803	3.826	3.819	3.837	3.819	3.809	3.809	3.794	3.687
8	3.805	3.833	3.857	3.881	3.766	3.833	3.838	3.818	3.749	3.749	3.804	3.695
9	3.808	3.833	3.854	3.809	3.759	3.833	3.838	3.819	3.741	3.721	3.801	3.694
10	3.803	3.832	3.854	3.809	3.767	3.832	3.837	3.818	3.751	3.819	3.837	3.693
11	3.805	3.825	3.851	3.807	3.782	3.825	3.836	3.820	3.743	3.819	3.798	3.692
12	3.740	3.833	3.861	3.818	3.752	3.832	3.838	3.816	3.739	3.826	3.806	3.699
13	3.733	3.837	3.855	3.815	3.727	3.837	3.836	3.821	3.787	3.798	3.788	3.686
14	3.792	3.818	3.848	3.804	3.809	3.818	3.842	3.818	3.725	3.699	3.802	3.699

* The numbers of the compounds correspond to Table 1

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2020-8-2-112-123

FINANCIAL SUPPORT AND SPONSORSHIP

This study did not have any financial support from outside organizations.

AUTHORS' CONTRIBUTION

All authors equally contributed to the research work.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

1. Myhre O, Utkilen H, Duale N, Brunborg G, Hofer T. Metal dyshomeostasis and inflammation in Alzheimer's and Parkinson's diseases: possible impact of environmental exposures. Oxidative Medicine and Cellular Longevity. 2013, 1–19. Oxidative Medicine and Cellular Longevity. 2013;2013:1–19. DOI: 10.1155/2013/726954.
2. Snodderly DM. Evidence for protection against age-relatedmacular degeneration by carotenoids and antioxidant vitamins. The American Journal of Clinical Nutrition. 1995;62:1448–1461. DOI: 10.1093/ajcn/62.6.1448s.
3. Thome J, Zhang, JJ, Davids E, Foley P, Weijers HG, Wiesbeck GA, Boning J, Riederer P, Gerlach M. Evidence forincreased oxidative stress in alcohol-dependent patients provided byquantification of in vivo salicylate hydroxylation products. Alcoholism, clinical and experimental research.1997;21:82–85. DOI: 10.1111/J.1530-0277.1997.TB03732X.
4. Agadzhanayan VS, Oganesyan ET. Primenenie kvantovo-himicheskih metodov analiza dlja interpretacii antiradikal'noj aktivnosti v rjadu gidroksiproizvodnyh korichnoj kisloty [The use of quantum chemical analysis methods for the interpretation of anti-radical activity in a series of hydroxy derivatives of cinnamic acid]. Chemical Pharmaceuticals. 2008;42(11):12–17. Russian. DOI: 10.30906/0023-1134-2008-42-11-12-17.
5. Oganesyan ET, Shatokhin SS, Glushko AA. Ispol'zovanie kvantovo-himicheskih parametrov dlja prognozirovanija antiradikal'noj (no·) aktivnosti rodstvennyh struktur, soderzhashhih cinnamoil'nyj fragment [The use of quantum chemical parameters to predict the antiradical (HO) activity of related structures containing a cinnamoyl fragment. I. Derivatives of cinnamic acid, chalcone and flavanone]. Pharmacy and Pharmacology. 2019;7(1):53–66. Russian. DOI: 10.19163/2307-9266-2019-7-1-53-66.
6. Litvinenko VI. Natural flavonoids. In the book "Technology and standardization of drugs". Rostov, LLC Rireg. 1996:784 p.
7. Handbook of a chemist in 6 volumes. M.: Chemistry. 1964;3:1005 p.
8. Neuvonen K, Neuvonen H, Koch A, Kleinpeter E. Taft equation in the light of NBO computations. Introduction of a novelpolar computational substituent constant scale σ*q for alkyl groups. Computational and Theoretical Chemistry. 2012;981:52–58. DOI: 10.1016/j.comptc.2011.11.044.
9. Minkin VI, Simkin BYa, Minyaev RM. Theory of the structure of molecules. Rostov-on-Don. 1997:560 p.
10. Krasnov KS. Molecules and chemical bonding. M. "Higher School". 1977:280 p.
11. Zhdanov YuA. Theory of the structure of organic compounds. M. "HigherSchool". 1971:288 p.
12. Aghajanayan VS, Oganesyan ET, Abaev VT. Celenapravlennyj poisk soedinenija-lidera v rjadu proizvodnyh korichnoj kisloty, obladajushhih antiradikal'noj aktivnost'ju [A purposeful search for a leader compound in a series of cinnamic acid derivatives with antiradical activity]. Khimiko-Farmatsevticheskii Zhurnal. 2010;44(7):21–26. Russian. DOI: 10.30906/0023-1134-2010-44-7-21-26.
13. Oganesyan ET, Dorkina EG, Khochava MR, Tuskaev VA, Maltsev YuA. Ispol'zovanie kvantovo-himicheskih metodov dlja obosnovanija antiradikal'nogo (ON˙) dejstvija poligidroksihalkonov [The use of quantum chemical methods to substantiate the antiradical (OH˙) action of polyhydroxychalcones]. Khimiko-Farmatsevticheskii Zhurnal. 2002;36(12):21–25. Russian.
14. Oganesyan ET, Maltsev YuA, Tvorovsky DE. Issledovanie mehanizma reakcii proizvodnyh flavona s gidroksil'nym radikalom polujempiricheskimi metodami [Investigation of the reaction mechanism of flavone derivatives with a hydroxyl radical by semi-empirical methods]. 2001;71(6):99–1005. Russian.
15. Voronkov AV, Abaev VT, Oganesyan ET, Pozdnyakov DI. Nekotorye aspekty cerebroprotektornoj aktivnosti 4-gidroksi-3,5-di-tretbutil korichnoj kisloty pri ishemicheskom povrezhdenii golovnogo mozga v jeksperimente [Some aspects of the cerebroprotective activity of 4-hydroxy-3,5-di-tert-butyl cinnamic acid in ischemic brain damage in the experiment]. Medicinskij vestnik Severnogo Kavkaza. 2018;13(1.1.):90–93. Russian.
16. Voronkov AV, Pozdnyakov DI, Khuri EI, Kulbekova YuA, Kobin AA. Ocenka antioksidantnoj aktivnosti 4-gidroksi-3,5-ditretbutil korichnoj kisloty, meksidola i tioktovoj kisloty na modeli fokal'noj ishemii golovnogo mozga [Evaluation of the antioxidant activity of 4-hydroxy-3,5-ditretbutyl cinnamic acid, mexidol and thioctic acid in a model of focal cerebral ischemia]. Voprosy biologicheskoj, medicinskoj i farmacevticheskoj himii. 2017;20(2):48–52. Russian.
17. Voronkov AV, Oganesyan ET, Pozdnyakov DI, Abaev VT. Izuchenie dozozavisimogo jendoteliotropnogo vlijanija soedinenija ATACL v uslovijah ishemicheskogo povrezhdenija golovnogo mozga u krys v jeksperimente [The study of the dose-dependent endotheliotropic effect of the ATACL compound under conditions of ischemic brain damage in rats in the experiment]. Vestnik VolgGMU. 2017;1(61):54–58. Russian.
18. Voronkov AV, Oganesyan ET, Gerashchenko AD. Aspekty aktoprotektornoj aktivnosti nekotoryh prirodnyh soedinenij razlichnoj himicheskoj struktury [Aspects of actoprotective activity of some natural compounds of different chemical structures]. Sportivnaja medicina: nauka i praktika. 2017;7(1):92–96. Russian. DOI:10.17238/ISSN2223-2524.2017.1.92.
19. Illyel E. Fundamentals of stereochemistry. – M: Binom; Laboratory of Knowledge. 2014:120 p.
20. Tsukerman SV, Surov YuN, Lavrushin VG. Dipol'nye momenty s IK-spektrami 4 i 4 '-monozameshhennyh halkonov [Dipole moments with IR spectra of 4 and 4'-monosubstituted chalcones]. Zhokh. 1968;38(38):524–529. Russian.
21. Tarakhovsky YuS, Kim YuA, Abdrasilov BS, Muzafarov EN. Flavonoids: biochemistry, biophysics, medicine. Synchrobook, Pushchino. 2013:310 p. Russian.

AUTHORS

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

Stanislav S. Shatokhin – post-graduate student of the Department of Organic Chemistry, Pyatigorsk Medical and Pharmaceutical – a branch of Volgograd State Medical University. ORCID 0000-0001-7891-8338. E-mail: Shatohin.stanislav95@yandex.ru

RESEARCH ARTICLE

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY

УДК 615.4

FILMS IN RUSSIAN MEDICINE AND COSMETOLOGY: DEVELOPMENT HISTORY, CLASSIFICATION, TECHNOLOGY

V.M. Kishchenko, V.V. Vernikovsky, I.M. Privalov, A.M. Shevchenko

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

E-mail: viktoriya.kishchenko@yandex.ru

Received 31 January 2020 Review (1) 5 April 2020 Review (2) 15 April 2020 Accepted 10 May 2020

Since the moment of their appearance in the second half of the 20th century, application forms have attracted the attention of the specialists involved in the skin application of pharmacologically active agents. Herewith, both localized exposure to the external integuments and the possibility of achieving a systemic effect, are of interest. The range of products used in modern films, is also wide – from pharmaceutical substances to biologically active components of cosmetics.

The aim of the present work is to study the current state of research in the field of the creation and improvement of medicinal and cosmeceutical films.

Materials and methods. The study was conducted on the base of patent information (fips.ru, findpatent.ru) and information and search databases – the State register of medicines (grls.rosminzdrav.ru) and the data from the Federal accreditation service (www.fsa.gov.ru), as well as scientific libraries (Google Scholar, eLIBRARY, PubMed) and reference literature.

Results. Native and foreign medicinal films have longer than a 50-year history of their existence in the pharmaceutical market. Modern scientists' interest in this application form, does not fade away due to a great number of its positive characteristics. In addition to pharmaceutical applications, films are widely used in cosmetics in the form of masks applied to the skin. Biologically active substances are widely used in cosmetics which, in recent years, has led to the emergence of a group of cosmeceutical products that combine medical and cosmetic films. The article also discusses film manufacturing technology, active substances, as well as polymers used for medicinal and cosmetic films presented in the Russian market.

Conclusion. The analysis of the literature data makes it possible to conclude that the development of films is promising in both medicine and cosmeceuticals.

Keywords: medicinal films, biologically active components, matrix, polymers, cosmetic films, cosmeceutics, classification of films, history of film development, film manufacturing technology

ПЛЕНКИ В РОССИЙСКОЙ МЕДИЦИНЕ И КОСМЕТОЛОГИИ: ИСТОРИЯ РАЗВИТИЯ, КЛАССИФИКАЦИЯ, ТЕХНОЛОГИЯ

В.М. Кищенко, В.В. Верниковский, И.М. Привалов, А.М. Шевченко

Пятигорский медико-фармацевтический институт – филиал федерального государственного бюджетного образовательного учреждения высшего образования «Волгоградский государственный медицинский университет» Министерства здравоохранения Российской Федерации
357532, Россия, Ставропольский край, г. Пятигорск, пр. Калинина, 11

E-mail: viktoriya.kishchenko@yandex.ru

Получено 31.01.2020 Рецензия (1) 5.04.2020 Рецензия (2) 15.04.2020 Принята к печати 10.05.2020

Аппликационные формы с момента своего появления во второй половине XX века привлекают внимание специалистов, занимающихся вопросами накожного применения фармакологически активных средств. При этом интерес вызывает как оказание локализованного воздействия на наружные покровы, так и возможность достижения систем-

For citation: Kishchenko V.M., Vernikovsky V.V., Privalov I.M., Shevchenko A.M. Films in russian medicine and cosmetology: development history, classification, technology. *Pharmacy & Pharmacology*. 2020;8(2):124-132. **DOI:** 10.19163/2307-9266-2020-8-2-124-132

Для цитирования: Кищенко В.М., Верниковский В.В., Привалов И.М., Шевченко А.М. Пленки в российской медицине и косметологии: история развития, классификация, технология. *Фармация и фармакология.* 2020;8(2): 98-124-132. **DOI:** 10.19163/2307-9266-2020-8-2-124-132

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2020-8-2-124-132

ного эффекта. Широк также и диапазон применяемых в составе современных пленок средств – от фармацевтических субстанций до биологически активных компонентов косметических средств.
Цель. Настоящая работа посвящена изучению современного состояния российских исследований в области создания и совершенствования лекарственных и косметических пленок.
Материалы и методы. Исследование проводилось с использованием патентно-информационных (fips.ru, findpatent.ru) и информационно-поисковых баз – Государственного реестра лекарственных средств (grls.rosminzdrav.ru) и данных Федеральной службы по аккредитации (www.fsa.gov.ru), а также научных библиотек (GoogleScholar, eLIBRARY, PubMed) и справочной литературы.
Результаты. В России и за рубежом лекарственные пленки насчитывают более чем 50-летнюю историю своего существования на фармацевтическом рынке. Интерес современных ученых к данной аппликационной форме не угасает благодаря большому количеству положительных характеристик. Помимо фармацевтической сферы применения пленки получили широкое распространение в косметике, где применяются в качестве масок, наносимых на кожу. В косметических средствах широко применяются биологически активные вещества, что в последние годы привело к появлению группы космецевтической продукции, объединяющей медицинские и косметические пленки. Также в статье рассмотрены методы получения пленок, действующие вещества и полимеры, применяемые для лекарственных и косметических пленок, представленных на российском рынке.
Заключение. Проведенный анализ данных литературы позволяет сделать вывод о перспективном развитии пленок в российской медицине и космецевтике.
Ключевые слова: пленки лекарственные, биологически активные компоненты, матрица, полимеры, пленки косметические, космецевтика, классификация пленок, история развития пленок, технология изготовления пленок

INTRODUCTION

Application (from the Latin. *applicatio* – application) as a way to use medicines and cosmetics and medical products, has been known since ancient times. Application preparations are considered dosage forms and other products applied to the skin, mucous membranes or wound surfaces and somehow fixed on them. This method of application, depending on the active ingredients used, excipients and design features of the application preparation, makes it possible to achieve both localized and systemic effects on the human body. The latter gives a possibility to consider application preparations not from the point of view of being traditional dosage forms, but as delivery systems [1]. One of the promising directions for the development of local-regional and transdermal drug delivery systems is self-fixing application dosage forms and, in particular, films.

THE AIM of the present work is to study the current state of research in the field of the creation and improvement of medicinal and cosmeceutical films.

MATERIALS AND METHODS

The study was conducted on the base of information (fips.ru, findpatent.ru) and information and search databases – the State register of medicines (grls.rosminzdrav.ru) and data from the Federal accreditation service (www.fsa.gov.ru), as well as scientific libraries (Google Scholar, eLIBRARY, PubMed) and reference literature. The search depth of literary sources was 21 years, and the patent search depth was 17 years. The following search terms were used during the search for materials: “medicinal films”, “films”, “cosmetic films”, “films”.

RESULTS AND DISCUSSION

The history of films as a production form of medicines and cosmetics, has more than 50 years. For the first time in our country, films as a dosage form appeared in the 60s of the XXth century, and their scope was limited to the ophthalmic practice [2]. In the foreign pharmaceutical market, a “film” dosage form was officially introduced in 1970 as a substitute for rapidly dissolving tablets [3]. In native pharmacy, the official definition of the “film” dosage form first appeared in the State Pharmacopoeia of the XIIIth edition in the general pharmacopeial article on ophthalmic dosage forms, where “ophthalmic films” were highlighted as “solid ophthalmic dosage forms for topical application” [4]. However, in already the XIVth edition of the State Pharmacopoeia, films as a separate general pharmacopeial article, were highlighted as an independent dosage form. According to the definition of the current pharmacopoeia, films are a solid dosage form, represented by thin plates of a suitable size, containing one or more active substances and some auxiliary substances, including film-forming ones. The scope of application of films as a dosage form of drugs was expanded; depending on the method of administration and the route of administration, ophthalmic films and films for oral use are distinguished [5].

As any dosage form, films have a number of positive and negative featurers (advantages and disadvantages) that determine the scope of their application (Table 1) [6].

Table 1 – Advantages and disadvantages of films as a dosage form

Advantages	Disadvantages
Technological: • film production does not require complex equipment; • the possibility of combining various groups of active substances; • ease of use because of the reduction of administration (with a prolonged release); • sufficient mobility for patients' self-use. Pharmacological: • prolongation possibility of active substances' action; • maintaining a constant concentration of active substances; • possible reduction in a therapeutically active dose; • if necessary, the dose of the active substance can be increased by applying an additional film; • reducing or eliminating side effects; • an active substance penetrates into the systemic bloodstream with a reduced effect of the first hepatic pass.	• It is difficult to include considerable amounts of active substances in the composition of films; • low in some cases, the rate of passive diffusion requires the use of special auxiliary substances – penetrators; • active substances of natural origin can form competent complexes with auxiliary substances reducing their pharmacological activity; • restraint of selection and a high cost of packaging; • during storage, films can change their properties if packaging has been chosen incorrectly (loss of moisture, dampness).

However, as Table 1 shows, in some cases, positive features of this dosage form can be also its disadvantages. For example, the size of the dosage form allows it to be quite mobile and convenient to use, but since a film is a compact form, due to its size, it cannot include a large number of active substances. Films can also contain combinations of active substances in their composition, which is undoubtedly their positive feature. However, in this case, the selection of the composition is complicated by the fact that not only the active substances must be indifferent to each other, but also the base polymer in the manufacturing process, is more likely to bind the active substances and thus prevent their release.

The analysis of the literature data has shown that the most developed classification issues are for films as a medicinal form, while in the case of cosmetic films, the classification has not been given a sufficient attention.

A growing interest of researchers and a vast scope of applications of "film" medicinal form, dictated the need to create classifications by various features.

For example, a detailed classification of films was presented by Professor E.A. Korzhavykh. According to the author, medicinal films can be classified according to four main characteristics:

1. by the route of administration: buccal, vaginal, ocular, dental, dermatological, intraocular;
2. by composition: collagen, fibrin, phyto-films;
3. by properties of the polymer: insoluble and rapidly dissolving;
4. by other features: impregnated, spray and modified release films.
5. One of the highlighted groups is a group of modified release films.

A prolonged release of active components from the films is achieved through the use of certain polymers and their combinations. Thus, the results of a biopharmaceutical study of remineralizing films with trimecaine hydrochloride and chlorhexidinebigluconate on four bases (sodium-carboxymethylcellulose, sodium alginate, Blanose 7MF, Blanose 7M8SF) and remineralizing films with calcium chloride, sodium phosphate bi-substituted, sodium fluoride based on methylcellulose, have been published. The results of this study showed that the rate of release of active substances from different bases is not the same: the base with sodium alginate had a prolonged release of the active substances, and Blanose 7M8SF provided an accelerated release, since this polymer base had a high swelling ability and, therefore, rapid dissolution [8]. A rapid release of active substances from the polymer base can be one of the advantages of films. An accelerated release, for example, is relevant for oral dispersed drugs containing nitroglycerin or loratidine [3, 9].

A mathematical analysis of the process of releasing the active component from a hydrophilic matrix (in particular, based on chitosan) placed in water, was carried out by A.O. Syromyasov and co-authors. As a result, the following mathematical model of the diffusion of substances from the same hydrophilic film, which takes into account the influence of various factors on this process, was proposed: the dependence of the properties of the matrix on the concentration of the active component in it, due to the phenomenon of partial binding of the active component inside the matrix, and the dependence of the properties of the matrix on the time associated with its swelling and possible dissolution in an aqueous medium [10].

Since their inception, the films have undergone significant modifications, for example, in terms of releasing an active component. Thus, the issues related to the development of new compositions and improvement of technologies, are some of the "pressing issues" of modern pharmacy [11, 12].

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2020-8-2-124-132

Currently, native scientists have developed a significant number of compositions of polymer medicinal films with various effects: antimicrobial, antiviral, immunomodulating, affecting the cardiovascular system and also used for local anesthesia [9, 13, 14]. Modern films may contain herbal remedies, enzymes and other medicines as active ingredients for the treatment of ophthalmic, dental, dermatological, otorhinolaryngological, gynecological, oncological diseases, burns, wounds, alcoholism, drug addiction, depression, angina pectoris, etc. [2, 15–18].

The greatest interest of researchers is attracted to the films for use in the oral cavity, containing active ingredients of natural, synthetic origin or a combination thereof. So, the composition was developed and the technology for producing dental films with bischofite having anti-edematous action was standardized [19].

A study on the creation of two-layer dental films with analgesic, anti-inflammatory and antimicrobial effects was conducted. As active ingredients, in the composition of these films, the authors used novocain, norsulfazolum-natrium and kalanchoe juice; a number of polymers were used to create model matrix formulations of the dosage form: gelatin, methyl cellulose, polyvinyl alcohol and sodium carboxymethyl cellulose. To select the optimal basis, the film formers were compared among themselves on the basis of their organoleptic properties. The study showed that the optimal basis for Novocain is a 3% solution of methyl cellulose, and for norsulfazole, a 6% solution of polyvinyl alcohol is most suitable [20].

The results on physico-chemical research and the production of dental films based on collagen and gelatin, have been published; they contain 30% chlorhexidine in their composition. It has been established that the gelation process is influenced by concentration, the initial temperature of gelation, the rate of the cooling process and the content of auxiliary additives [21]. The gelation rate increased if the process started at a lower temperature. In the free cooling mode (from 38 °C in the air to the ambient temperature of 24 °C), the structure of the gelatin solution changed with the endpoint of gelation. The gelatin mass precipitation time during solidification was 60 minutes. The structure formation of collagen from a thin layer of a solution in the initial period was determined by the nature of the evaporation of pure solvents. This process is quite lengthy – the authors indicate that in 100 minutes only 23% of the liquid phase was removed from the total mass [21].

For the prevention and treatment of periodontal disease, a medical-preventive film with a matrix based on polyvinyl alcohol and vitamin D_3 as the active component, has been developed. For this composition, the studies of the physical integrity of the developed composition have been conducted. They showed that the physical integrity of the film increases by 10% with the joint introduction of a film former (polyvinyl alcohol), plasticizer (glycerine) and vitamin D_3 as the active substance. The effect of vitamin D_3 on the functional activity of the cells isolated from the periodontal pocket, was also studied. The studies have shown that vitamin D3 released from the film, significantly limited the production of inflammatory mediators [22].

To correct gingivitis, periodontosis and periodontitis, compositions of medicinal films with chaga melanin (*Inonotus obliquus* (Ach. ex Pers.) Pil.) and chlorhexidine based on the composition of polymers – polyvinyl alcohol and zoster polysaccharide, were developed. The developed dental films were subjected to tests on the indicator of “the moisture content” and the time of dissolution. The results of the experiments showed that the optimal moisture content values for dental films, were in the range of 6–12%. It was also established that the introduction of chlorhexidine into the composition reduced its solubility by 8% [23]. A polyvinyl alcohol-based matrix was also used to develop the composition of dental medicinal films containing magnesium chloride and zinc-substituted calcium hydroxyapatite as active substances [24].

For the deposition of drugs on the surface of the nasal mucosa and maxillary sinus, an adhesive polymer soluble film containing a composition of lidocaine hydrochloride and polysorb MP, was developed. A blend of polymers oxypropylmethylcellulose and pectin, was chosen as a matrix. Clinical studies of the specific activity of the proposed composition conducted on a group of patients, are of interest. As a result, it was determined that the developed film contributes to a faster epithelization of the nasal mucosa and maxillary sinus [25]. The results on the development of an optimal film composition based on phytocomposition (a mixture of dry extracts of calendula and yarrow) with the addition of propolis tincture for the treatment of traumatic lesions of the oral mucosa, have been published; in this composition gelatin was used as a film-forming agent with the addition of glycerol as a plasticizer [26].

Medicinal films are also used in pediatric dental practice. So, the compositions and technologies of films with anesthetic and anti-inflammatory effects have been developed. In these compositions, the active ingredients were the substance of trimecaine and the aqueous extraction from chamomille flowers; the matrix was a composition of sodium carboxymethylcellulose, gelatin, and polyethylene oxide-600 [27, 28].

Besides the dental field, films are also widely used in ophthalmology. For example, the study results of the choice of the ophthalmic films composition with a liquid extract of aloe for the correction of inflammatory diseases of the conjunctiva oculi have been published. Polyvinylpyrrolidone, carboxymethylcellulose, polyethylene oxide-400 were used as polymers to create the film base. The optimal composition selection makes it possible to evaluate the following parameters: a moisture content, linear dimensions, pH, as well as the attractiveness of the film appearance. As a result of the studies, it was found

out that the best characteristics had been obtained for the film based on carboxymethylcellulose [29].

Medicinal films are used not only for the treatment of humans, but also for the correction of diseases in animals. For treating animals' eyes, polymeric drug films with moxifloxacin and a base of a polyvinyl alcohol and arabinogalactan composition, have been proposed. A study of the kinetics of the drug release from the model bases carried out by a spectrophotometric method showed that the studied composite base has a more pronounced prolonging effect compared to a film based on pure polyvinyl alcohol [30, 31].

Despite the active research conducted by native scientists on the development of medicinal films, this form of production is presented very modestly in the pharmaceutical market. The State register of medicines contains only five registered medicines produced in this form. These are two versions of films with nitroglycerine "Trinitrolong" glued to the gum, intended for the prevention and relief of angina attacks. It is based on a matrix of a polymer that is bio-soluble for medicinal films, which is a copolymer of acrylamide, N-polyvinylpyrrolidone and ethylacrylate. Two other drugs are oral dispersed films with sildenafil "Invida ODP" and "Dynamico Forward", intended for the correction of an erectile dysfunction. As a film-forming agent, in these medicines a food polysaccharidepullulan is used. In these cases, the film form is a kind of analog of tablets, having the advantage over the latter in the form of a simple technology that makes possible a more flexible regulation of the release kinetics. As for the fifth drug registered in the form of films, these are eye films "Taurine", used for the correction of dystrophy and injuries of the cornea, also created on the basis of a matrix of biopolymer soluble for medicinal films.

The simplicity and high manufacturability of films, provides the possibility to use them not only as medicines, but also as dressings. At different times, scientists conducted research in the market of dressings and medicinal films, thus showing the relevance of their use and the continuing interest in improving this form of medicines production [2, 18, 32, 33].

Currently, in Russia films are used not only in the native medical practice; in the native cosmetic market they have also taken a fairly stable position. So, according to the Russian Accreditation dated August 21, 2019, in the perfumery and cosmetic market, cosmetics included more than 162,000 product names (100%). The subgroup of "skin care products" consisted of more than 22,700 items, which represented approximately 14% of all cosmetics. In turn, among the skin care products, "face masks" stand out (more than 3500 items, or 2.2% of the total number of cosmetics); among them "mask-films" were allocated in the amount of about 0.01% of the total the number of cosmetics in the Russian market. Despite such a small share in relative indices, in absolute terms, there are more than 150 types of film masks in the Russian cosmetic market, which is many times greater than the number of drugs sold in this form in Russia.

Being not medicinal products, cosmetic masks-films can contain the same biologically active substances as medicinal forms, but in a much lower concentration, thereby having a favorable effect on the skin. Such masks help to eliminate dryness and peeling of the skin, regulate the work of the sebaceous glands, etc. At the same time, they do not have a toxic effect on the consumer's body due to the content of active substances in concentrations much lower than in drugs (in most cases, the concentration is about 0.5% or less) [34–36]. The use of biologically active components in cosmetics has led to the emergence of the term "cosmeceutics", which refers to cosmetic products containing components that have a pronounced biological activity. More than 20 years ago, this term combining the concepts of "pharmacy" and "cosmetics", was introduced by an American dermatologist Albert Kligman. Cosmeceuticals differ from cosmetics mainly in the following: they do not mask skin imperfections, but eliminate the cause of their appearance. Cosmeceutical agents can also affect the hypoderma, while cosmetic ones are usually able to penetrate no further than the derma [37, 38].

The analysis of the range of biologically active substances included in the composition of cosmeceuticals, has shown that they are mainly of natural origin, while synthetic compounds are practically not used. The components of animal origin included in cosmetic products are, for example, a number of bee products, such as bee pollen, which has an antioxidant, anti-inflammatory, anti-carcinogenic, anti-bacterial, anti-fungal effect [39]; drone brood, which slows down the aging process of the skin; Royal jelly, used as a means with a high regenerative index [40]. A fairly common active component is snail mucin, which can be used for the treatment of various types of burns, dermatitis, eczema, diaper rash and wounds [41–43]. A study of a product research of cosmetic masks with collagen acting not only as a film-forming agent but also as an active ingredient, has been published [46]. However, in addition to these rather specific components, cosmeceutical films contain components found in almost every cosmetic product – guanine, keratin, etc. [44, 45].

In addition to biologically active substances of animal origin, vegetable components are widely used in cosmetics: rose water, extracts of chamomile, cornflower, calendula, etc., as well as vegetable oils (including essential oils). Products derived from aloe vera and tree aloe, are among the most commonly found in the composition of dermatological masks-films. Aloe juice and extracts are used in the cosmetic industry to stimulate skin regeneration and prevent dermatitis of various origins.

In cosmetics, a study of the application frequency of polymers containing natural mineral salts was conducted; it showed that polyvinylpyrrolidone, xanthan gum,

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2020-8-2-124-132

cellulose derivatives and carbomers are the most applicable for these compositions [47].

Polymers of natural, semi-synthetic and synthetic origins, are used as a matrix for creating films; it gives a possibility to divide films into the following groups [48]:

- of animal origin (collagen, gelatin, elastin, chitosan);
- of vegetable (alginates, cellulose);
- of microbial origin (agar-agar, dextrin, pullulan);
- semi-synthetic (methyl cellulose, sodium carboxymethylcellulose, (hydroxypropyl)ethyl cellulose, modified starches);
- synthetic (polyvinylpyrrolidone, polyvinyl alcohol, polyethylene oxides, polyacrylamides).

Most often, in films as drugs manufacturing, cellulose derivatives (methyl cellulose, etc.), gelatin and agar are used [29, 49, 50]. In cosmeceutical masks-films, sodium alginate and polyvinyl alcohol are most often found as base polymers [51].

In addition to the auxiliary substances making up the base, films include plasticizers (glycerin, propylene glycol, polyethylene glycol, castor oil, tweens), preserving agents (ethyl alcohol, nipagin, benzalconium chloride), penetrators (dimethyl sulfoxide, dimethylformamide), odor and taste flavoring agents, pH regulators, solubilizers (tween 80, polyethylene glycol 1500, glycyram) and others. They provide optimal technological, chemical, physico-chemical and pharmacological parameters.

In addition to form-forming and auxiliary ingredients, medicinal and cosmetic films combine manufacturing processes of film matrices. Currently, the following methods of forming films are used:

- spraying;
- pouring;
- extrusion.

A pulverization method consists in the distribution of the polymer base over the substrate with constant drying in an intensive flow of warm air using a spray gun. The films obtained in this way, dry up faster, but the film mass can be also distributed unevenly, and at the drying stage, the finished films may not correspond to the organoleptic characteristics. In films manufacturing by the method of pouring, the polymer solution is distributed on the substrate, and then dried up either in chamber driers or at the room temperature. The disadvantage of this method is uneven drying of the film: during the drying process, the layer located on the surface dries quickly and prevents the removal of moisture from the underlying layers, which can result in an uneven film. This disadvantage can be avoided by using the equipment set up at the level of the form, as well as dryers to speed up the drying process. During extrusion molding, the film mass is pressed under pressure through the forming nozzle, obtaining a film of the required thickness [5], but the disadvantage of this method is the formation of inclusions of air bubbles in the film mass. This disadvantage can be corrected by including the vacuum stage in the production process.

CONCLUSION

A review of the studies made it possible to draw a conclusion about the rapid improvement of the films and their sufficient representation in the classification of medicines and cosmetics. The initial data from literary sources allow us to conclude that films are not only relevant and highly-demanded, they are also a popular dosage form. However, in the pharmaceutical market they are extremely limited. Based on a number of proven advantages of films, it can be assumed that this form is optimal for use in cosmetology.

FINANCIAL SUPPORT AND SPONSORSHIP

This study did not have any financial support from outside organizations.

AUTHOR`S CONTRIBUTION

All authors equally contributed to the research work.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Krivosheev SA, Devjatkina IA, Demina NB. Applikacionnye lekarstvennyeformy: Plastyri [Application dosage forms: Plasters] Pod obshh. red. V.A. Bykova. Moscow: MAKS Press. 2005:104. Russian.
2. Erofeeva LN. Lekarstvennye plenki. Istorija i sovremennost' [Medicinal films. History and modernity] Universitetskaja nauka: vzgljad v budushhee: materialy Mezhdunar. nauch. konf., posvjashh. 83-letiju Kurskogo gosudarstvennogo medicinskogo universiteta. Pod red. rektora KGMU, Zasluzhennogo vracha RF, professora, d.m.n. V.A. Lazarenko. Kursk: FGBOU VO KGMU MinzdravaRossii. 2018;2(2): 52–27. Russian.
3. Narayana PR, Kumar MS, Reddy M, Ravishankar K. Formulation and Evaluation of Fast Dissolving Films of Loratidine by Solvent Casting method. The Pharm Innovayion J. 2013;2(2):31–35.
4. Gosudarstvennaja Farmakopeja RF – 13 izd. [State Pharmacopoeia of the Russian Federation. – 13 ed.] [Internet]. Moscow, 2015. [cited 2019 November19] Available from: http://femb.ru/femb/pharmacopea13.php
5. Gosudarstvennaja Farmakopeja RF – 14 izd. [State Pharmacopoeia of the Russian Federation. – 14 ed.] [Internet]. Moscow, 2018. [cited 2019 December 12] Available from: http://www.femb.ru/femb/pharmacopea.php
6. Kishhenko VM. Razrabotka sostava i standartizacija dermatologicheskih plenok s aloje i aktoveginom [Development of composition and standardization of dermatologi-

cal films with aloe and Actovegin]. Sb. mater. Mezhdunar. nauch. konf. «Molodyeuchenye – medicine» (20–21 May). 2016;(1):136–139. Russian.
7. Kafedra hirurgiimediko-profilakticheskogo fakul'teta Pervogo Moskovskogo Medicinskogo Universiteta im. I.M. Sechenova [Department of surgery of the medical-preventive faculty of the First Moscow Medical University. I.M. Sechenova] [Internet]. Moscow, 2014. [cited 2019 November 29] Available from: http://www.surgerympf.com.
8. Rjumina TE, Golovanenko AL. Biofarmacevticheskie issledovanija plenok lekarstvennyh anestezirujushhego i remineralizirujushhego dejstvija [Biopharmaceutical studies of films of medicinal anesthetic and remineralizing action]. Sovremennyeproblemynaukiiobrazovanija. – [Internet]. Moscow. 2012. (1). [cited 2019 November 14] Available from: http://www.science-education.ru/ru/article/view?id=5430.
9. Mizina PG. Fitoplenki v farmacii i medicine [Phytofilms in pharmacy and medicine]. Farmacija. 2000;5-6:38–40. Russian.
10. Syromjasov AO, Shurshina AS, Galkin DV. Model' diffuzii lekarstvennogo veshhestva s uchetom ego svjazyvanija v organicheskoj plenke [Diffusion model of a medicinal substance taking into account its binding in an organic film]. V sbornike: Matematicheskoe modelirovanie, chislennye metody i kompleksy programmimen i E.V. Voskresenskogo VIII Mezhdunarodnaja nauchnaja molodezhnaja shkola-seminar. 2018:150–155. Russian.
11. Stepanova EF, Losenkova SO, Morozov JA. Sozdanie i farmakotehnologic heskie issledovanija innovacionnyh lekarstvennyh form meksidola [Creation and pharmacotechnological research of innovative medicinal forms of Mexidol]. Razrabotka I registracija lekarstvennyh sredstv. 2018;4(25):37–43. Russian.
12. Sysuev BB, Pletneva IV. Sovremennoe sostojanie issledovanij razrabotok v oblasti innovacionnyh lekarstvennyh form i ihmodifikacij [Current state of research and development in the field of innovative dosage forms and their modifications]. Vestnik Volgogradskogo gosudarstvennogo medicinskogo universiteta. 2014;4(52):7–12. Russian.
13. Oleshko LN. Vybor sostava stomatologicheskih plenok anestezirujushhego dejstvija [The choice of the composition of the dental films of anesthetic action]. Farmacija. 1999;6:30–32. Russian.
14. Pankrusheva TA. Polimernye lekarstvennye plenki dlja lechenija zabolevanijslizistyh obolochek [Polymeric drug films for the treatment of diseases of the mucous membranes]. Uchenye zapiski Orlovskogogo sud. un-ta. Serija: «Estestvennye nauki». 2014;7(63):211–212. Russian.
15. Saushkina AS, Savchenko LN, Chakchir BA, Marinina TF. Perspektivy ispol'zovanija stomatologicheskih lekarstvennyh plenok s askorbinovoj kislotojiI rutinom dlja lechenija i profilaktiki zabolevanij parodonta [Prospects for the use of dental medicinal films with ascorbic acid and rutin for the treatment and prevention of periodontal disease]. Vestnik Rossijskoj voenno-medicinskoj akademii. 2013;3(43):118–125. Russian.
16. Piskunov SZ, Erofeeva LN. Razrabotka i issledovanie plenok dlja lechenija rinitov [Development and research of films for the treatment of rhinitis]. Rossijskaja rinologija. 2015;3:54–56. Russian.
17. Kamaeva SS, Pocelueva LA., Safiullin RS., Egorova EV. Razrabotka sostava lekarstvennyh plenok s hlorgeksidina bigljukonatom [Development of the composition of medicinal films with chlorhexidine bigluconate]. Farmacija. 2007;2 20–22. Russian.
18. Vinnik JuS. Sovremennye ranevye pokrytija v lechenii gnojnyh ran [Modern wound dressings in the treatment of purulent wounds]. Novosti hirurgii. 2015;5(23):552–558. Russian.
19. Vdovina, GP, Ganicheva LM, Merkulova EV. Vlijanie novoj lekarstvennoj formy bishofita na skorost' krovosnabzhenija v mjagkih tkanjah parodonta u krys v uslovijah jeksperimental'nogo vospalenija [The effect of the new dosage form of bischofite on the rate of blood supply in the soft periodontal tissues in rats under experimental inflammation]. Permski jmedicinskij zhurnal. 2006;2:58–60. Russian.
20. Marinina TF, Gjul'bjakova H.N. Razrabotka tehnologii i analiz dvuhslojnyh stomatologicheskih plenok protivovospalitel'nogo i anestezirujushhego dejstvija [Development of technology and analysis of two-layer dental films of anti-inflammatory and anesthetic effects]. Sovremennye problem nauki i obrazovanija. – [Internet]. Moscow. 2014;(4). [cited 2020 January 04] Available from: https://science-education.ru/ru/article/view?id=13902.
21. Vasil'ev MP, Alekseeva G.A. Poluchenie i issledovanie kollagenovyh plenochnyh materialov dlja stomatologii [Obtaining and research of collagen film materials for dentistry]. Vestnik molodyh uchenyh Sankt-Peterburgskogo gosudarstvennogo universiteta tehnologii i dizajna. 2017;3:56–60. Russian.
22. Ostrovskaja LJu. Sostav dlja poluchenija stomatologicheskoj lechebno-profilakticheskoj plenki [Composition for obtaining a dental therapeutic film]. V sbornike: Molodezh' i XXI vek – 2015 materialy V Mezhdunarodnoj molodezhnoj nauchnoj konferencii: v 3-h tomah. 2015:79–81. Russian.
23. Latipova AD, Sysoeva EV, Sysoeva MA. Razrabotka sostava lekarstvennyh plenok dlja stomatologii [Development of the composition of medicinal films for dentistry]. Vestnik tehnologicheskogo universiteta. 2016;19(22):168–171. Russian.
24. Bulkina NV, Vulah NA, Kropotina AJu, Kadykov AL, Popova OV, Pichhidze SJa. Costav i sposob dlja poluchenija bioaktivnoj stomatologicheskoj lechebno-profilakticheskoj plenki [Composition and method for producing bioactive dental therapeutic film]. Russian Federation patent (RF) 2651041. No. 2016143505. 03.11.2016. No. 11. Russian.
25. Sirak SV, Koshel' IV, Koshel' VI. Adgezivnaja polimernaja rastvorimaja plenka dlja deponirovanija lekarstvennyh veshhestv na poverhnosti slizistoj obolochki nosa i verhnecheljustnogo sinusa [Adhesive polymer soluble film for the deposition of drugs on the surface of the nasal mucosa and maxillary sinus]. Russian Federation patent (RF) 2634259. No. 2016115425. 20.04.2016. No. 30. Russian.
26. Aver'janov SV. Primenenie stomatologicheskoj plenki dlja lechenija porazhenij slizistoj obolochki polosti rta [The use of dental film for the treatment of lesions of the oral mucosa]. Dental Forum. 2018;4:11. Russian.
27. Bespalova AV. Razrabotka tehnologicheskoj shemy poluchenija detskih stomatologicheskih plenok anestezirujushhego i protivovospalitel'nogo dejstvija [Development of a technological scheme for producing children's dental films of anesthetic and anti-inflammatory action]. V

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2020-8-2-124-132

sbornike: Applied and Fundamental Studies proceedings of the 11th International Academic Conference. 2017:118–125. Russian.

28. Sampiev AM, Bespalova AV, Nikiforova AV. Razrabotka sostava i tehnologii detskih stomatologicheskih plenok anestezirujushhego i protivovospalitel'nogo dejstvija [Development of the composition and technology of children's dental films of anesthetic and anti-inflammatory action]. Zaporozhskij medicinskij zhurnal. 2017;19;5(104):668–674. Russian. DOI: 10.14739/2310–1210.2017.5.110230.
29. Shikova YV, Lichoded VA., Brazhenko AV, Ishmakova ZR, Girfanov IF. Devolopment of composition and technology of eye medicinal films with aloe extract. Pharmacy & Pharmacology. 2016;4(4):48-54. Russian. DOI: 10.19163/2307-9266-2016-4-4-48–54.
30. Badykova LA. Primenenie polimernyh kompozicij v kachestve glaznyh lekarstvennyh plenok v veterinarii [The use of polymer compositions as ophthalmic medicinal films in veterinary medicine]. Dostizhenija himii v agropromyshlennom komplekse: materialy Vserossijskoj molodezhnoj konferencii-shkoly s mezhdunarodnym uchastiem. Ufa: Bashkirskij GAU. 2015:63–64. Russian.
31. Badykova LA, Mudarisova RH. Primenenie glaznyh lekarstvennyh plenok v veterinarii [The use of ophthalmic medicinal films in veterinary medicine]. Dostizhenija himii v agropromyshlennom komplekse: materialy Vserossijsko jmolodezhnoj konferencii-shkoly s mezhdunarodnym uchastiem. Ufa: Bashkirskij GAU. 2018:144–147. Russian.
32. Sampiev AM, Nikiforova EB, Sopovskaja AV. Sovremennoe sostojanie issledovanij v oblasti sozdanija stomatologicheskih plenok [Current state of research in the field of dental films]. Mezhdunarodnyj zhurnal prikladnyh i fundamental'nyh issledovanij. 2016;3–2:293–297. Russian.
33. Mayorova AV., Syisuev BB., Hanalieva IA., Vihrova IV. Modern assortment, properties and perspectives of medical dressings improvement of wound treatment. Pharmacy & Pharmacology. 2018;6(1):4–32. Russian. DOI: 10.19163/2307-9266-2018-6-1-4-32
34. Baldynova FP, Byzgaeva AV. Issledovanie i razrabotka kosmeticheskoj maski na osnove fermentirovannyh cvetkov romashki aptechnoj [Research and development of a cosmetic mask based on fermented chamomile flowers]. Himija i himicheskaja tehnologija pererabotki rastitel'nogo syr'ja :materialy dokladov Mezhdunarod. nauch.-tehnich. konf. Minsk: BGTU. 2018:222–225. Russian.
35. Bejenor, HD, Chupyatova NA, Mayorova PV, Gutnikova MS. The development of the composition and technology of cosmetic cleansing mask-film based on badyagi [Development of the composition and technology of cosmetic cleansing mask film based on badiaga]. Youth, science, medicine: materials of the 63rd All-Russian Interuniversity. studen. scientific conf. from the international participation / Tver. state honey. Un-t; ed. col.: M.N. Kalinkin [et al.]. – Tver: Ed. center of Tver. state honey. University. 2017:636–637. Russian.
36. Dribnohod JuJu. Kosmetologija [Cosmetology]. Izd. 13-e. Rostov. Feniks. 2017:798 s. Russian.
37. Shadrina, VO. Specifika realizacii kosmecevtiki v RF [The specifics of the implementation of cosmeceuticals in the Russian Federation]. Biznes-obrazovanie v jekonomike znanij [Internet]. Moscow. 2016;3(5):70–73. [cited 2019 December 23]. Available from: https://cyberleninka.ru/article/n/spetsifika-realizatsii-kosmetsevtiki-v-rf. Russian.
38. Lintner K, Mas-Chamberlin C, Mondon P, Peschard O, Lamy L. Cosmeceuticals and active ingredients. Clin. Dermatol. 2009;27(5):461–468. DOI: 10.1016/j.clindermatol.2009.05.009
39. Xi X, Li J, Guo S, Li Y, Xu F, Zheng M, Cao H, Cui X, Guo H, Han C. The Potential of Using Bee Pollen in Cosmetics: a Review. Journal of Oleo Science. 2018; 67(9):1071-1082. DOI: 10.5650/jos.ess18048.
40. Lazarjan DS, Sotnikova EM, Linnikova VA, Lazarjan GD. Kosmeticheskaja lechebno – profilakticheskaja krem-maska s trutnevym rasplodom i matochnym molochkom, obladajushhaja protivovospalitel'nym i ranozazhivljajushhim dejstviem [Cosmetic treatment – prophylactic cream – mask with drone brood and royal jelly, which has anti-inflammatory and wound healing effects]. Russian Federation patent (RF) 2208434. No.2001114383/14. 24.05.2001. No. 20. Russian.
41. Sajapova AI. Svojstvaj ekstrakta ulitki v sostave kosmeticheskih sredstv [Properties of snail extract in cosmetics] Innovaciiinauka: problemyiperspektivysbornikstatej. [Internet] Moscow. Impul's. 2018:180-183. [cited 2020 January 04] Available from: http://impulse-science.ru/wp-content/uploads/2018/12/%D0%9A-29.pdf. Russian.
42. Newar, J. Studies on the Adhesive Property of Snail Adhesive Mucus. China journal of Chinese materiamedica. 2014:14–17. DOI: 10.1021/acs.langmuir.5b03498.
43. Ruiz MA, Clares B, Morales ME, Cazalla S. Preparation and stability of cosmetic formulations with an anti-aging peptid. Journal of Cosmetic Science. 2017:157–171.
44. Volkov KV. Aktivnaja protiv akne dobavka k parfjumerno-kosmeticheskim produktam [Active against acne additive to perfumes and cosmetics]. Russian Federation patent (RF) 2678307. No. 2018139909. 13.11.2018. No. 3. Russian.
45. Mokrejs P, Hutta, M, Pavlackova J, Egner P, Benicek L. The cosmetic and dermatological potential of keratin hydrolysate. J CosmetDermatol. 2017;16(4):21–27. DOI: 10.1111 / jocd.12319.
46. Sapozhnikova AI, Pehtasheva EL, Shhukina EV. Ocenka potrebitel'skih svojstv kosmeticheskih masok s kollagenom [Assessment of consumer properties of cosmetic masks with collagen]. Vestnik rossijskogo jekonomicheskogo universiteta im. G.V. Plehanova. 2010;1(31):118–124. Russian.
47. Sysuev BB, Evseeva SB. The opportunities and specifics of the polymers application as auxiliary substance in the cosmetics compositions based on natural mineral salts. Pharmacy & Pharmacology. 2017;5(2):98–116. Russian. DOI: 10.19163/2307-9266-2017-5-2-98-116.
48. Handbook of Pharmaceutical Excipients. Sixth edition. Edited by Raymond C Rowe, Paul J. Sheskey and Marian E. Quinn. P. 129–133.
49. Rubilar, JF, Zuniga, RN, Osorio F, Pedreschi F. Physical properties of emulsion based hydroxypropyl methylcellulose/whey protein isolate (HPMC/WPI) edible films. CarbohydrPolym. 2015;123:27–38. DOI: 10.1016 / j.carbpol.2015.01.010.
50. Aver'janov CV, Hajrzamanova KA, Ishakov IR, Isaeva AI. Primenenie stomatologicheskih plenok prizabolevanijah slizistoj polosti rta [The use of dental films for diseases of the oral mucosa]. Uspeh isovremennoj nauki. 2017;5(1):99–104. Russian.
51. Naumova NV. Kosmeticheskaja maska-plenka [Cosmetic mask film]. Russian Federation patent (RF) 2702907. No. 2018145902. 21.12.2018. No. 29. Russian.

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY

AUTHORS

Victoria M. Kishchenko – post-graduate student of the Department of Pharmaceutical Technology with a course in medical biotechnology at Pyatigorsk Medical and Pharmaceutical Institute – a branch of Volgograd State Medical University. ORCID 0000-0002-6947-7662. E-mail: viktoriya.kishchenko@yandex.ru

Vladislav V. Vernikovsky – Candidate of Sciences (Biology), associate Professor of the Department of Pharmaceutical Technology with a course in medical biotechnology of Pyatigorsk Medical and Pharmaceutical Institute– a branch of Volgograd State Medical University.. ORCID 0000-0002-0324-1999. E-mail: v.v.vernikovsky@mail.ru

Igor M. Privalov – Candidate of Sciences (Biology), associate Professor of the Department of Pharmaceutical Technology with a course in medical biotechnology of Pyatigorsk Medical and Pharmaceutical Institute, – a branch of Volgograd State Medical University. ORCID 0000-0001-9797-4060. E-mail: igor.privacy@gmail.com

Aleksandr M. Shevchenko – Doctor of Sciences (Pharmacy), associate Professor, the head of the Department of Pharmaceutical Technology with a course in medical biotechnology of Pyatigorsk Medical and Pharmaceutical Institute, a branch of Volgograd State Medical University. ORCID 0000-0002-7541-2558. E-mail: nplfarmak-50@ya.ru

Научно-практический журнал
ФАРМАЦИЯ И ФАРМАКОЛОГИЯ

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2020-8-2-133-146

CHEMICAL CONSTITUENTS OF GEUM RIVALE L. AND THEIR BIOLOGICAL ACTIVITY

A.A. Orlova, M.N. Povydysh

Saint Petersburg State Chemical Pharmaceutical University
14 lit. A, Professor Popov St., St. Petersburg, Russia,197022

E-mail: anastasiya.lebedkova@spcpu.ru

Received 25 February 2020 Review (1) 30 April 2020 Review (2) 10 May 2020 Accepted 15 May 2020

The aim of the study is to review the literature data on the chemical constituents of arial and underground parts of *Geum rivale* L. (*Rosaceae*) and the pharmacological activity of its extracts and individual compounds.
Materials and methods. The study was carried out using Internet resources (Google Scholar, PubMed) and library databases (e-Library, Scopus, Web of Science). The main research methods were a review and analysis of the literature data on the topic for the period from 1958 up to the present.
Results. For the period from 1958 up to the present more than 80 components in the arial and underground parts of *G. rivale* have been identified. Among them there were components of the essential oil, phenolic acids and coumarins, aglycones of flavonoids, including luteolin, apigenin, quercetin and kaempferol, as well as a number of their glycosides and glucuronides, ellagitannins (hemin A, B, C, D, pedunculagin, stachiurin/casuarinin, tellimagrandin I). Some aspects of the pharmacological activity of total extracts and individual secondary metabolites of *G. rivale* have been studied, anti-inflammatory, antioxidant, antimicrobial, antiviral activities have been experimentally confirmed.
Conclusion. The analysis of the literature data showed that a further study of the composition of metabolites of *G. rivale* and their pharmacological activity is an urgent task, the solution of which will expand the range of use of this plant in medical practice and consider *G. rivale* as a promising source of pharmaceutical substances for the creation of new drugs and biologically active additives.
Keywords: river gravilat, *Geum rivale* L., phenolic compounds, essential oils, tannins, pharmacological activity

ХИМИЧЕСКИЕ КОМПОНЕНТЫ GEUM RIVALE L. И ИХ БИОЛОГИЧЕСКАЯ АКТИВНОСТЬ

А.А. Орлова, М.Н. Повыдыш

Федеральное государственное бюджетное образовательное учреждение высшего образования
«Санкт-Петербургский государственный химико-фармацевтический университет»
Министерства здравоохранения Российской Федерации
197376, Россия, Санкт-Петербург, ул. Профессора Попова, 14, лит. А

E-mail: anastasiya.lebedkova@spcpu.ru

Получено 25.02.2020 Рецензия (1) 30.04.2020 Рецензия (2) 10.05.2020 Принята к печати 15.05.2020

Целью исследования является обзор данных литературы о составе биологически активных веществ надземных и подземных органов гравилата речного (*Geum rivale* L.) и фармакологической активности его извлечений и индивидуальных соединений.

Для цитирования: Орлова А.А., Повыдыш М.Н. Химические компоненты Geum rivale L. и их биологическая активность. *Фармация и фармакология.* 2020;8(2):133-146. **DOI:** 10.19163/2307-9266-2020-8-2-133-146

For citation: Orlova A.A., Povydysh M.N. Chemical constituents of Geum rivale L. and their biological activity. *Pharmacy & Pharmacology*. 2020;8(2):133-146. **DOI:** 10.19163/2307-9266-2020-8-2-133-146

Материалы и методы. Исследование проводили с использованием Интернет-ресурсов (Google Scholar, PubMed) и библиотечных баз данных (e-Library, Scopus, Web of Science). Основными методами исследования являлись обзор и анализ литературных данных по тематике исследования за период с 1958 года по настоящее время.
Результаты. В период с 1958 года по настоящее время в надземных и подземных частях гравилата речного идентифицировано более 80 компонентов в составе эфирного масла, ряд фенольных кислот и кумаринов, агликоны флавоноидов, в том числе лютеолин, апигенин, кверцетин и кемпферол, а также ряд их гликозидов и глюкуронидов, эллаготанины (гемин A, B, C, D, педункулагин, стахиурин/казуаринин, теллимаграндин I). Изучены некоторые аспекты фармакологической активности суммарных извлечений и индивидуальных вторичных метаболитов гравилата речного, экспериментально подтверждены противовоспалительная, антиоксидантная, противомикробная, противовирусная активности.
Заключение. Анализ литературных данных показал, что дальнейшее изучение состава метаболитов гравилата речного и их фармакологической активности является актуальной задачей, решение которой позволит расширить спектр использования данного растения в медицинской практике и рассматривать гравилат речной как перспективный источник фармацевтических субстанций для создания новых лекарственных препаратов и биологически активных добавок к пище.
Ключевые слова: гравилат речной, *Geum rivale* L., фенольные соединения, эфирные масла, танины, фармакологическая активность

INTRODUCTION

The genus *Geum* L. (*Rosaceae*) is represented by 58 species [1], about 20 of which grow on the territory of the Russian Federation [2-5]. *G. rivale*is is a perennial plant, the distribution area includes most of Europe up to the Ural Mountains, with the exception of the West of France, Spain and the Mediterranean region, as well as Western Siberia, Central Asia, some regions of North America [6, 7].

G. rivale is widely used in folk medicine for prevention and treatment of gastrointestinal diseases, including lack of appetite and diarrhea, malaria [8], for febrile diseases, muscle pain, hemorrhoids, for inflammatory diseases of the mucous membranes and skin integuments, as an antiseptic and astringent agent [9, 10]. In homeopathy, it is used for inflammatory diseases of the bladder and urinary tract, as well as for arthritis [9, 11, 12].

To date, a number of studies have been carried out to study the qualitative and quantitative composition of biologically active substances in the arial and underground parts of *G. rivale*, and some aspects of the pharmacological activity of extracts and individual groups of biologically active substances have been experimentally revealed.

The study of widespread plants as sources of pharmaceutical substances for the production of medicines and biologically active additives is an urgent task, since they show high efficiency along with low toxicity and allergenicity.

Based on this, the aim of the study was to review the literature data on the chemical composition of biologically active constituents of *G. rivale* and their pharmacological activity.

MATERIALS AND METHODS

The Internet resources (Google Scholar, PubMed) and library databases (e-Library, Scopus, Web of Science) as sources of information were used. The main research methods were the review and analysis of the literature data on the research topic for the period from 1958 up to the present.

RESULTS

Chemical components of Geum rivale L.

To date, a lot of data have been obtained on various groups of secondary metabolites contained in the arial and underground parts of the *G. rivale*. Thus, using the method of gas chromatography combined with a mass spectrometric detector (GC-MS), the component composition of the essential oil has been studied in sufficient detail [13, 14]. In the experiments, the essential oil was isolated from various parts of plant material by hydrodistillation. The components of a complex mixture of the essential oil were separated by gas chromatography with a flame ionization detector (GC-FID). The component identification was based on a comparison of mass spectra of the essential oil components with mass spectra of commercial libraries. The identification of isomers was based on a comparison of the retention index (RI) with the literature data. In the course of the experiment, more than 80 components were found in the samples of the essential oil of the arial and underground parts of *G. rivale* (compounds 51–143 in Table 1). The dominant components in *G. rivale* essential oil are 3-octen-1-ol (33.9%) and 3-hexenol (16.2%). In addition, the essential oil contains a large amount of sesquiterpenoids (32 compounds), a certain amount of monoterpenoids has been found [14]. Vollmann, C. et al. (1995) conducted a comparative analysis of the qualitative and quantitative composition of the essential oil of various species of the genus *Geum* L. As a result of the experiment, all species of the genus were divided into 2 large groups: the first group comprised the species containing a high percentage of eugenol (66–92%) and a low content pinene derivatives – *G. urbanum, G. fauriei* Levl. and *G. macrophyllum* Willd.; the second group comprised the species with

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2020-8-2-133-146

a high content of pinene derivatives and a low content of eugenol (0.3–4.1%) – *G. rivale* L., *G. rhodopeum* Stoj. et Stefanov, *G. bulgaricum* Pancic, *G. borisii* Kellerer ex Siindermann, and *G. chiloense* Balb. [13].

Panizzi et al. (2000) analyzed the composition of triterpenoids in the arial part of *G. rivale* in the extracts obtained by extracting raw materials in a Soxhlet apparatus with n-hexane, chloroform, and an alcohol-chloroform mixture (1: 9). The isolation of compounds in pure form was carried out by sequential purification on Sephadex, silica gel, thin layer chromatography and reverse phase chromatography. The structure was confirmed using IR and UV spectroscopy, as well as ^{1}H and ^{13}C NMR methods. The compounds identified during the study are shown in Fig. 1 and numbered 1-10 in Table 1 [15, 16].

The most extensively represented group of secondary metabolites in the arial and underground parts of G. rivaleis are polyphenolic compounds. Obtaining extracts using solvents of different polarity makes it possible to study the qualitative and quantitative composition of various groups of polyphenolic compounds. The analysis of phenolic acids and coumarins is based on obtaining extracts with a methanol-chloroform mixture [15, 16, 18], petroleum ether [17] and n-butanol [22]. By means of IR spectroscopy methods and 1H- and 13C-NMR, HPLC-UV in comparison with standard samples, GC-MS, their component composition in the arial and underground parts of the river gravel was determined. The compounds identified in the work of several scientific groups, are shown in Fig. 2 and Table 1 under numbers 11-26 (Fig. 2). According to the estimates by Owczarek et al. (2013), the content of phenolic acids in the arial part is 5.9 mg/g, and in the underground part it is 18.9 mg/g [17]. In addition, Owczarek et al. (2014) determined the content of free ellagic acid (0.52± 0.01mg/g) in the arial part of the river gravity (gallic acid was not detected in this case); in the underground part there was 0.43±0.002 mg/g of ellagic acid, and gallic acid was not found there either [20].

Panizzi et al. (2000) also carried out extensive work on the study of the composition of flavonoids of the aerial part of G. rivale L. The extraction of this group of compounds was carried out from a mixture of the plant material pretreated with n-hexane, chloroform, and chloroform-methanol (9:1) by maceration with methanol at room temperature with subsequent purification on Sephadex and silica gel and separation on a C18 reverse phase column. In the study, 13 compounds were isolated, the structures of which were established by IR and UV spectroscopy, ^{1}H and ^{13}C NMR (Fig.3, Table 1) [16]. Owczarek et al. (2013) evaluated the quantitative content of flavonoids according to the method described in the Polish Pharmacopoeia of the VIII edition: in the underground part – 0.3 mg/g; in the aerial part – 3.0 mg/g [17].

Another group of polyphenolic compounds – tannins – is of great interest. The main methods of analysis of this group and the experimental data on the pharmacological activity were described in our previously published review [21]. In *G.* rivale, the composition of ellagitannins was also widely studied in the works by Moilanen et al. (2008, 2015). After the extraction of raw materials with 70% acetone with the addition of 0.1% ascorbic acid to prevent the oxidation of the compounds, the composition of ellagitannins (44–50) [22-23] was established by using HPLC-ESI-MS. The total acid content was determined by Owczarek et al. (2014) after hydrolysis of tannins with a 25% hydrochloric acid solution: ellagic acid – 40.31±1.08 mg/g in the arial part, 60.64±0.87 mg/g in the underground part; gallic acid – 7.45±0.08 mg/g in the arial part and 9.57±0.27 mg/g in the underground part (in terms of dry plant material). On the basis of the obtained results the authors made a conclusion about the greater prevalence of ellagitannins in comparison with gallotannins, both in the arial and underground parts of the studied species [20].

Rare sulfonated derivatives of ellagic acid obtained by precipitation from the aqueous extraction with boiling methanol, were studied by Owczarek et al. (2017). The following structures were established by UV spectroscopy, mass spectrometry, and ^{1}H- and ^{13}C-NMR: potassium salt of 3,3'-dimethoxy-4-sulfoxyellagic acid (29) and 3,3', 4'-trimethoxy-4-sulfoxyellagic acid potassium salt (30) (Fig. 4) [25].

Determination of the antioxidant activity of the extracts showed that the roots of *G. rivale* have a high antioxidant potential. According to the results, the authors of the work suggest that polyphenolic compounds bear the main responsibility for the antioxidant activity thanks to the transfer of a hydrogen atom during the reaction (HAT mechanism) [30].

Oszmianski et al. (2007) screened the antioxidant activity of tannins in the roots of *G. rivale*. During the research, the following experiments were carried out: thiolysis of proanthocyanidins according to the method described by Guyot et al. (2001) [37]; reverse phase HPLC after thiolysis; the content of proanthocyanidins (10.5 g/kg) and phenolic compounds (3.0 g/kg) in the feed were determined, as well as the degree of polymerization of proanthocyanidins – 3. For screening of the antioxidant activity, two methods were used by the authors: the DPPH test according to Yen et al.'s method (1995) [38] and the ABTS test by Re et al.'s the method (1999) [39]. This study demonstrated a significant antioxidant potential of the extract containing phenolic compounds [36].

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY

Pharmacological activity of extracts and components of G. rivale

Simultaneously with the study of the component composition of the secondary metabolites in the arial and underground parts of *G. rivale*, extensive studies of the pharmacological activity of the total extracts obtained using solvents of different polarities, as well as individual metabolites, were carried out. Thus, Tunon et al. (1995) conducted a study of the anti-inflammatory activity of the total water extract from the arial part of *G. rivale*, obtained by a two-stage extraction at room temperature, in tests of the effect on prostaglandin synthesis and PAF-induced exocytosis. The extract showed a high inhibitory activity in the PAF test, while an inhibitory effect on the biosynthesis of prostaglandins was not found [27]. In addition, the use of extracts from *G. rivale* as an anti-inflammatory agent in traditional medical practice is reported in the works by Birnesser et al. and Parimala et al. [28, 29].

Owczarek et al. (2015) investigated the total extracts of different polarity, obtained by the extraction of the methanol extract from the arial and underground parts of the *G. rivale*, in tests for antioxidant activity: DPPH test by method of Brand Williams, Cuvier and Berset [31] with the previously described modifications [32]; the FRAP test described by Pulido et al. [33] with some modifications [34]; the test for linoleic acid peroxidation according to Azuma et al.'s modified method [35, 32].

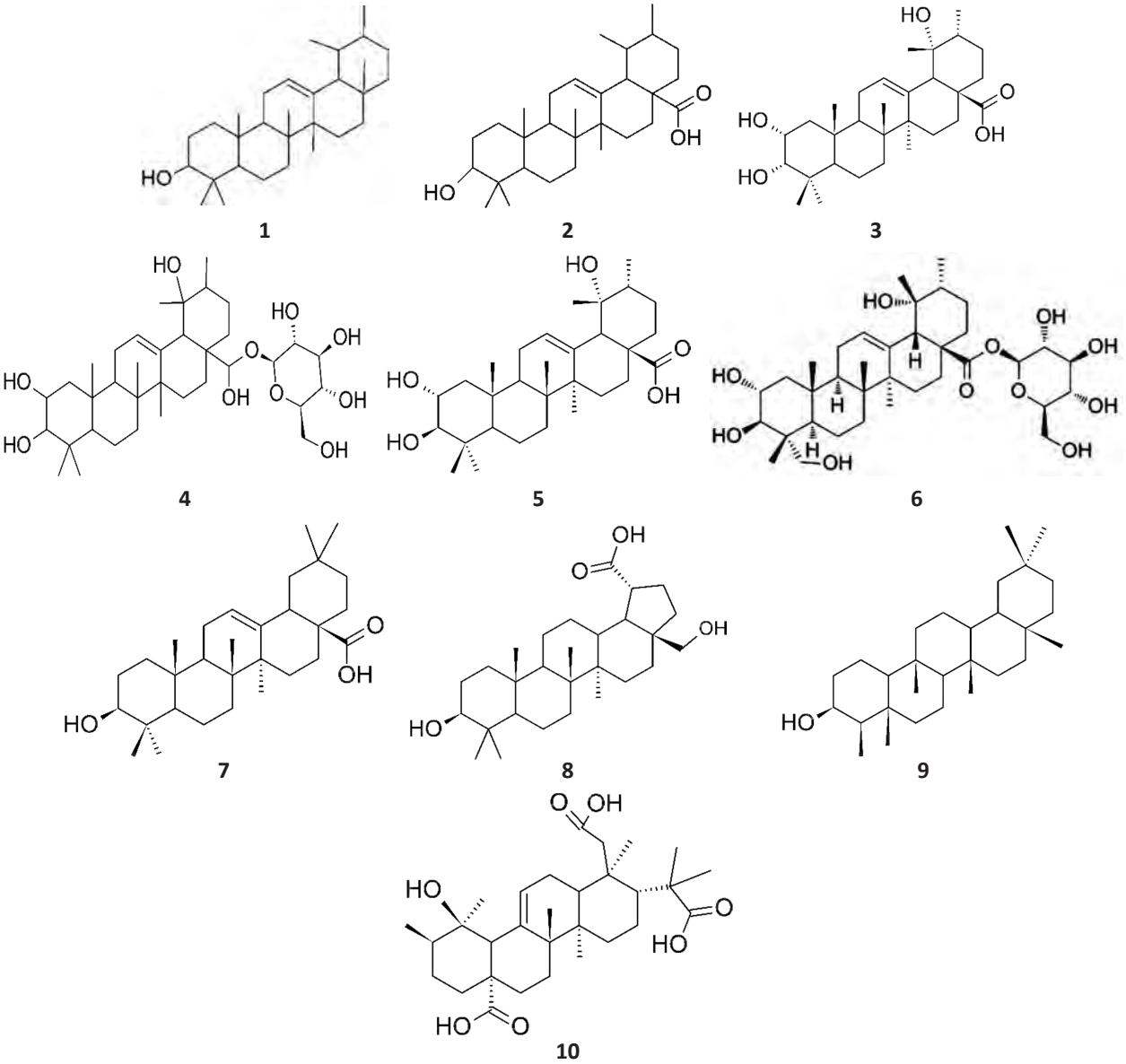


Figure 1 – Triterpenoids of the arial part of G. rivale (Panizzi, L. et al., 2000)

Note: 1 – α-amyrin; 2 – ursolic acid; 3 – euskafic acid; 4 – euskafic acid 28-glucoside; 5 – tormentic acid; 6 – nigaishigoside F1; 7 – oleic acid; 8 – betulin; 9 – epifriedelonol; 10 – cescropic acid

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2020-8-2-133-146

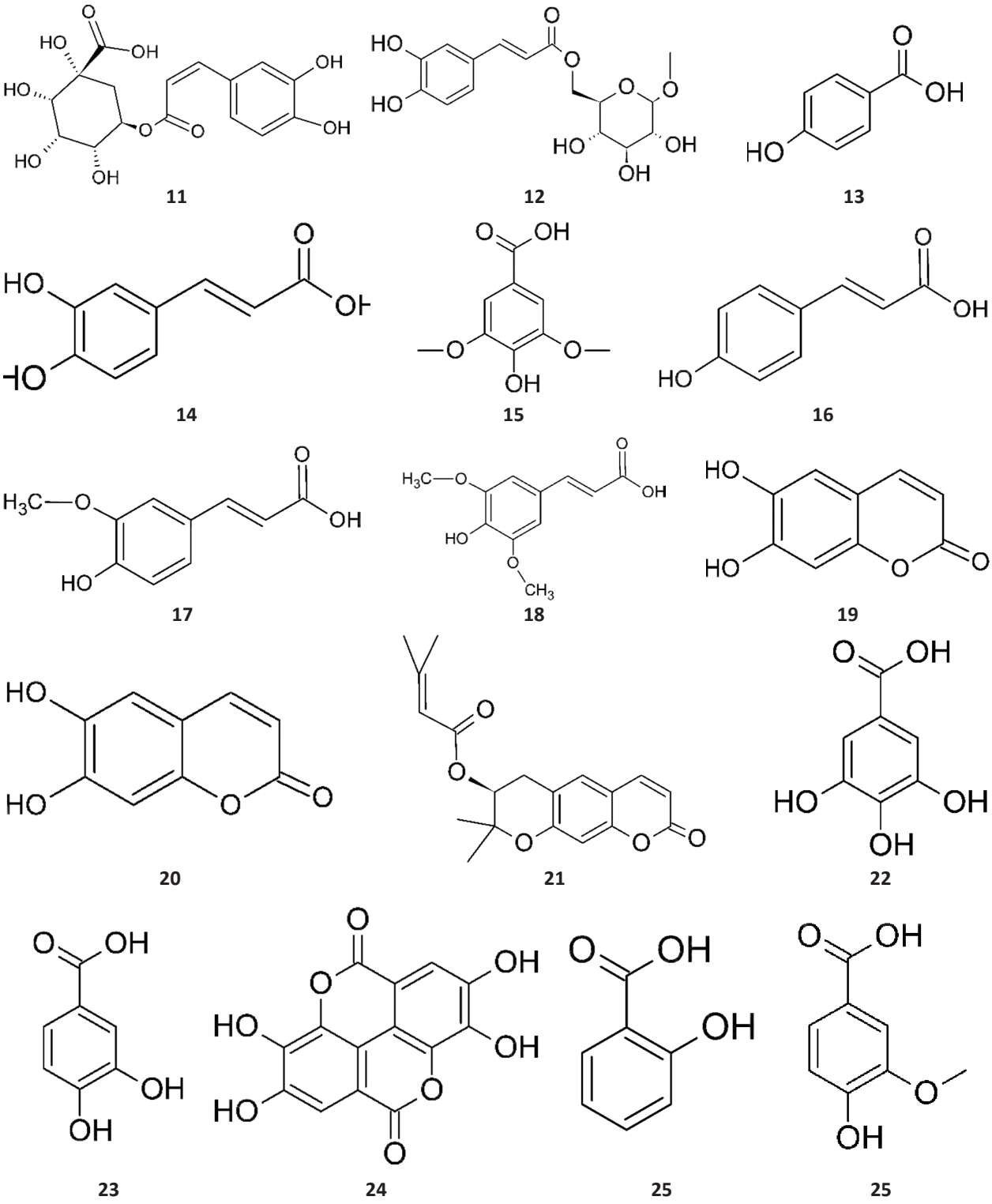


Figure 2 – Phenolic acids and coumarins of G. rivale (Panizzi et al.,2000; Owczarek et al., 2013)

Note: 11 – chlorogenic acid; 12 – 6-O-caffeyl-1-O-methyl-β-D-glucopyranose; 13 – p-hydroxybenzoic acid; 14 – caffeic acid; 15 – lilac acid; 16 – p-coumaric acid; 17 – ferulic acid; 18 – sinapic acid; 19 – scopoletin; 20 – esculetin; 21 – decursin; 22 – gallic acid; 23 – protocatechuic acid; 24 – ellagic acid; 25 – salicylic acid; 26 – vanillin

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY

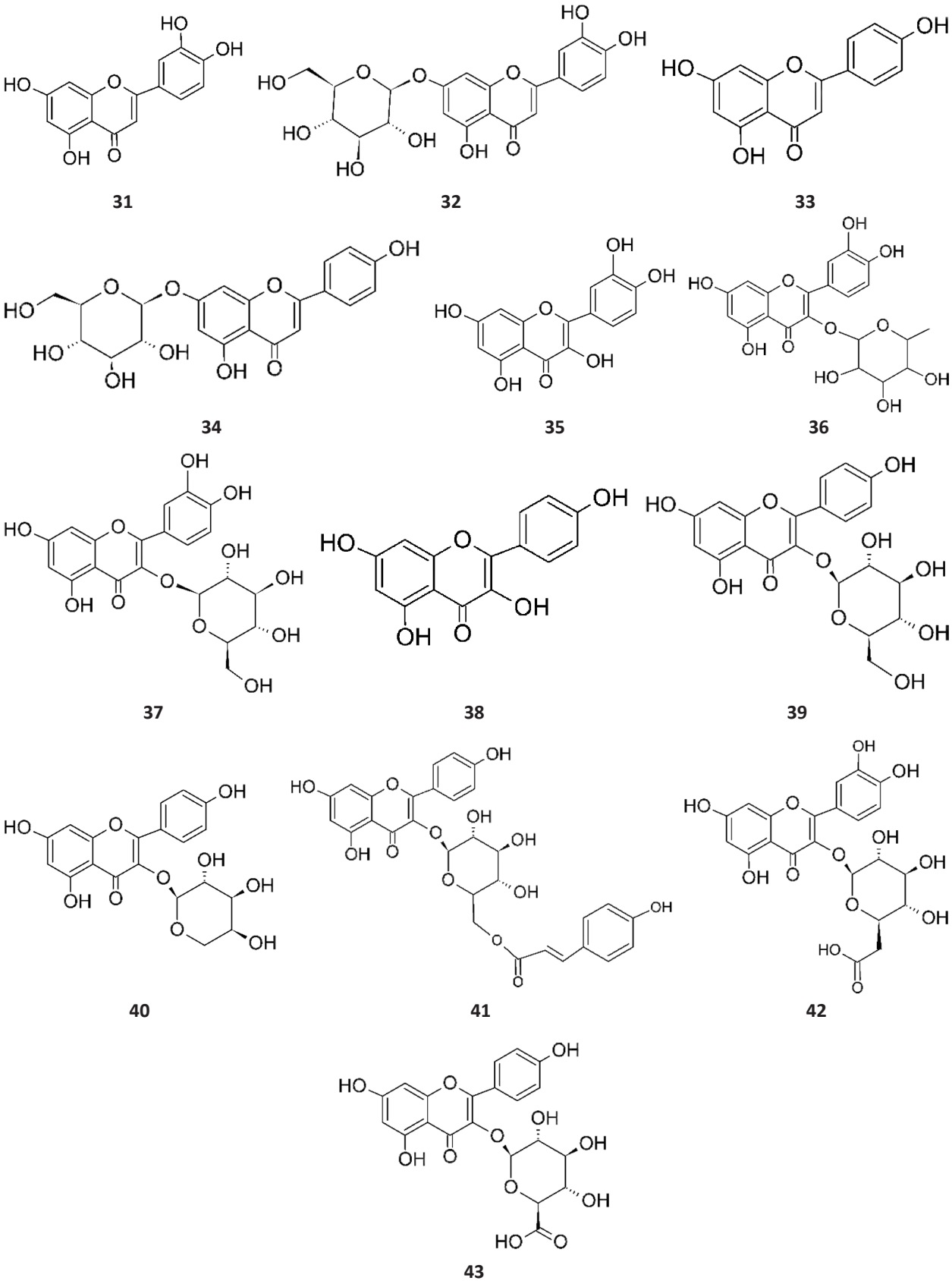


Figure 3 – Flavonoids of *G. rivale* (Panizzi et al., 2000)

Note: 31 – luteolin; 32 – luteolin-7-O-glucoside; 33 – apigenin; 34 – apigenin-7-O-glucoside; 35 – quercetin; 36 – quercetin-3-Oramnoside; 37 – quercetin-3-O-glucoside; 38 – kaempferol; 39 – kaempferol-3-O-glucoside; 40 – kaempferol-3-O-arabinoside; 41 – tilyroside; 42 – quercetin-3-O-glucuronide; 43 – kaempferol-3-O-glucuronide

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2020-8-2-133-146

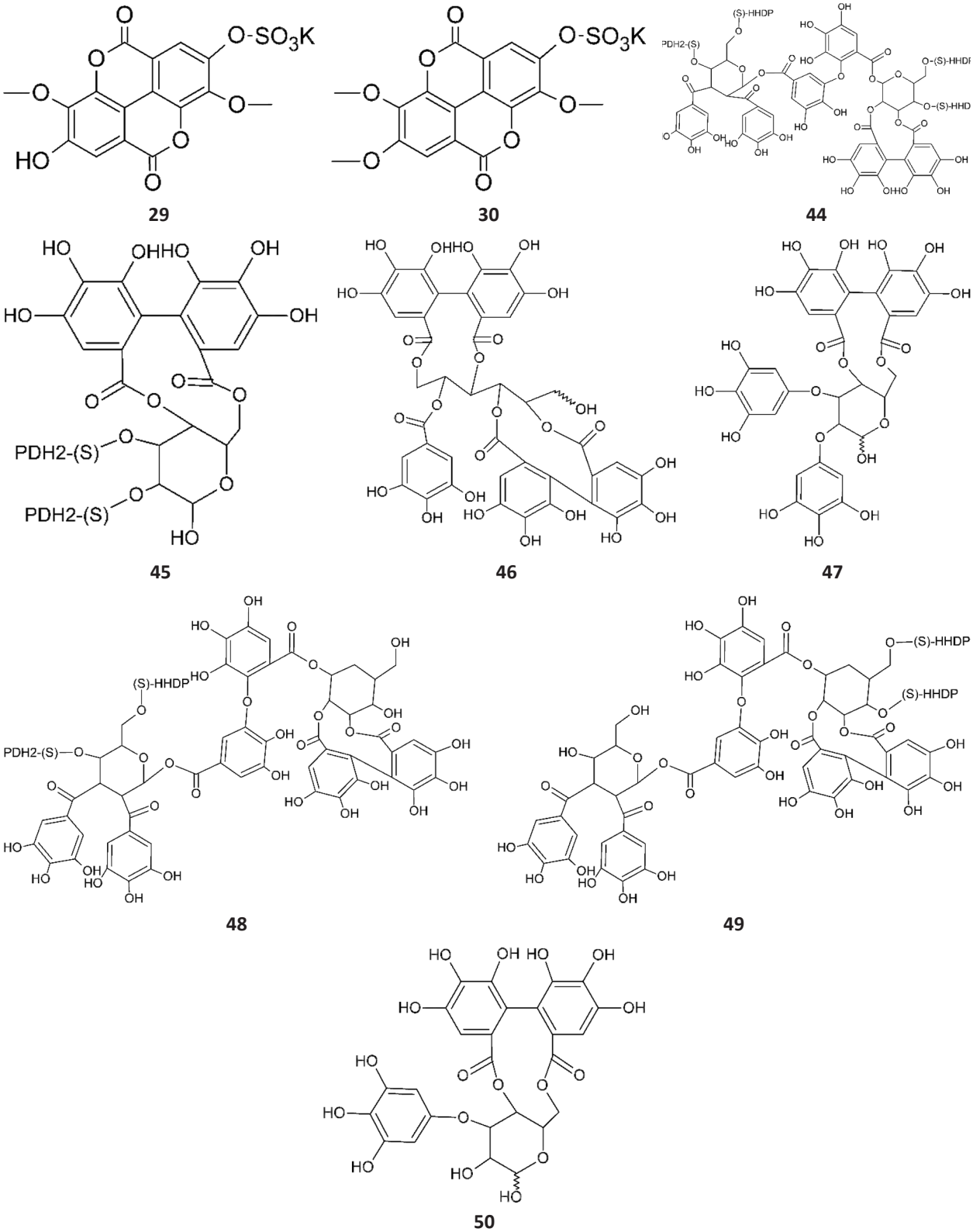


Figure 4 – Ellagitannins of *G. rivale* (Moilanen et al.,2008, 2015; Owczarek et al., 2017)

Note: 29 – 3,3'-dimethoxy-4-sulfoxyellagic acid potassium salt; 30 – 3,3',4'-trimethoxy-4-sulfoxyellagic acid potassium salt; 44 – Gemin A; 45 – Pedunculagin; 46 – Stachiurin / casuarinin; 47 – Tellimagrandin 1; 48 – Gemin B; 49 – Gemin C; 50 – Gemin D

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY

Table 1 – Biologically active compounds of G. rivale

№	Compounds	Morphological parts	References
Triterpenoids (Ursanes)			
1	α-amyrin	Arial part	14, 15
2	Ursolic acid		
3	Euskafic acid		
4	Euskafic acid 28-glucoside		15
5	Tormentic acid		14, 15
6	Nigaishigoside F1		14, 15, 22, 67
Other Triterpenoids			
7	Oleanolic acid	Arial part	14, 15
8	Betulin		
9	Epifriedelonol		
10	Cescropic acid		14, 15, 22, 67
Phenylpropanoids			
11	Chlorogenic acid	Arial and underground parts	14, 15, 17, 45
12	6-O-caffeyl-1-O-methyl-β-D-glucopyranose		14, 15, 22, 67
13	p-hydroxybenzoic acid		16
14	Caffeic acid	Arial parts	14, 15, 17, 45
15	Lilac acid	Arial and underground parts	16, 17
16	p-coumaric acid	Arial parts	16, 45
17	Ferulic acid	Arial and underground parts	16, 17
18	Sinapic acid		16, 17
19	Skopoletin		14, 15, 16
20	Esculetin		
21	Decursin		
Other constituents			
22	Gallic acid	Arial and underground parts	14, 15, 16, 19, 23, 45, 67
23	Protocatechuic acid		14, 15, 16
24	Ellagic acid		14, 15, 16, 19, 45
25	Salicylic acid		14, 15, 16
26	Vanillin	Arial parts	14, 16, 67
27	1-O-protocatechioyl glucose	Arial parts	22
28	Sucrose	Arial parts	
29	3,3'-dimethoxy-4-sulfoxyellagic acid potassium salt	Underground parts	23
30	3,3 ', 4'-trimethoxy-4-sulfoxyellagic acid potassium salt		
Flavonoids			
31	Luteolin	Arial part	14, 15
32	Luteolin 7-O-glucoside		
33	Apigenin		
34	Apigenin 7-O-glucoside		
35	Quercetin		
36	Quercetin 3-O-rhamnoside		
37	Quercetin 3-O-glucoside		
38	Kaempferol		
39	Kaempferol 3-O-glucoside		
40	Kaempferol 3-O-arabinoside		
41	Tiliroside		
42	Quercetin 3-O-glucuronide		
43	Kaempferol 3-O-glucuronide		
Ellagitannins			
44	Gemin A	Arial part	20, 21
45	Pedunculagin		21
46	Stachiurin / casuarinin		
47	Tellimagrandin 1		
48	Gemin B		
49	Gemin C		
50	Gemin D		

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2020-8-2-133-146

№	Compounds	Morphological parts	References
Essential oil constituents			
51	(E) -2-hexenal	Arial part	13, 14
52	(Z), (E) -3-hexene-1-ol		
53	Hexanol		
54	Heptanol		
55	6-methyl-5-hepten-2-ol		
56	(Z) -3-hexenyl acetate		
57	α-pellandrene		
58	β-pellandrene		
59	(E) -β-ocimene		
60	(E) -2-octene-1-ol		
61	Octanol		
62	Terpinolen		
63	Nonanal		
64	Nonanol		
65	Terpinen-4-ol		
66	Deanal		
67	β-cyclocitral		
68	Dodecane		
69	(Z) -3-hexenyl-2-methylbutanoate		
70	(Z) -3-hexenyl isovalerate		
71	Tridecan		
72	(Z) -3-hexenyl crucible		
73	δ-element		
74	α-cubeben		
75	β-damascenone		
76	α-ylangen		
77	β-bourbonene		
78	β-cubeben		
79	β-caryophyllene		
80	β-copen		
81	α-humulene		
82	Alloaromadendren		
83	β-ionone		
84	γ-muurelen		
85	Germacren D		
86	(Z, E) – α-farnesene		
87	α-muurelen		
88	(E, E) – α-farnesene		
89	γ-cadinen		
90	α-calacoren		
91	Trans-nerolidol		
92	(Z) -3-hexenyl benzoate		
93	Caryophyllene oxide		
94	Viridiflorol		
95	Humulene epoxy II		
96	Farnesene epoxy		
97	Cubenol		
98	T-muurolol		
99	α-cadinol		
100	Pentadecanal		
101	Heptadecan		
102	Benzyl benzoate		
103	Octadecan		
104	Fitol		
105	Tricosan		
106	Tetracosan		
107	Hexacosan		
108	(Z) -hexenyl butyrate		

№	Compounds	Morphological parts	References
109	1-zopropylcyclohex-1-ene	Underground part	13, 14
110	Trans-linalool oxide		
111	Trans-myrtanal		
112	Palmitic acid		
113	Oct-1-en-ol		
114	α-guayenne		
115	Cumin aldehyde		
116	Nerol		
117	trans-anethole		
118	Geraniol		
119	2-methoxy-6-vinylphenol		
120	Isoeugenol		
121	Eugenol		
122	Perilla aldehyde		
123	Fellandral		
124	Perilla alcohol		
125	Mirtenal		
126	trans-pinocarveol		
127	Camphene	Arial and underground part	13, 14
128	1-octene-3-ol		
129	3-octanol		
130	Limonen		
131	Cis-linalool oxide		
132	Camphor		
133	Citronellol		
134	p-cymene		
135	δ-cadinen		
136	α-copen		
137	cis-myrtanol		
138	trans-myrtanol		
139	α-terpineol		
140	Mirtenol		
141	Linalool		
142	Nopinone		
143	cis-myrtanal		

Table 2 – Pharmacological effects of the main groups of constituents of *G. rivale*

Pharmacological effect	Extraction type or group of biologically active substances	References
Anti-inflammatory activity due to PAF-induced exocytosis	Total water extract	24, 25, 26
Antioxidant activity (DPPH-, FRAP-tests, linoleic acid peroxidation test)	Polyphenolic compounds	27
Antioxidant activity (DPPH and ABTS tests)	Phenolic acids and proanthocyanidins	33
Antimicrobial activity:		14, 15, 38, 68
a) antimicrobial activity against gram-positive and gram-negative microorganisms	Total polar extracts, triterpene fraction, flavonoid fraction, tannin fraction, ursolic acid, caffeic acid	
b) antifungal activity	Total polar extract, triterpene fraction, caffeic acid	
c) *Candida albicans*	Chloroform extract, total polar extracts, triterpene fraction, caffeic acid	
d) *Staphilococcus aureus, Pseudomonas aeruginosa*	Triterpene fraction, quercetin, kaempferol, caffeic acid, gallic acid	
Antiviral activity (influenza virus types A and B)	Ethanol extracts from the arial part	40

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2020-8-2-133-146

Panizzi et al. (2000) investigated the antimicrobial activity of extracts of different polarity from the arial part of *G. rivale,* and some individual compounds. The dried raw material was extracted in a Soxhlet apparatus with n-hexane, chloroform, a mixture of chloroform-methanol 9:1, and then by maceration with methanol at room temperature. Then, the obtained total extracts were purified by column chromatography to individual compounds, their identification was carried out by IR and UV spectroscopy, ^{1}H and ^{13}C NMR. All the investigated fractions were dissolved in DMSO and screened for the antimicrobial activity by the agar diffusion method described by Clark, et al. (1981), using test microorganisms: *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Candida albicans* and *Aspergillus niger* [40]. The study showed that the total methanol extract has a high antimicrobial and antifungal activity, while the n-hexane extract showed a weak activity against bacteria and *Aspergillus niger*; chloroform extract had a pronounced activity against *Candida albicans*; and chloroform-methanol and water-methanol extracts were found active against all the tested organisms. When analyzing the purified extracts and individual compounds, the following results were obtained: triterpene fraction showed an efficiency comparable to methanol and chloroform-methanol extracts against all studied microorganisms; a mixture of flavonoids was found active against gram-positive and gram-negative bacteria in the absence of antifungal activity; the tannin fraction was active only against bacteria, but its effectiveness was lower than that of the flavonoid fraction; ursolic acid had zones of inhibition very similar to those obtained using a chloroform-methanol extract in the absence of antifungal effect; among the flavonoid aglycones, kaempferol and quercetin affected only *Staphylococcus aureus* and *Pseudomonas aeruginosa*, respectively, while apigenin had no antimicrobial and antifungal activity; caffeic acid showed a moderate activity against all test organisms, while gallic acid showed a pronounced effectiveness against *Staphylococcus aureus, Escherichia coli* and *Candida albicans* [16].

The identification of natural metabolites and synthetic agents that are effective in the prevention and treatment of diseases caused by influenza viruses of various types, is an urgent problem of the last decade. Researchers suggest that total native complexes of metabolites, as well as individual natural compounds of various natures, such as polyphenols, triterpenoids, alkaloids, organic acids, and some others, can be used as agents for inhibiting infections at various stages [42]. Therefore, in the work by Lobanov et al. (2016) the antiviral activity of 70 plant species belonging to 14 different families, including the aerial part of the *G. rivale, was* considered. The study was carried out using ethanol extracts obtained by the method described in the work by Kostina et al. (2013) [44]; avian influenza virus A / chicken / Kurgan / 05/2005 (H5N1) and a strain of human influenza virus A / Aichi / 2/68 (H3N2) adapted to laboratory mice, the titer of which was calculated by Spearman-Kerber's method using statistical processing according to Sachs, L. (1976) [45]. In the course of the study it was revealed that the ethanol extract from the arial part of *G. rivale* has a pronounced antiviral activity against both studied viral strains and can be recommended for a further research in this area in order to create phytopreparations for the prevention and treatment of influenza caused by these virus strains [43].

Ellagic acid is a metabolite of higher plants, it is in sufficiently large quantities in the arial and underground parts of *G. rivale*, both in free and bound forms as parts of ellagitannins. Due to its wide distribution, the possibilities of using this compound in medical practice are well studied. Thus, for the first time, a systematic review of the literature was conducted by García-Niño et al. (2015) and the following possible pharmacological effects of ellagic acid were described in detail [47]: antimutagenic [48], antigenotoxic [49–50], antiapoptotic [51], anticarcinogenic [52], antibacterial [53], antiviral [54], antimalarial [55], antiallergic [56], anti-inflammatory [57], antiatherogenic [58], antidiabetic [59], antiepileptic [60], antidepressant [61], antinociceptive [62], neuroprotective [63], nephroprotective [64], cardioprotective [65] and hepatoprotective [66] activities. However, the work notes: the contribution of ellagic acid to the pharmacological effects of the extracts obtained from the arial parts of *G. rivale,* has not been revealed.

CONCLUSIONS

The analysis of the literature showed that *Geum rivale* L. has been subjected to phytochemical studies for a long period of time. This is due to both the rich raw material base of the plants and its widespread use in folk medicine.

For the period from 1958 to the present, more than 80 components have been identified in the arial and underground parts of the river gravity. The main groups of secondary metabolites have been characterized, including the essential oil, triterpenoids and phenolic compounds of the arial and underground parts of *Geum rivale* L. The most extensively represented group of secondary metabolites is polyphenolic compounds. Despite the sufficient knowledge of the chemical composition, the plant is not official in Russia.

The rich composition of polyphenolic compounds determines characteristic pharmacological effects of the plant, including anti-inflammatory, antioxidant, antimicrobial and antiviral activity. The pharmacological activity has been experimentally confirmed, both the extraction obtained by the extraction with solvents of polarity or fractionation, and some compounds. These types of activity may be useful against some socially significant pathologies, for example, antioxidant activity in the prevention and treatment of diseases of the cardiovascular, urinary and nervous systems, the antimicrobial and antiviral ac-

tivities in the treatment of the diseases caused by resistant strains of microorganisms and viruses.

However, the currently available data on the chemical composition and activity of *Geum rivale* L. do not give a general picture of the potential for using a plant as a source of new pharmaceutical substances of natural origin for the creation of medicines and biologically active additives.

Modern analytical methods in phytochemistry, dictate the development of the allocation of natural resources and compounds with the establishment of their exact structures using one of the methods of analytical magnetic resonance and infrared spectroscopy with a further study of their pharmacological potential. Therefore, it is advisable to continue the study of the composition of secondary metabolites of the arial and underground parts of this plant using modern methods of analysis to identify both – previously not discovered, as well as new for science natural compounds. The identification of specific compounds responsible for the development of types of biological activity valuable for medicine using *in silico* methods, the analysis of possible synergistic or additive effects of combinations of secondary metabolites, as well as the prediction of the mechanisms associated with the manifestation of a certain effect, may become a promising direction for further studies of *Geum rivale* L. The data obtained will make it possible to expand the range of use of *Geum rivale* L. in medicine.

FUNDING

This study did not have any financial support from other organizations.

AUTHOR'S CONTRIBUTION

All authors equally contributed to the research work.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

REFERENCES

1. Tsvelev NN. Flora vostochnoi Evropi. Tom X. [Flora Europae Orientalis. Volume 10]. SPb.: World and family. Ed. SPHFA. 2001;10:460–466. Russian.
2. Gubanov IA, Kiseleva KV, Novikov VS, Tikhomirov VS. Illyustrirovannyy opredelitel rasteniy Sredney Rossii. T. 2. [An illustrated guide to plants of Central Russia. T. 2]. M.: KMK Scientific Publishing Partnership, Institute of technological research. 2003:2190. Russian.
3. Mayevsky PF. Flora sredney polosy evropeyskoy chasti Rossii. 10-e izd [Flora of the middle zone of the European part of Russia. 10th ed.]. M.: KMK Scientific Publishing Partnership. 2006:379–400. Russian.
4. Elenevsky AG, Radygina VI, Chaadaeva NN. Rasteniya Belgorodskoy oblasti (konspekt flory) [Plants of the Belgorod region (flora synopsis)]. M.: Moscow State Pedagogical University. 2004:120 p. Russian.
5. Lazarev AV, Burchenko TV. Rod Geum v sovremennykh ekologicheskikh usloviyakh sredney Rossii [Genus Geum in the modern ecological conditions of Central Russia]. Scientific Bulletin. 2009;3(58):34–38. Russian.
6. Hulten E. The Amphi-Atlantic Plants and their Phytogeographical Connections. Kongl. Svenska Vetens. – Akad. Handl. 1958;7(1):1–340.
7. Taylor K. Geum rivale L. Journal of Ecology. 1997;85(5):721–731.
8. Hulden L. The first Finnish malariologist, Johan Haartman, and the discussion about malaria in 18th century Turku, Finland. Malaria Journal. 2011;10:43. DOI:10.1186/1475-2875-10-43.
9. Thomson Healthcare. Physicians' desk reference for herbal medicines. Thomson Healthcare (Firm), 4th ed. Montvale, NJ: Thomson. 2007:71–72.
10. Vollmann C, Scbultze W. Composition of the Root Essential Oils of Several Geum Species and Related Members of the Subtribus Geinae (Rosaceae). Flavour and Fragrance Journal. 1995;10:173–178.
11. Birnesser H, Stolt P. The Homeopathic antiarthritic preparation Zeel comp. N: Review of Molecular and Clinical Data. Explore. 2007;3(1):16–22. DOI: 10.1016/j.explore.2006.10.002.
12. Egoshina TL, Luginina EA. Medicinal plants in folk medicine of taiga zone of Russia: peculiarities of use and resources. Plant, fungal and habitat diversity investigation and conservation. Proceedings of IV BBC – Sofia, 20-26 June 2006:624-631. DOI: 10.13140/2.1.4303.9044.
13. Vollmann C, Scbultze W. Composition of the Root Essential Oils of Several Geum Species and Related Members of the Subtribus Geinae (Rosaceae). Flavour and Fragrance Journal. 1995;10:173–178.
14. Owczarek A, Gudej J, Kicel A. Composition of Essential Oil from Aerial and Underground Parts of Geum rivale and G. urbanum Growing in Poland. Natural Product Communications. 2013;8(4):505–508. DOI: 10.1177/1934578X1300800425.
15. Cheng X-R, Jin H-Z, Qin J-J, Fu J-J, Zhang W-D Chemical Constituents of Plants from the Genus Geum. Chemistry and biodiversity. 2011;8(2):203–222. DOI: 10.1002/cbdv.200900347.
16. Panizzi L, Catalano S, Miarelli C, Cioni P. L, Campeol E. In vitro Antimicrobial Activity of Extracts and Isolated Constituents of Geum rivale. Phytotherapy Research. 2000;14(7):561–653. DOI: 10.1002/1099-1573(200011)14:7<561::AID-PTR651>3.0.CO;2-H.
17. Owczarek A, Gudej J. Investigation into biological active constituents of Geum rivale L. Acta Poloniae Pharmaceutica – Drug research. 2013;70(1):111–114.
18. Morozova EV, Chemesova II, Yakovlev GP. Soderzhaniye i sostav fenolkarbonovykh kislot v Geum rivale, G. urbanum i G. aleppicum (Rosaceae) [Content and composition of phenolcarboxylic acids in Geum rivale, G. urbanum, and G. aleppicum (Rosaceae)]. Plant resources. 2009;45(4):54–55. Russian.
19. Polukhina TS, Pogudina SF, Inizarova DR. Kolichestvennoye

ОРИГИНАЛЬНАЯ СТАТЬЯ
DOI: 10.19163/2307-9266-2020-8-2-133-146

opredeleniye flavonoidov v nadzemnoy chasti gravilata rechnogo (Geum rivale L.) [Quantitative determination of flavonoids in the aboveground part of the water avens (Geum rivale L.)]. Modern scientific research: Actual problems, achievements and innovations. Collection of articles by the winners of the III International Scientific and Practical Conference. Publ.: Science and Education. 2017:225–227. Russian.

20. Owczarek A, Olszewska MA, Gudej J. Quantitative determination of ellagic acid and gallic acid in Geum rivale L. and G. urbanum L. Acta Biologica Cracoviensia Sertes Botanica. 2014;56/2:74–78. DOI: 10.2478/abcsb-2014-0021.
21. Orlova A, Povydysh M. Obzor metodov kachestvennogo i kolichestvennogo analiza taninov v rastitelnom syrye [Review of methods of qualitative and quantitative analysis of tannins in raw plant materials]. Chemistry of plant raw materials. 2019;4:29–45. Russian. DOI: 10.14258 / jcprm.2019045459.
22. Moilanen J, Salminen J-P. Ecologically neglected tannins and their biologically relevant activity: chemical structures of plant ellagitannins reveal their in vitro oxidative activity at high pH. Chemoecology. 2008;18:73–83. DOI: 10.1007/s00049-007-0395-7.
23. Moilanen J, Koskinen P, Salminen J-P. Distribution and content of ellagitannins in Finnish plant species. Phytochemistry. 2015;116:188–197. DOI: 10.1016/j.phytochem.2015.03.002.
24. Ming D-Sh, Jiang R-W, But P P-H, Towers GH N, Yu D-Q. A new compound from Geum rivale L. Journal of Asian Natural Products Research. 2002;4(3):217–220. DOI: 10.1080/10286020290024022.
25. Owczarek A, Ró˙zalski M, Krajewska U, Olszewska MA. Rare Ellagic Acid Sulphate Derivatives from the Rhizome of Geum rivale L. – Structure, Cytotoxicity, and Validated HPLC-PDA Assay. Applied Science. 2017;7(4):400. DOI: 10.3390/app7040400.
26. Budantsev AL. Rastitelnyye resursy Rossii. Tom 2. Semeystva Actinidiaceae – Malvaceae. Euphorbiaceae – Haloragaceae [Plant resources of Russia. Volume 2. Families Actinidiaceae – Malvaceae, Euphorbiaceae – Haloragaceae]. Ed. AL Budantsev. SPb: KMK Scientific Publishing Partnership. 2009:513. Russian.
27. Tunon H, Olavsdotter C, Bohlin L. Evaluation of anti-inflammatory activity of some Swedish medicinal plants. Inhibition of prostaglandin biosynthesis and PAF-induced exocytosis. Journal of Ethnopharmacology. 1995;48:61–76. DOI: 10.1016/0378-8741(95)01285-l.
28. Birnesser H, Stolt P. The Homeopathic Antiarthitic Preparation Zeel comp. N: A Review of Molecular and Clinical Data. Explore. 2007;3(1):16–22. DOI: 10.1016/j.explore.2006.10.002.
29. Parimala Devi B, Tamilchelvan N, Ramasubramaniaraja R. Inflammation and Medicinal Plants – An Ethnomedicinal Approach. 2010;2(2):49–56.
30. Owczarek A, Gudej J, Olszewska MA. Antioxidant activity of Geum rivale L. and Geum urbanum L. Acta Poloniae Pharmaceutica – Drug research. 2015;72(6):1239–1244.
31. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. LWT – Food Science and Technology. 1995;28(1):25–30. DOI: 10.1016/S0023-6438(95)80008-5.
32. Olszewska MA, Presler A, Michel P. Profiling of Phenolic Compounds and Antioxidant Activity of Dry Extracts from the Selected Sorbus Species. Molecules. 2012;17(3):3093–3113. DOI: 10.3390/molecules17033093.
33. Pulido R, Bravo L, Saura-Calixto F. Antioxidant Activity of Dietary Polyphenols as Determined by a Modified Ferric Reducing/Antioxidant Power Assay. Journal of Agricultural and Food Chemistry. 2000;48(8):3396–3402. DOI: 10.1021/jf9913458.
34. Olszewska MA, Michel P. Antioxidant activity of inflorescences, leaves and fruits of three Sorbus species in relation to their polyphenolic composition. Natural Product Research. 2009; 23(16): 1507–1521. DOI: 10.1021/jf9913458.
35. Azuma K, Nakayama M, Koshioka M, Ippoushi K, Yamaguchi Y, Kohata K, Yamauchi Y, Ito H, Higashio H. Phenolic Antioxidants from the Leaves of Corchorus olitorius L. Journal of Agricultural and Food Chemistry. 1999;47(10):3963–3966. DOI: 10.1021/jf990347p.
36. Oszmianski J, Wojdylo A, Lamer-Zarawska E, Swiader K. Antioxidant tannins from Rosaceae plant roots. Food chemistry. 2007;100:579–583. DOI: 10.1016/j.foodchem.2005.09.086.
37. Guyot S, Marnet N, Sanoner P, Drilleau JF. Direct thiolysis on crude apple materials for HPLC characterization and quantification of polyphenols in cider apple tissues and juices. Methods in Enzymology. 2001;335:57–70. DOI: 10.1016/S0076-6879(01)35231-X.
38. Yen GC, Chen HY. Antioxidant activity of various tea extracts in relation to their antimutagenicity. Journal of Agricultural and Food Chemistry. 1995;43:27–32. DOI: 10.1021/jf00049a007.
39. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology & Medicine. 1999;26(9/10):1231–1237. DOI: 10.1016/S0891-5849(98)00315-3.
40. Clark AM, El-Feraly FS, Li WS. Antimicrobial activity of phenolic constituents of Magnolia grandiflora L. Journal of Pharmaceutical Science. 1981;70:951–952. DOI: 10.1002/jps.2600700833.
41. Varaprasad B. A Search for Antibacterial Agents Edited by Varaprasad Bobbarala. Published by InTech Janeza Trdine 9, 51000 Rijeka, Croatia. 2012:8.
42. Levina AS, Repkova MN, Mazurkova NA, Makarevich EV, Ismagilov ZR, Zarytova VF. Knockdown of different influenza A virus subtypes in cell culture by a single antisense oligodeoxyribonucleotide. International Journal of Antimicrobial Agents. 2015;46(1):125–128. DOI: 10.1016/j.ijantimicag.2015.03.004.
43. Lobanova IE, Filippova EI, Vysochina GI, Mazurkova NA. Protivovirusnyye svoystva dikorastushchikh i kultiviruyemykh rasteniy yugo-zapadnoy Sibiri. Rastitelnyy mir aziatskoy Rossii. 2016;2(22):64–72. Russian.
44. Kostina NE, Ibragimova ZhB, Protsenko MA, Makarevich EV, Skarnovich MA, Filippova EI, Gorbunova IA, Vlasenko VA, Troshkova GP, Mazurkova NA, Shishkina LN. Vydeleniye, kharakteristika i protivovirusnyye svoystva biologicheski aktivnykh veshchestv iz vysshikh gribov Zapadnoy Sibiri [Isolation, characterization and antiviral properties of biologically active substances from higher fungi of Western Siberia]. Rational nutrition, food additives and biostimulants. 2014;2:25–26. Russian.
45. Zaks L. Statisticheskoye otsenivaniye. Seriya: Zarubezhnyye statisticheskiye issledovaniya. Perevod s nemetskogo [Sta-

tistical estimation. Series: Foreign Statistical Studies. Translated from German] M.: Statistics. 1976: 598. Russian.
46. Kozira SA, Kulagina MA, Serbin AG. Khimichniy sklad ta vikoristannya v meditsini roslin rodu Geum L. (Obzor literaturi) [Chemistry warehouse and vikorystannya in the medical plant of the genus Geum L. (Literature review)]. Zaporozhye medical journal. 2008;2(47):80–82.
47. Garcia-Nino WR, Zazueta C. Ellagic acid: Pharmacological activities and molecular mechanisms involved in liver protection. Pharmacological Research. 2015; 97: 84–103. DOI: http://dx.doi.org/10.1016/j.phrs.2015.04.008.
48. Zahin M, Ahmad I, Gupta RC, Aqil F. Punicalagin and ellagic acid demonstrate antimutagenic activity and inhibition of benzo[a]pyrene induced DNA adducts. BioMed Research International. 2014:467465. DOI: 10.1155/2014/467465,467465.
49. Rehman MU, Tahir M, Ali F. Cyclophosphamide-induced nephrotoxicity, genotoxicity, and damage in kidney genomic DNA of Swiss albino mice: the protective effect of ellagic acid. Molecular and Cellular Biochemistry. 2012;365(1–2):119–127. DOI: 10.1007/s11010-012-1250-x.
50. Abraham SK. Anti-genotoxic effects in mice after the interaction between coffee and dietary constituents. Food Chemical Toxicology. 1996;34:15–20. DOI: 10.1016/0278-6915(95)00085-2.
51. Khanduja KL, Avti PK, Kumar S, Mittal N, Sohi KK, Pathak CM. Anti-apoptotic activity of caffeic acid, ellagic acid and ferulic acid in normal human peripheral blood mononucleararcells: a Bcl-2 independent mechanism. Biochimica et Biophysica Acta (BBA) – General Subjects. 2006;1760(2):283–289. DOI: 10.1016/j.bbagen.2005.12.017.
52. Li T, Chen G, Su C, Lin J-G, Yen C, Cheng K., Chung J. Ellagic acid induced p53/p21 expression, G1 arrestand apoptosis in human bladder cancer T24 cells. Anticancer Research. 2005;25(2A):971–979.
53. Abuelsaad AS, Mohamed I, Allam G, Al-solumani AA. Antimicrobial andimmunomodulating activities of hesperidin and ellagic acid against diarrheic Aeromonas hydrophila in a murine model. Life Science. 2013;93(20):714–722. DOI: 10.1016/j.lfs.2013.09.019.
54. Park SW, Kwon MJ, Yoo JY, Choi HJ, Ahn YJ. Antiviral activity and possible mode ofaction of ellagic acid identified in Lagerstroemia speciosa leaves toward human rhinoviruses. BMC Complementary and Alternative Medicine. 2014;14:171. DOI: 10.1186/1472-6882-14-171.
55. Soh PN, Witkowski B, Olagnier D, Nicolau M-L, Garcia-Alvares M-C, Benoit-Vical F. In vitro and in vivo properties ofellagic acid in malaria treatment. Antimicrobial Agents and Chemotherapy. 2009;53(3):1100–1106. DOI: 10.1128/AAC.01175-08.
56. Choi YH, Yan GH. Ellagic Acid attenuates immunoglobulin E-mediated allergic response in mast cells. Biological and Pharmaceutical Bulletin. 2009;32(6):1118–1121. DOI: 10.1248/bpb.32.1118.
57. Promsong A. Wo C, Satthakarn S, Nittayananta W. Ellagic acid modulates the expression of oral innate immune mediators: potential role in mucosal protection. Journal of Oral Pathology Medicine. 2014;44(3):214–221. DOI: 10.1111/jop.12223.
58. Kuo M-Y, Ou H-C, Lee W-J, Kuo W-W, Hwang L-L, Song T-Y, Huang C-Y, Chiu T-H, Tsai K-L, Tsai C-S, Sheu WH-H. Ellagic acid inhibits oxidized low-density lipoprotein (OxLDL)-induced metalloproteinase (MMP) expression by modulating the protein kinase C-α/extracellular signal-regulated kinase/peroxisome proliferator-activated receptor γ/ nuclear factor-κB (PKC-α/ERK/ PPAR-γ/NF-κB) Signaling Pathway in Endothelial Cells. Journal of Agricultural and Food Chemistry. 2011;59(9):5100–5108. DOI: 10.1021/jf1041867.
59. Pinto M da S, de Carvalho JE, Lajolo FM, Genovece MI, Shetty K. Evaluation of antiproliferative, anti-type 2 diabetes, and antihypertension potentials of ellagitannins from strawberries (Fragaria × ananassa Duch.) using in vitro models. Journal of Medicinal Food. 2010;13(5):1027–1035. DOI: 10.1089/jmf.2009.0257.
60. Dhingra D, Jangra A. Antiepileptic activity of ellagic acid, a naturally occurring polyphenolic compound, in mice. Journal of Functional Foods. 2014;10:364–369. DOI: 10.1016/j.jff.2014.07.011.
61. Dhingra D, Chhillar R. Antidepressant-like activity of ellagic acid in unstressed and acute immobilization-induced stressed mice. Pharmacological Reports. 2012;64(4):796–807. DOI: 10.1016/S1734-1140(12)70875-7.
62. Girish C, Raj V, Arya J, Balakrishnan S. Involvement of the GABAergic sys-tem in the anxiolytic-like effect of the flavonoid ellagic acid in mice. European Journal of Pharmacology. 2013;710(1–3):49–58. DOI: 10.1016/j.ejphar.2013.04.003.
63. Kwak H, Jeon S, Sohng B, Kim J, Lee J, Lee K, Jeong H, Hur J, Kang Y, Song K. β-Secretase (BACE1) inhibitors from pomegranate (Punica granatum) husk. Archives of Pharmacal Research. 2005;28(12):1328–1332. DOI: 10.1007/BF02977896.
64. El-Garhy AM, Abd El-Raouf OM, El-Sayeh BM, Fawzy HM, Abdallah DM. Ellagic acid antiinflammatory and antiapoptotic potential mediate renoprotection in cisplatin nephrotoxic rats. Journal of Biochemical and Molecular Toxicology. 2014;28(10):472–479. DOI: 10.1002/jbt.21587.
65. Kannan MM, Quine SD. Ellagic acid inhibits cardiac arrhythmias, hypertrophy and hyperlipidaemia during myocardial infarction in rats. Metabolism. 2013;62(1):52–61. DOI: 10.1016/j.metabol.2012.06.003.
66. Lee JH, Won JH, Choi JM, Cha HH, Jang YJ, Park S, Kim HG, Kim HC, Kim DK. Protective effect of ellagic acid on concanavalin A-induced hepatitis via Toll-like receptor and mitogen-activated protein kinase/nuclear factor κB signaling pathways. Journal of Agricultural and Food Chemistry. 2014;62(41):10110–10117. DOI: 10.1021/jf503188c.

AUTHORS

Anastasia A. Orlova – postgraduate student of the Department of Pharmacognosy, Junior Researcher of the Research Department of the Saint Petersburg State Chemical Pharmaceutical University. ORCID 0000-0002-7836-5785. E-mail: anastasiya.lebedkova@spcpu.ru

Maria N. Povydysh – Doctor of Sciences (Biology), Candidate of Sciences (Pharmacy), Associate Professor of the Department of Pharmacognosy, Head of the Research Department of the St. Petersburg State Chemical-Pharmaceutical University. ORCID 0000-0002-7768-9059. E-mail: maria.povydysh@pharminnotech.com


1943
MENTE ET ANIMO
ПМФИ
Филиал ВолгГМУ