GEORGIAN MEDICAL NEWS

ISSN 1512-0112

NO 6 (351) Июнь 2024

ТБИЛИСИ - NEW YORK

ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ

Медицинские новости Грузии

საქართველოს სამედიცინო სიახლენი

GEORGIAN MEDICAL NEWS

Monthly Georgia-US joint scientific journal published both in electronic and paper formats of the Agency of Medical Information of the Georgian Association of Business Press. Published since 1994. Distributed in NIS, EU and USA.

GMN: Georgian Medical News is peer-reviewed, published monthly journal committed to promoting the science and art of medicine and the betterment of public health, published by the GMN Editorial Board since 1994. GMN carries original scientific articles on medicine, biology and pharmacy, which are of experimental, theoretical and practical character; publishes original research, reviews, commentaries, editorials, essays, medical news, and correspondence in English and Russian.

GMN is indexed in MEDLINE, SCOPUS, PubMed and VINITI Russian Academy of Sciences. The full text content is available through EBSCO databases.

GMN: Медицинские новости Грузии - ежемесячный рецензируемый научный журнал, издаётся Редакционной коллегией с 1994 года на русском и английском языках в целях поддержки медицинской науки и улучшения здравоохранения. В журнале публикуются оригинальные научные статьи в области медицины, биологии и фармации, статьи обзорного характера, научные сообщения, новости медицины и здравоохранения. Журнал индексируется в MEDLINE, отражён в базе данных SCOPUS, PubMed и ВИНИТИ РАН. Полнотекстовые статьи журнала доступны через БД EBSCO.

GMN: Georgian Medical News – საქართველოს სამედიცინო სიახლენი – არის ყოველთვიური სამეცნიერო სამედიცინო რეცენზირებადი ჟურნალი, გამოიცემა 1994 წლიდან, წარმოადგენს სარედაქციო კოლეგიისა და აშშ-ის მეცნიერების, განათლების, ინდუსტრიის, ხელოვნებისა და ბუნებისმეტყველების საერთაშორისო აკადემიის ერთობლივ გამოცემას. GMN-ში რუსულ და ინგლისურ ენებზე ქვეყნდება ექსპერიმენტული, თეორიული და პრაქტიკული ხასიათის ორიგინალური სამეცნიერო სტატიები მედიცინის, ბიოლოგიისა და ფარმაციის სფეროში, მიმოხილვითი ხასიათის სტატიები.

ჟურნალი ინდექსირებულია MEDLINE-ის საერთაშორისო სისტემაში, ასახულია SCOPUS-ის, PubMed-ის და ВИНИТИ РАН-ის მონაცემთა ბაზებში. სტატიების სრული ტექსტი ხელმისაწვდომია EBSCO-ს მონაცემთა ბაზებიდან.

WEBSITE

www.geomednews.com

К СВЕДЕНИЮ АВТОРОВ!

При направлении статьи в редакцию необходимо соблюдать следующие правила:

1. Статья должна быть представлена в двух экземплярах, на русском или английском языках, напечатанная через **полтора интервала на одной стороне стандартного листа с шириной левого поля в три сантиметра**. Используемый компьютерный шрифт для текста на русском и английском языках - **Times New Roman** (**Кириллица**), для текста на грузинском языке следует использовать **AcadNusx.** Размер шрифта - **12**. К рукописи, напечатанной на компьютере, должен быть приложен CD со статьей.

2. Размер статьи должен быть не менее десяти и не более двадцати страниц машинописи, включая указатель литературы и резюме на английском, русском и грузинском языках.

3. В статье должны быть освещены актуальность данного материала, методы и результаты исследования и их обсуждение.

При представлении в печать научных экспериментальных работ авторы должны указывать вид и количество экспериментальных животных, применявшиеся методы обезболивания и усыпления (в ходе острых опытов).

4. К статье должны быть приложены краткое (на полстраницы) резюме на английском, русском и грузинском языках (включающее следующие разделы: цель исследования, материал и методы, результаты и заключение) и список ключевых слов (key words).

5. Таблицы необходимо представлять в печатной форме. Фотокопии не принимаются. **Все цифровые, итоговые и процентные данные в таблицах должны соответствовать таковым в тексте статьи**. Таблицы и графики должны быть озаглавлены.

6. Фотографии должны быть контрастными, фотокопии с рентгенограмм - в позитивном изображении. Рисунки, чертежи и диаграммы следует озаглавить, пронумеровать и вставить в соответствующее место текста **в tiff формате**.

В подписях к микрофотографиям следует указывать степень увеличения через окуляр или объектив и метод окраски или импрегнации срезов.

7. Фамилии отечественных авторов приводятся в оригинальной транскрипции.

8. При оформлении и направлении статей в журнал МНГ просим авторов соблюдать правила, изложенные в «Единых требованиях к рукописям, представляемым в биомедицинские журналы», принятых Международным комитетом редакторов медицинских журналов - http://www.spinesurgery.ru/files/publish.pdf и http://www.nlm.nih.gov/bsd/uniform_requirements.html
В конце каждой оригинальной статьи приводится библиографический список. В список литературы включаются все материалы, на которые имеются ссылки в тексте. Список составляется в алфавитном порядке и нумеруется. Литературный источник приводится на языке оригинала. В списке литературы сначала приводятся работы, написанные знаками грузинского алфавита, затем кириллицей и латиницей. Ссылки на цитируемые работы в тексте статьи даются в квадратных скобках в виде номера, соответствующего номеру данной работы в списке литературы. Большинство цитированных источников должны быть за последние 5-7 лет.

9. Для получения права на публикацию статья должна иметь от руководителя работы или учреждения визу и сопроводительное отношение, написанные или напечатанные на бланке и заверенные подписью и печатью.

10. В конце статьи должны быть подписи всех авторов, полностью приведены их фамилии, имена и отчества, указаны служебный и домашний номера телефонов и адреса или иные координаты. Количество авторов (соавторов) не должно превышать пяти человек.

11. Редакция оставляет за собой право сокращать и исправлять статьи. Корректура авторам не высылается, вся работа и сверка проводится по авторскому оригиналу.

12. Недопустимо направление в редакцию работ, представленных к печати в иных издательствах или опубликованных в других изданиях.

При нарушении указанных правил статьи не рассматриваются.

REQUIREMENTS

Please note, materials submitted to the Editorial Office Staff are supposed to meet the following requirements:

1. Articles must be provided with a double copy, in English or Russian languages and typed or compu-ter-printed on a single side of standard typing paper, with the left margin of **3** centimeters width, and **1.5** spacing between the lines, typeface - **Times New Roman** (**Cyrillic**), print size - **12** (referring to Georgian and Russian materials). With computer-printed texts please enclose a CD carrying the same file titled with Latin symbols.

2. Size of the article, including index and resume in English, Russian and Georgian languages must be at least 10 pages and not exceed the limit of 20 pages of typed or computer-printed text.

3. Submitted material must include a coverage of a topical subject, research methods, results, and review.

Authors of the scientific-research works must indicate the number of experimental biological species drawn in, list the employed methods of anesthetization and soporific means used during acute tests.

4. Articles must have a short (half page) abstract in English, Russian and Georgian (including the following sections: aim of study, material and methods, results and conclusions) and a list of key words.

5. Tables must be presented in an original typed or computer-printed form, instead of a photocopied version. **Numbers, totals, percentile data on the tables must coincide with those in the texts of the articles.** Tables and graphs must be headed.

6. Photographs are required to be contrasted and must be submitted with doubles. Please number each photograph with a pencil on its back, indicate author's name, title of the article (short version), and mark out its top and bottom parts. Drawings must be accurate, drafts and diagrams drawn in Indian ink (or black ink). Photocopies of the X-ray photographs must be presented in a positive image in **tiff format**.

Accurately numbered subtitles for each illustration must be listed on a separate sheet of paper. In the subtitles for the microphotographs please indicate the ocular and objective lens magnification power, method of coloring or impregnation of the microscopic sections (preparations).

7. Please indicate last names, first and middle initials of the native authors, present names and initials of the foreign authors in the transcription of the original language, enclose in parenthesis corresponding number under which the author is listed in the reference materials.

8. Please follow guidance offered to authors by The International Committee of Medical Journal Editors guidance in its Uniform Requirements for Manuscripts Submitted to Biomedical Journals publication available online at: http://www.nlm.nih.gov/bsd/uniform_requirements.html
http://www.icmje.org/urm_full.pdf
In GMN style for each work cited in the text, a bibliographic reference is given, and this is located at the end of the article under the title "References". All references cited in the text must be listed. The list of references should be arranged alphabetically and then numbered. References are numbered in the text [numbers in square brackets] and in the reference list and numbers are repeated throughout the text as needed. The bibliographic description is given in the language of publication (citations in Georgian script are followed by Cyrillic and Latin).

9. To obtain the rights of publication articles must be accompanied by a visa from the project instructor or the establishment, where the work has been performed, and a reference letter, both written or typed on a special signed form, certified by a stamp or a seal.

10. Articles must be signed by all of the authors at the end, and they must be provided with a list of full names, office and home phone numbers and addresses or other non-office locations where the authors could be reached. The number of the authors (co-authors) must not exceed the limit of 5 people.

11. Editorial Staff reserves the rights to cut down in size and correct the articles. Proof-sheets are not sent out to the authors. The entire editorial and collation work is performed according to the author's original text.

12. Sending in the works that have already been assigned to the press by other Editorial Staffs or have been printed by other publishers is not permissible.

Articles that Fail to Meet the Aforementioned Requirements are not Assigned to be Reviewed.

ავტორთა საყურადღებოდ!

რედაქციაში სტატიის წარმოდგენისას საჭიროა დავიცვათ შემდეგი წესები:

1. სტატია უნდა წარმოადგინოთ 2 ცალად, რუსულ ან ინგლისურ ენებზე,დაბეჭდილი სტანდარტული ფურცლის 1 გვერდზე, 3 სმ სიგანის მარცხენა ველისა და სტრიქონებს შორის 1,5 ინტერვალის დაცვით. გამოყენებული კომპიუტერული შრიფტი რუსულ და ინგლისურენოვან ტექსტებში - **Times New Roman (Кириллица)**, ხოლო ქართულენოვან ტექსტში საჭიროა გამოვიყენოთ **AcadNusx.** შრიფტის ზომა – 12. სტატიას თან უნდა ახლდეს CD სტატიით.

2. სტატიის მოცულობა არ უნდა შეადგენდეს 10 გვერდზე ნაკლებს და 20 გვერდზე მეტს ლიტერატურის სიის და რეზიუმეების (ინგლისურ, რუსულ და ქართულ ენებზე) ჩათვლით.

3. სტატიაში საჭიროა გაშუქდეს: საკითხის აქტუალობა; კვლევის მიზანი; საკვლევი მასალა და გამოყენებული მეთოდები; მიღებული შედეგები და მათი განსჯა. ექსპერიმენტული ხასიათის სტატიების წარმოდგენისას ავტორებმა უნდა მიუთითონ საექსპერიმენტო ცხოველების სახეობა და რაოდენობა; გაუტკივარებისა და დაძინების მეთოდები (მწვავე ცდების პირობებში).

4. სტატიას თან უნდა ახლდეს რეზიუმე ინგლისურ, რუსულ და ქართულ ენებზე არანაკლებ ნახევარი გვერდის მოცულობისა (სათაურის, ავტორების, დაწესებულების მითითებით და უნდა შეიცავდეს შემდეგ განყოფილებებს: მიზანი, მასალა და მეთოდები, შედეგები და დასკვნები; ტექსტუალური ნაწილი არ უნდა იყოს 15 სტრიქონზე ნაკლები) და საკვანძო სიტყვების ჩამონათვალი (key words).

5. ცხრილები საჭიროა წარმოადგინოთ ნაბეჭდი სახით. ყველა ციფრული, შემაჯამებელი და პროცენტული მონაცემები უნდა შეესაბამებოდეს ტექსტში მოყვანილს.

6. ფოტოსურათები უნდა იყოს კონტრასტული; სურათები, ნახაზები, დიაგრამები - დასათაურებული, დანომრილი და სათანადო ადგილას ჩასმული. რენტგენოგრამების ფოტოასლები წარმოადგინეთ პოზიტიური გამოსახულებით **tiff** ფორმატში. მიკროფოტოსურათების წარწერებში საჭიროა მიუთითოთ ოკულარის ან ობიექტივის საშუალებით გადიდების ხარისხი, ანათალების შეღებვის ან იმპრეგნაციის მეთოდი და აღნიშნოთ სურათის ზედა და ქვედა ნაწილები.

7. სამამულო ავტორების გვარები სტატიაში აღინიშნება ინიციალების თანდართვით, უცხოურისა – უცხოური ტრანსკრიპციით.

8. სტატიას თან უნდა ახლდეს ავტორის მიერ გამოყენებული სამამულო და უცხოური შრომების ბიბლიოგრაფიული სია (ბოლო 5-8 წლის სიღრმით). ანბანური წყობით წარმოდგენილ ბიბლიოგრაფიულ სიაში მიუთითეთ ჯერ სამამულო, შემდეგ უცხოელი ავტორები (გვარი, ინიციალები, სტატიის სათაური, ჟურნალის დასახელება, გამოცემის ადგილი, წელი, ჟურნალის №, პირველი და ბოლო გვერდები). მონოგრაფიის შემთხვევაში მიუთითეთ გამოცემის წელი, ადგილი და გვერდების საერთო რაოდენობა. ტექსტში კვადრატულ ფჩხილებში უნდა მიუთითოთ ავტორის შესაბამისი N ლიტერატურის სიის მიხედვით. მიზანშეწონილია, რომ ციტირებული წყაროების უმეტესი ნაწილი იყოს 5-6 წლის სიღრმის.

9. სტატიას თან უნდა ახლდეს: ა) დაწესებულების ან სამეცნიერო ხელმძღვანელის წარდგინება, დამოწმებული ხელმოწერითა და ბეჭდით; ბ) დარგის სპეციალისტის დამოწმებული რეცენზია, რომელშიც მითითებული იქნება საკითხის აქტუალობა, მასალის საკმაობა, მეთოდის სანდოობა, შედეგების სამეცნიერო-პრაქტიკული მნიშვნელობა.

10. სტატიის ბოლოს საჭიროა ყველა ავტორის ხელმოწერა, რომელთა რაოდენობა არ უნდა აღემატებოდეს 5-ს.

11. რედაქცია იტოვებს უფლებას შეასწოროს სტატია. ტექსტზე მუშაობა და შეჯერება ხდება საავტორო ორიგინალის მიხედვით.

12. დაუშვებელია რედაქციაში ისეთი სტატიის წარდგენა, რომელიც დასაბეჭდად წარდგენილი იყო სხვა რედაქციაში ან გამოქვეყნებული იყო სხვა გამოცემებში.

აღნიშნული წესების დარღვევის შემთხვევაში სტატიები არ განიხილება.

GEORGIAN MEDICAL NEWS
No 6 (351) 2024

Содержание:

GEORGIAN MEDICAL NEWS
No 6 (351) 2024

PROTECTIVE EFFECT OF A NEW SUPEROXIDE-PRODUCING ENZYME COMPLEX FROM RASPBERRY IN RATS WITH THIRD-DEGREE THERMAL BURNS

Karapetyan A.G[1], Danielyan M.H[1]*, Badalyan B.Yu[4], Simonyan K.V[1], Grigoryan V.S[1,2], Simonyan M.A[3], Dallakyan A.M[1], Simonyan G.M[3], Simonyan R.M[3].

[1]*Orbeli Institute of Physiology NAS RA, 0028, Yerevan, Armenia.*

[2]*University of Traditional Medicine, 0022, Yerevan, Armenia.*

[3]*Institute of Biochemistry after H. Buniatyan NAS RA, 0014, Yerevan, Armenia.*

[4]*Yerevan State Medical University after Mkhitar Heratsi, 0025, Yerevan, Armenia.*

Abstract.

Thermal burns are the most common type of burn injuries. Medical treatment for burns is crucial, especially for third-degree burns and when a significant surface area of the body is affected. One of the most pressing issues in modern medicine is the search for new effective means to accelerate the healing of burn wounds. Oxygen radicals play a significant role in maintaining homeostasis, forming the body's resistance to infection, and ensuring the regeneration of organs and tissues. In this study, a superoxide (O_2^-)-producing enzyme (SPE) from raspberries was applied (topically to the skin, injected under the wound surface, with solution concentrations of 12.75% and 5%) after a third-degree thermal burn to determine its reparative effects on the skin. To assess the condition of the animals that had suffered burn injuries and the healing process, blood parameters were analyzed, and cytogenetic indices of bone marrow from the femur of the animals were studied: mitotic index, number of polyploid cells, and chromosomal aberrations. When analyzing hematological, cytogenetic, and histological parameters, significant differences were found between the «clean burn» groups and the groups in which SPE was used in different concentrations and methods of application. The use of SPE in both concentrations contributed to a reduction in the area of burn wounds compared to a «clean burn». The survival rate of animals for 30 days (before the end of the experiment) was 100% when using a 12.75% SPE solution and 50% when using a 5% SPE solution. The use of SPE led to significant differences in hematological parameters from the «clean burn» group throughout the entire duration of the experiment, showing a tendency to normalize the parameters. Under the influence of the 12.75% SPE solution, there was a tendency toward normalization of the mitotic index, along with a significant reduction in the percentage of polyploid cells and chromosomal aberrations, which may indicate its beneficial effects. This study found that a 12.75% SPE solution derived from raspberries was more effective and had healing properties on third-degree thermal burns, promoting rapid healing of the burn wound.

Key words. Third-degree thermal burn, superoxide (O_2^-)-producing enzyme (SPE), raspberry, hematological parameters, polyploid cells, mitotic index.

Introduction.

According to the World Health Organization, burns rank fourth among all injuries. Each year, thermal burns cause 6.6 million injuries and 300.000 deaths worldwide. They are a significant cause of morbidity and mortality globally, leading to debilitating, lifelong injuries and having serious psychological and economic consequences [1,2]. Thermal burns are the most common type of burn injury, accounting for about 86% of burn patients requiring hospitalization in burn centers. Thermal burns are skin injuries caused by excessive heat, typically from contact with hot surfaces, hot liquids, steam, or flame [3]. They are classified by the depth of tissue damage. There are four classifications established by the American Burn Association. The most traumatic and difficult to heal are third-degree and, especially, fourth-degree burns. Third-degree burns penetrate the entire dermal layer and often damage subcutaneous tissue. Medical treatment for burns is crucial, especially when they are third-degree burns and when a significant surface area of the body is affected [4].

Extensive deep burns cause a complex of pathological functional and morphological changes in the internal organs and systems of the body. It is known that severe burn injuries lead to the loss of skin functions, plasma loss, and metabolic disturbances [5]. Burn injuries also cause profound changes in hematological parameters, including leukocytosis, hyperkalemia, hypoproteinemia, and increased hematocrit. As blood flows through tissues after a burn, thermal damage and erythrocyte destruction occur, resulting in the release of free hemoglobin into the plasma [6,7]. Burn disease also induces a pronounced leukocyte reaction, which has been described by many researchers in both clinical and experimental settings [8]. One of the most pressing issues in modern medicine is the search for new effective means to accelerate the healing of burn wounds. Such preparations should be highly effective and long-lasting, not cause significant side effects, be non-toxic, have no even short-term negative effects on the body, and come in a convenient form for oral administration or injection. Additionally, these preparations should retain their pharmacological properties for a long time [9]. Burn healing agents are used for their anti-inflammatory, antiseptic, analgesic, and regenerative properties [10,11].

In recent years, the perspective on free radical oxidation and the formation of reactive oxygen species (ROS) has significantly changed. ROS possess biological effects that, depending on their concentration, can be either regulatory or toxic. Despite their reactivity and potential toxicity, oxygen radicals in small concentrations are normal participants in many metabolic reactions within cells. Today, at least three main roles of ROS in the body are recognized. First, the formation of ROS is a natural physiological process that constantly occurs in the body. Second, ROS produced in increased quantities act as a damaging factor. Finally, ROS are considered a signaling system involved in key regulatory mechanisms of living cells.

As a multi-component system, ROS ensure the transmission of external signals to the cell nucleus, followed by the activation of translation and protein synthesis. Oxygen radicals play a crucial role in maintaining homeostasis, forming the body's resistance to infection, and ensuring the regeneration of organs and tissues [12]. This interest in studying the role of ROS and free radical oxidation in the mechanisms of action of therapeutic physical factors aims at correcting free radical processes and disrupted ROS-mediated metabolic pathways, as well as stimulating apoptosis and necrosis of pathologically altered tissues [13].

Superoxide radicals (O_2^-) are free radicals formed in cellular metabolic reactions either through auto-oxidation or the action of enzymes such as oxidases. In our bodies, the superoxide radical is a primary agent of the bactericidal action of phagocytes, but it can also be a harmful mediator of inflammation and cause damage to normal tissues [14]. The areas of application of superoxide radicals are very large, in particular, in wound healing, in experiment [15].

New thermostable enzymes have been isolated from biomembranes and biofluids of animal and plant origin, which continuously produce monocomponent superoxide radicals under aerobic conditions. The latter are stabilized and transferred by molecular oxygen [16]. Monocomponent superoxide radicals are important components of aerobic metabolic processes, stimulate the proliferation of bacteria in the gastrointestinal tract [17] and in empirical effective concentrations stimulate or suppress the proliferation of cells of various types [18].

The aim of the work is to find empirically determined effective concentrations of O2-producing thermostable complex – enzyme, isolated and purified from raspberries during use in treatment of thermal burns in the experiment, as an enzyme that continuously produces monocomponent O_2^-, to identify the effectiveness of the reparative effect on the skin. Since burn disease causes a pronounced leukocyte reaction [19], it was deemed appropriate to analyze blood parameters to assess the condition of animals that had suffered burn injuries and the healing process. To detect any changes between groups of animals with untreated burns and those treated with 12.75% and 5% SPE solutions and injections, the following blood parameters were examined: blood clotting time, leukocyte count, platelet count, erythrocyte count, hemoglobin level, and hematocrit. Additionally, cytogenetic indices of bone marrow from the femur of the animals were studied, including the mitotic index, the number of polyploid cells, and chromosomal aberrations. Skin layers at the burn site and with the injection of the preparation under the burn wound, stained with hematoxylin-eosin, were also examined.

Materials and Methods.

Before discovering the healing properties for thermal burns, it was necessary to isolate the SPE from raspberries, as well as to determine the continuity of production of monocomponent superoxides by SPE and their stationary concentration.

1. Isolation of superoxide (O_2^-)-producing enzyme (SPE) from raspberries:

SPE was isolated from raspberries using a licensed method [16,20]. In particular, after incubation of aqueous raspberry homogenate at pH 9.5 and precipitation of the SPE fraction, its ion-exchange chromatography on DE-52 cellulose (Whatman), heat treatment in boiled water, deionization and vacuum lyophilization, it was stored under anaerobic conditions at -10°C.

2. Determination of the continuity of production of monocomponent superoxides by SPE and their stationary concentration:

Only monocomponent superoxides (superoxides produced by SPE) reduce cytochrome C (from mitochondria of the bovine heart), without of it denaturing. However, non-monocomponent superoxides (formed during the cleavage of peroxides) do not reduce cytochrome C but denature it [21]. This feature is used to determine the continuity of production of monocomponent superoxides by the SPE method. To determine the stationary concentration of monocomponent produced superoxides, it is necessary to divide the absorption density of the alpha band of the introduced cytochrome C (at 550 nm) by the molar absorption of cytochrome C (30000 $M^{-1}cm^{-1}$).

To obtain SPE, this study, cellulose DE-52 (Whatman, England), Sephadex G-200 (Pharmacia, Sweden), adrenaline (Sigma, USA), cytochrome C (electrophoretically pure protein obtained from bovine heart), the spectrophotometer Cary 60 (USA), the spectrofluorimeter Cary Eclipse (USA), and centrifuge K-70D (Germany) were used.

Before the experiments, the SPE powder obtained as a result of lyophilization was dissolved in physiological solution and solutions of appropriate concentrations (12.75% and 5%) were prepared.

Male albino rats (Sprague-Dawley) weighing 175-180 g were used as experimental animals. A thermal third-degree burn covering 30% of the body surface was applied to the epilated skin on the back of the animals with a hot object. Then the damaged skin surface was covered with a solution of SPE, or a subcutaneous injection was given.

The animals were divided into five groups (6 rats in each group):

1. Group I: Thermal burn + SPE (12.75% solution, topical application).
2. Group II: Thermal burn + SPE (12.75% solution, subcutaneous injection).
3. Group III: Thermal burn + SPE (5% solution, topical application).
4. Group IV: Thermal burn only («clean burn», control group).
5. Group V: Norm (intact animals).

In group I of animals, a 12.75% SPE solution was applied to the wound surface 1 hour after the burn and then once a day for the next 6 days. Animals of group II were given an injection of 0.5 ml of a 12.75% SPE solution under the wound surface once a day 1 hour after the burn, and then for the next 6 days. In group III, a 5% SPE solution was applied to the wound surface 1 hour after the burn and then once a day for the next 6 days.

The activity of these compounds was evaluated based on survival, showing the dynamics of mortality in the test rats over a 30-day monitoring period. Visual monitoring of the burn wound was also conducted, with observations continuing until complete healing and hair regrowth on the healed surface. Hematological parameters were also studied. For

hematological analyses, blood was drawn from the tail vein at specific intervals (on days 3, 7, 14, 21, and 30). The following parameters were determined: blood clotting time (BCT); the number of leukocytes, erythrocytes, and platelets (using the classical method with a Goryaev chamber); hemoglobin levels, and hematocrit using standard laboratory equipment [22]. The material for cytogenetic studies was the bone marrow from the femur of the animals. The cytogenetic study included the analysis of chromosomes stained with Giemsa dye. Cytogenetic parameters were studied according to the Ford-Wollam method [23], determining the mitotic index (MI) in %, chromosomal aberrations (CA) in %, and polyploid cells (PC) in % in bone marrow (counting 1000 cells in each preparation), according to G. McGregor [24]. Metaphase searches were conducted under a microscope with magnifications of 900 times.

Additionally, microscopic analysis of the skin was performed. The study material was obtained from animals under anesthesia (Pentobarbital, 40 mg/kg, intraperitoneal). Samples were taken from the epilated skin surface on the back of intact animals and the affected area of experimental animals, fixed in a 10% neutral formalin solution for 72 hours, and then processed for paraffin embedding. Sections 5 µm thick were cut using a microtome and stained with hematoxylin-eosin [25]. The prepared sections were analyzed under a light microscope. The count of cells in the basal layer of the skin was conducted in a 1 cm² field of view at a magnification of 320x. Subsequent imaging of the prepared sections was carried out using a digital camera (Amscope MU500 5 MP, USA) with an OPTON microscope (West Germany).

Statistical analysis of the obtained data was conducted using various computer programs designed for statistical processing of numerical data arrays. Specialized statistical packages Statsoft-7, SPSS-10.0, MedCalc, and StatGraphics Plus were used. Correlation, regression, and multivariate regression analysis methods were applied [26]. Multivariate regression analysis is a method that allows building a model with a large number of factors and determining the influence of each individually as well as their combined effect on the modeled index [27]. $p<0.05$ is typically considered to be statistically significant.

Results and Discussion.

Results of the effect of SPE from raspberry on burn wound area.

When using the SPE solution, throughout the observation period, the burn surface remained dry, without signs of pus formation. Over the 30-day monitoring period, there was a noticeable trend in wound healing influenced by SPE, with a reduction in the wound area compared to the control group (group IV) (Table 1). The results show that the use of SPE in both concentrations contributed to a reduction in burn wound area compared to the control group.

According to the obtained exponential curve equations, the best result for wound healing of the skin surface was recorded in group II (with subcutaneous injection of 12.75% SPE solution) (Figure 1).

Survival over 30 days (until the end of the experiment) was 100% in groups I and II, 80% in group III and 60% in group IV, which confirms the correct choice of solution concentration and the effectiveness of the 12.75% SPE. This is supported by the fastest healing rates observed in group II.

Results of the hematological study.

As seen from the hematological indices (Table 2 and Figure 2), the application of SPE results in a significant difference from the «clean burn» group throughout the experiment and a tendency towards normalization of the BCT. There is also a significant increase in the level of leukocytes (leukocytosis) relative to the «clean burn» group. As for platelets, all three groups show a significant decrease compared to the "«clean burn» group and normalization. The level of erythrocytes throughout the experiment in groups II and III is significantly different from the «clean burn» group (indicating a beneficial effect). Both with a clean burn and with the use of SPE during the entire analysis period, there is unreliable variability in hemoglobin and hematocrit between all groups of experimental animals, which is reflected in Table 2. This indicates that this drug does not significantly affect these red blood parameters. Literature sources note that with the restoration of the skin, the levels of hemoglobin, erythrocytes, and leukocytes normalize [28]. It should be noted that because of SPE from raspberry, the healing of the skin occurred faster.

In the animals, a significant correlational relationship was found between hematocrit, hemoglobin, and erythrocytes ($r=0.89–0.93$), indicating a restoration of hemoconcentration by the 30th day of the experiment. Using multiple regression analysis, we determined the interdependence of these three indices (Figure 3).

Simultaneously, even in the early period, there is significant activation of bone marrow hematopoiesis, which somewhat compensates for the loss of erythrocytes. Alongside this, leukocytosis is observed, which characterizes not only the stage of burn shock but also the subsequent acute period—toxemia. Initially, leukocytosis represents, to some extent, blood thickening, while later, during the toxemia stage, it develops due to the intense tissue breakdown and purulent process on the burned skin. In groups I and III, an increase in leukocyte levels was observed not only compared to the burned-only group but also compared to the normal intact group. Only in group II did the leukocyte count reach 16,900 ± 1,700 N/µL by day

Table 1. *Dynamics of wound surface area (in cm²).*

Groups \ Timelines (in days)	1	3	7	14	21	30
I	49.5	45.2	43.4	38.5	30.5	26.6
II	50.2	49.9	42.5	34	28.6	24.8
III	50.2	46.8	40	37.2	28.8	25.2
IV	50.8	50.1	44.8	39.2	30.7	27.2

Table 2. *Dynamics of changes in blood parameters (*p<0.05).*

Timelines (in days) / **Group Indices**		3	7	14	21	30
Blood Coagulation Time (seconds) **Normal Range** 311.0±19.0	**IVgr**	264.0 ±12.88	215.0 ±14.32	225.6 ±13.09	277.8 ±15.77	330.0 ±18.44
	Igr	387.5 ±22.05*	278.7 ±34.23	280.8 ±25.71*	290.7 ±14.93	251.7 ±33.38*
	IIgr	475.0±5.0*	175.0±10.0*	138.0±12.0*	156.5±6.5*	246.5±16.5*
	IIIgr	388.0±2.0*	327.5±47.5*	320.0±30.8*	210.0±7.2*	243.0±11.1*
Leukocytes (N/μL) **Normal Range** 11500.0 ±420	**IVgr**	14720.0 ±810.0	16920.0 ±618.0	11320.0 ±890.0	13640 ±670.0	15960.0 ±460.0
	Igr	15100.0 ±1688.19	21366.7 ±1416.49*	20066.7 ±2115.76*	22433.3 ±940.09*	28600.0 ±2552.38*
	IIgr	11800.0 ±800.0*	15500.0 ±5500.0	11500.0 ±1500.0	16900.0 ±1700.0	19100.0 ±4700.0
	IIIgr	15300.0 ±100.0	17900.0 ±100.0	13200.0 ±107.0*	21600.0 ±102.0*	24000.0 ±3100.0*
Platelets (N/μL) **Normal Range** 522000,0 ±10560.0	**IVgr**	627000.0 ±10793.52	681400.0 ±62572.38	609000.0 ±52115.26	590000 ±37524.98	571000 ±22934.69
	Igr	375000.0 ±49006.8*	346666.7 ±28509.26*	442500.0 ±24212.6*	395000.0 ±59651.77*	445883.3 ±30507.29*
	IIgr	327500.0 ±2500.0*	500000.0 ±35000.0*	410000.0 ±11000.0*	512500.0 ±12500.0*	330000.0 ±45000.0*
	IIIgr	382500.0 ±32500.0*	412500.0 ±27500.0*	440000.0 ±17600.0*	425000.0 ±15500.0*	395000 ±40000*
Red Blood Cells (N/μL) **Normal Range** 5823000.0 ±278800.0	**IVgr**	5920000 ±130000	3130000 ±1000000	3560000 ±180000	6470000 ±104000	6380000 ±190000
	Igr	5961666.7 ±413790.7	5814833.3 ±421322.5	5598333.3 ±390558.72*	6275000.0 ±212818.39	5850000.0 ±221163.59
	IIgr	5825000.0 ±825000.0	5425000.0 ±25000.0*	5125000.0 ±825000.0*	4500000.0 ±100000.0*	5200000 ±200000*
	IIIgr	6075000.0 ±25000.0	5715000.0 ±685000.0*	5200000.0 ±50000.0*	6800000 ±100000.0*	4450000 ±327000 *
Hemoglobin (g/L) **Normal Range** 138.1±5.82	**IVgr**	135.7±3.81	135.9±5.4	144.4±6.02	144.6±5.43	143.6±5.28
	Igr	140.6±4.1	128.5±3.74	130.0±6.45	138.2±5.89	140.1±5.3
	IIgr	135.2±3.0	129.9±0.7	130.3±0.9*	138.1±4.1	140.9±9.15
	IIIgr	137.4±14.0	134.3±5.9	136.0±6.8	140.0±7.9	145.6±9.5
Hematocrit (%) **Normal Range** 57.2±1.75	**IVgr**	46.7±1.4	49.1±0.91	47.3±1.11	48.3±1.18	44.5±1.71
	Igr	45.2±2.6	43.4±1.96*	42.0±1.5*	43.3±2.62	44.2±2.01
	IIgr	42.6±5.75	39.5±1.3*	42.1±0.1*	45.4±2.85	46.7±3.3
	IIIgr	46.9±2.15	40.8±0.8*	42.3±1.1*	45.6±2.1	48.3±3.4

Table 3. *Changes in cytogenetic parameters in groups I and II relative to groups IV and V, *p<0.05.*

Indices	**Norm (V gr)**	**«Clean burn» (IV gr)**	**Application to skin (I gr 12.75%)**	**Injection under the wound (II gr 12.75%)**	**Application to skin (III gr 5%)**
Mitotic Index (%)	20.35±2.8	9.8±0.96	17.8±0.6*	17.35±0.45*	18.0±1.7*
Polyploid cells (%)	0.5±0.08	3.7±0.1*	2.0±0.01*	1.3±0.3*	2.2±0.21*
Chromosomal aberrations (%)	2.6±0.26	6.8±0.74	3.7±0.1*	3.5±0.1*	3.6±0.3*

Table 4. *Number of epidermal layer cells in the microscopic field of view for third-degree burns and burns with injection of SPE solution under the wound on the 30th day post-burn. Counting under magnification: ×320.*

Number of cells		
Norm	**«Clean burn»**	**Injection SPE under the burn wound**
415.0±4.9	328.0±38.5	397.0±46.0

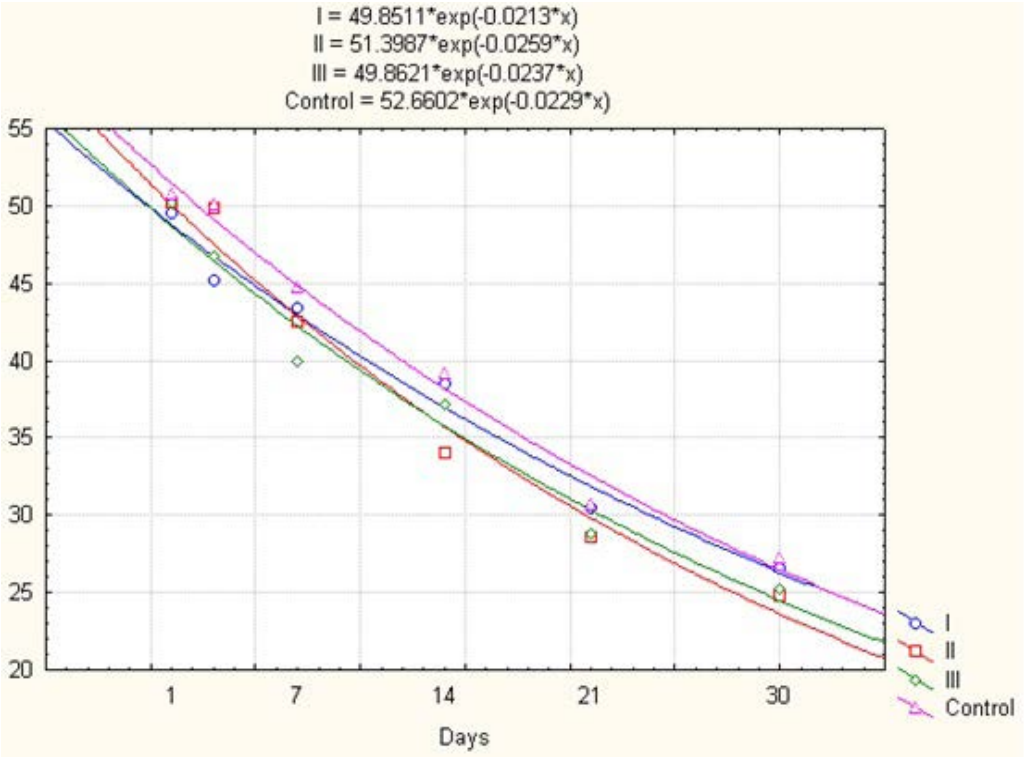

Figure 1. *Dynamics of decrease in the area of damaged skin surface in 4 groups (I, II, III and Control (IV) groups). x-axis: number of days elapsed since the start of the experiments; y-axis: area of the damaged skin surface.*

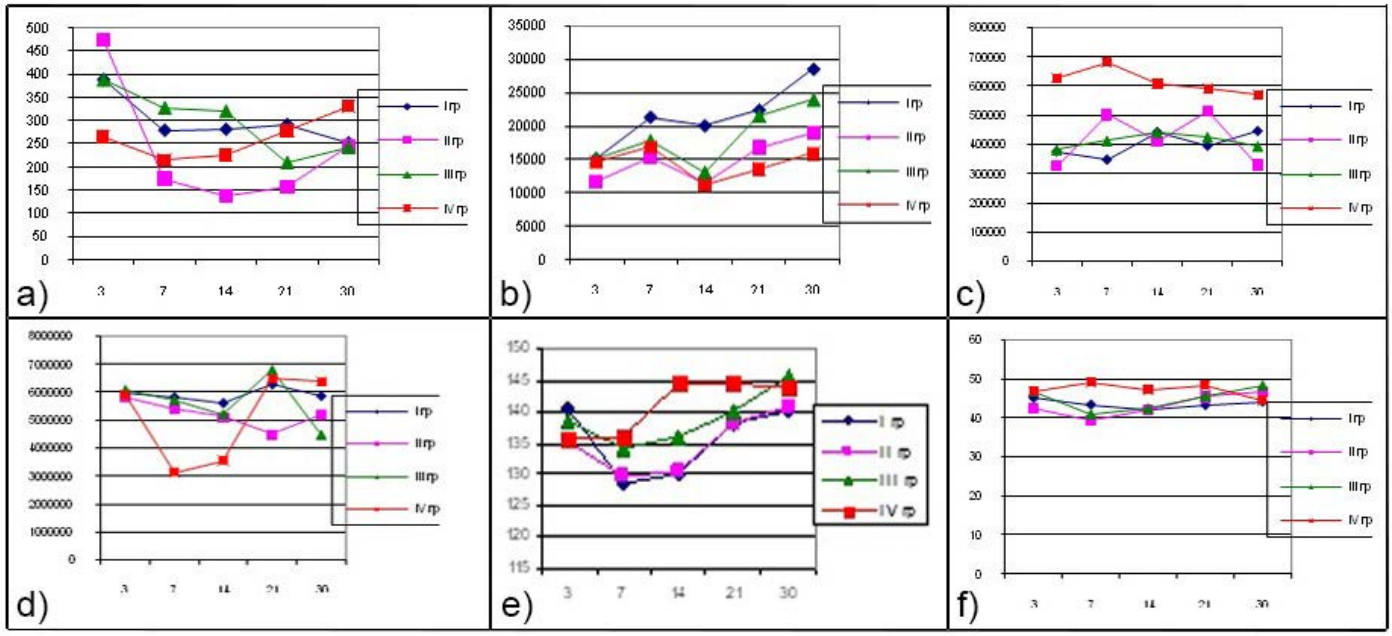

Figure 2. *Dynamics of changes in blood indices under the influence of SPE: a) Blood coagulation time (BCT); b) Leukocytes; c) Platelets; d) Erythrocytes; e) Hemoglobin; f) Hematocrit.*

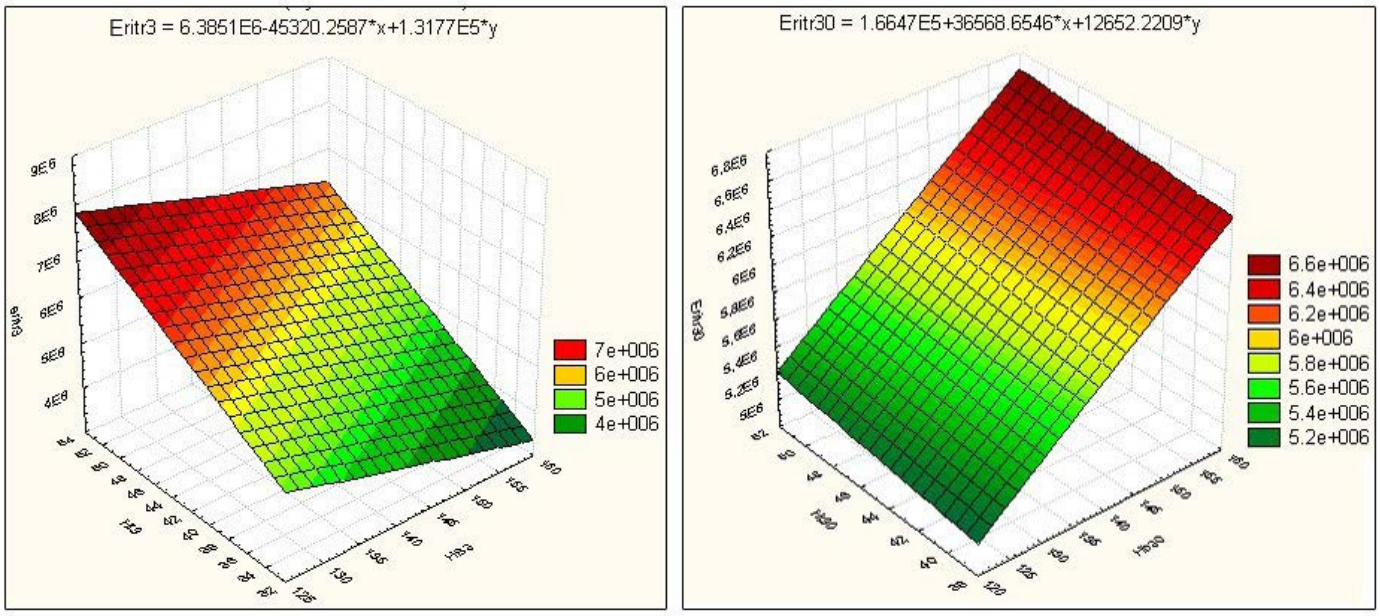

Figure 3. *Multiple regression relationship of indices characterizing the degree of hemoconcentration on the 3rd and 30th days after skin damage due to burns with the application of SPE. The corresponding equations are z=6.385E6-45320.25*x+1.3177E5*y and z= 1,6647E5+36568.65*x+ 12652.22*y, where x- he hemoglobin level, y- the hematocrit level, and z - the erythrocyte level.*

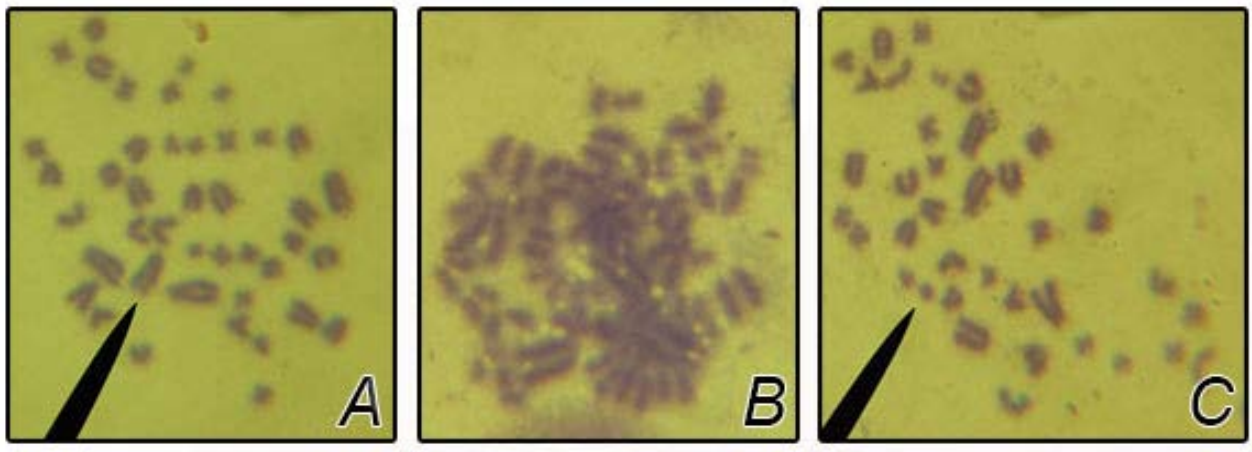

Figure 4. *Bone marrow cells. Cytogenetic abnormalities: a) Deletion, b) Polyploid cell, c) Double fragment. Magnification: ×900.*

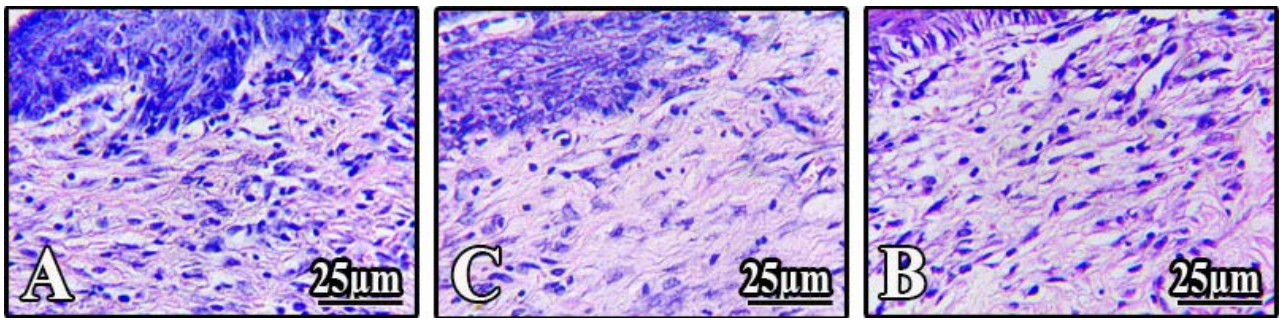

Figure 5. *Histology (hematoxylin-eosin staining) of the skin in intact animals (A), in a «clean burn» (B, and in a burn with injection of the SPE solution under the burn wound (C). Magnification: ×400.*

21, and 19,100 ± 4,700 N/μL by day 30, which is statistically significantly higher than normal values (11,500 ± 420 N/μL), likely due to different mechanisms of action of SPE (Table 2).

Results of the cytogenetic study.

Karyotype analysis showed that almost all cytogenetic parameters on the 30th day of the experiment in the experimental groups (groups IV («clean burn») and I, II (application of 12.75% SPE solution to the skin and when injecting it under the wound, respectively)) were statistically significantly different from the karyotype data of the intact animals (group V).

The results of the cytogenetic study on the 30th day of the experiment showed that animals with «clean burns», compared to intact animals (PC = 0.5±0.08; MI = 20.35±2.8), exhibited a significant increase in PC (3.7±0.1) and a decrease in MI (9.8±0.96). These changes are typically observed during inflammatory processes and are accompanied by suppression of bone marrow hematopoiesis [29].

Under the influence of SPE (groups I, II and III), there was a significant reduction in the percentage of PC (2.0±0.01; 1.3±0.3 and 2.2±0.21, respectively), which possibly indicates a beneficial effect of the treatment. There was also a trend towards normalization of MI (17.8±0.6; 17.35±0.45 and 18.0±1.7, respectively) under the influence of the SPE (Table 3). As for chromosomal aberrations, similar to PC, the percentage of chromosomal aberrations normalized under the SPE (3.7±0.1; 3.5±0.1 and 3.6±0.3, respectively) compared to «clean burn» (6.8±0.74). A reverse correlation was observed between MI and the number of PC and chromosomal aberrations. Chromosomal aberrations observed on the 30th day after a third-degree thermal burn were most commonly single and double fragments (Figure 4).

Thus, comparing the cytogenetic indices at the 30th day of the experiment, a statistically significant difference was observed between group IV («clean burn») and groups I, II and III (application of the 12.75% and 5% SPE solutions to the skin and injection the 12.75% SPE solution under the wound, respectively), indicating the beneficial effect of SPE at a concentration of 12.75% and 5%t (Table 3).

Results of the morphological study.

Macroscopic analysis of the skin after a third-degree thermal burn showed that that all layers of the skin were damaged and massive blisters with a thick shell filled with bloody contents formed at the burn site, then a wound covered with a scab formed. Morphometric analysis of the skin showed that on the 30th day after the burn in the epidermal layer of the skin, the density of granular and spinous cells becomes less than in intact animals (Figure 5). And when using a 12.75% solution of SPE solution, there is a tendency to increase the number of cells in epidermal layer of the skin compared to a «clean burn», although this did not reach statistical significance (Table 4).

Conclusion.

This study showed that raspberry-derived SPE has healing properties for thermal burns and promotes rapid healing of the burn wound. By evaluating hematological, cytogenetic, and histological parameters, significant differences were observed between the IV group («clean burn») and the I, II, and III groups, where SPE was applied in various concentrations and methods of application. The fastest healing rates were observed when using a 12.75% SPE solution subcutaneously, under the wound surface. In addition, the survival rate of animals was one hundred percent when using a 12.75% SPE solution, and when using a 5% SPE solution, the survival rate of animals was lower. Thus, the 12.75% SPE solution proved to be more effective both when applied to the skin and when injected subcutaneously. This suggests that SPE from raspberry berries has therapeutic properties and may have the potential to prevent or mitigate the effects of deep burns in animals, warranting further research into its properties and biological activity as a promising and effective agent for burn treatment.

Author contributions.

KAG, DMH, BBYu, SKV, GVS, SMA, DAM, SGM and SRM performed the experiments and data analysis. DMH and SKV provided histological interpretation. KAG, DMH, BBY, SKV, GVS, SMA, DAM, SGM and SRM provided advice on data interpretation. DMH, SKV, SMA, SRM and KAG wrote the manuscript. All of the authors have contributed substantially to the manuscript.

Funding.

The work was supported by the Higher Education and Science Committee of MESCS RA, in the frames of the research project № 21T-1F126.

Availability of data and materials.

Raw data can be provided upon request to the corresponding author.

Declarations.

Competing interests: The authors declare no competing interests.

Conflict of interest: The authors declare no conflict of interest.

Ethical approval and consent to participate.

The experimental protocol corresponded to the conditions of the European Communities Council Directive (2010/63/ UE) and was approved by the Ethics Committee of Yerevan State Medical University after Mkhitar Heratsi (IRB Approval N4, November 15, 2018).

REFERENCES

1. Moore EC, Pilcher DV, Bailey MJ, et al. A simple tool for mortality prediction in burns patients: APACHE III score and FTSA. Burns. 2010;36:1086-91.
2. Glik J, Cholewka A, Stanek A, et al. Thermal imaging and planimetry evaluation of the results of chronic wounds treatment with hyperbaric oxygen therapy. Adv Clin Exp Med. 2019;28:229-236.
3. Schaefer TJ, Tannan SC. Thermal Burns. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024.
4. Edwards M, Cooper JS. Hyperbaric Treatment of Thermal Burns. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024.
5. Walker NJ, King KC. Acute and Chronic Thermal Burn Evaluation and Management. [Updated 2023 May 29]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024.

6. Loginov LP, Smirnov SV, Khvatov VB, et al. Blood transfusions in burnt patients in the process of restoring the skin. Sat. scientific tr. II Congress of Combustiologists of Russia, Moscow. 2008:108-109.
7. Posluszny JA Jr, Gamelli RL. Anemia of thermal injury: combined acute blood loss anemia and anemia of critical illness. J Burn Care Res. 2010;31:229-42.
8. Mulder PPG, Vlig M, Boekema BKHL, et al. Persistent systemic inflammation in patients with severe burn injury is accompanied by influx of immature neutrophils and shifts in t cell subsets and cytokine profiles. Front Immunol. 2021;11:621222.
9. Torjesen I. Drug development: the journey of a medicine from lab to shelf. Pharmaceutical Journal. 2015.
10. Buloyan SA. The effect of Dermafen ointment on the regenerative processes of the skin of white rats after an experimental thermal burn. International Journal of Applied and Fundamental Research. 2015;10-2:268-272.
11. Ermini ML, Summa M, Zamborlin AV, et al. Copper nanoarchitecture topical cream for the accelerated recovery of burnt skin Nanoscale Adv. 2023;5:1212-1219.
12. Ulashchik VS. Active oxygen species, antioxidants, and the action of therapeutic physical factors. Problems of Balneology, Physiotherapy and Exercise Therapy. 2013;90:60-69.
13. Pham-Huy LA, He H, Pham-Huy C. Free radicals, antioxidants in disease and health. Int J Biomed Sci. 2008;4:89-96.
14. Sazontova T.G, Archipenko Yu.V. The importance of the balance of pro-oxidants and antioxidants - equivalent participants in metabolism. Pathological physiology and experimental therapy. 2007;3:2-18.
15. Naum Goldstein. Application of gas-phase superoxide ion-radicals in the clinic. Russian Open Medical Journal. 2003;4:49-53.
16. Simonyan RM, Simonyan MA. Method of the preparation of superoxide-producing thermostable systems from biomembranes and biofluids. The license of invention N 618 Y. Yerevan, Armenia. 2021.
17. Simonyan RM, Feschyan SM, Madoyan RA, et al. Superoxide-producing associates from gastrointestinal bacteria: stimulation of its growth by exogenous superoxide-producing complex from raspberries. Free Radical Research. 2024:1-14.
18. Simonyan MA, Karapetyan AV, Galstyan DA, et al. Superoxide-producing lipoprotein as a factor that suppresses tumor growth, increases leukocyte count, and accelerates proliferation of the cultured cells. Biochemistry (Moscow). 1996;61:1578-1583.
19. Velnar T, Bailey T, Smrkolj V. The wound healing process: An overview of the cellular and molecular mechanisms. J Int Med Res. 2009;37:1528-1542.
20. Feschyan SM, Simonyan RM, Simonyan GM, et al. NADPH containing superoxide-producing thermostable complex from raspberry, apricot, grape, and grape seeds: isolation, purification, and properties. Plant Methods. 2023;19:1.
21. Simonyan RM, Babayan MA, Oxuzyan GR, et al. Generation of superoxide and hydroxyle radicals during decay of hydrogen peroxide by ammonium hydroxide. Biol. J. of Armenia. 2017;2:53-58.
22. Kishkun AA. Guidelines for laboratory diagnostic methods. Moscow: GEOTAR-Media; 2007.
23. Ford EHR, Wollam DHM. A study of the mitotic chromosomes of mice of the strong a line. Experimental Cell Research. 1963;32:320-326.
24. McGregor G, Varley J. Techniques of chromosome studies. "Mir", Moscow. 1986:262.
25. Fischer AH, Jacobson KA, Rose J, et al. Hematoxylin and eosin staining of tissue and cell sections. CSH Protoc. 2008;2008:pdb.prot4986.
26. Vukolov EA. Fundamentals of Statistical Analysis. Workshop on statistical methods and operations research using Statistica and Excel packages. Forum, Moscow. 2008:464.
27. Siegel AF, Wagner MR. Chapter 12 - Multiple Regression: Predicting One Variable From Several Others. Practical Business Statistics (Eighth Edition), Academic Press. 2022:371-431.
28. Borisov VS, Smirnov SV. Burn anemia: causes and difficulties of treatment at the present stage. Russian Sklifosovsky Journal of "Emergency Medical Care". 2013;3:28-34.
29. Tukhtaeva KhKh. Comparative assessment of cytogenetic changes in bone marrow cells during chronic and acute experimental irradiation. Voprosi nauki i obrazovaniya. 2021;29:91-106.

ЗАЩИТНОЕ ДЕЙСТВИЕ НОВОГО СУПЕРОКСИД-ПРОДУКТИРУЮЩЕГО ФЕРМЕНТНОГО КОМПЛЕКСА ИЗ МАЛИНЫ НА КРЫС С ТЕРМИЧЕСКИМИ ОЖОГАМИ ТРЕТЬЕЙ СТЕПЕНИ

Карапетян А.Г[1], Даниелян М.А[1*], Бадалян Б.Ю[4], Симонян К.В[1], Григорян В.С[1,2], Симонян М.А[3], Даллакян А.М[1], Симонян Г.М[3], Симонян Р.М[3].

[1]Институт физиологии им. Л.А. Орбели НАН РА, 0028, Ереван, Армения.

[2]Университет традиционной медицины, 0022, Ереван, Армения.

[3]Институт биохимии имени Г. Бунятяна, 0014, Ереван, Армения.

[4]ЕГМУ имени Мхитара Гераци, 0025, Ереван, Армения.

Резюме

Термические ожоги являются наиболее распространенным типом ожоговых травм. Медицинское лечение ожогов имеет решающее значение, особенно когда они третьей степени и когда затронута значительная площадь поверхности тела. Одной из наиболее актуальных проблем современной медицины является поиск новых эффективных средств, ускоряющих заживление ожоговых ран. Кислородные радикалы играют значительную роль в поддержании гомеостаза, формировании устойчивости организма к инфекциям, обеспечении регенерации органов и тканей. В данном исследовании был применен супероксид (O_2^-)-продуцирующий фермент (superoxide (O_2^-)-producing enzyme, SPE) из ягод малины (нанесение на кожную поверхность, инъекции под раневую поверхность; концентрации 12.75% и 5%) после термического ожога третьей степени) для выявления эффективности его репаративного воздействия на кожу. Для оценки состояния организма животных, перенесших ожоговую

травму и процесс заживления, был проведен анализ параметров крови, а также изучали цитогенетические показатели костного мозга из бедренной кости животных: митотический индекс, количество полиплоидных клеток, хромосомные аберрации. При анализе гематологических, цитогенетических и гистологических показателей были обнаружены значимые различия между группами «чистый ожог» и группами, в которых применялся SPE в различных концентрациях и способах нанесения. Применение SPE в обеих концентрациях способствовало уменьшению площади ожоговых ран по сравнению с чистым ожогом. Выживаемость животных за 30 дней (до окончания эксперимента) составила 100% при применении 12.75% SPE раствора и 50% при применении 5% SPE раствора. Применение SPE приводит к достоверному отличию гематологических показателей от группы «чистый ожог» на протяжении всего времени эксперимента и наблюдается тенденция к нормализации этих показателей. Под влиянием 12.75% раствора SPE при нанесении на рану и после инъекции под рану отмечена тенденция к нормализации митотического индекса, и получено достоверное снижение процента полиплоидных клеток и хромосомных аберраций, что возможно, указывает на его благотворное действие. Это исследование показало, что 12.75% раствор SPE, полученный из малины, оказался более эффективным и обладает целебными свойствами при термических ожогах третьей степени, способствуя быстрому заживлению ожоговой раны.

Ключевые слова. Термический ожог третьей степени, супероксид (O_2^-)-продуцирующй фермент, малина, гематологические показатели, полиплоидные клетки, митотический индекс.