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ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ

Медицинские новости Грузии

საქართველოს სამედიცინო სიახლენი

GEORGIAN MEDICAL NEWS

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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.

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MORPHOLOGICAL AND BEHAVIORAL ANALYSIS OF THE PROTECTIVE EFFECTS OF BACTERIAL MELANIN IN A RAT MODEL OF PARKINSON'S DISEASE

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Abstract.

A comparative study of the morphological and functional state of the microvasculature of the substantia nigra pars compacta of the brain (SNc) and bone marrow of rats was carried out using the rotenone model of Parkinson's disease (PD) and with subsequent administration of bacterial melanin (BM). The detection of microvasculature was carried out according to the histoangiological method of Chilingaryan. Animal behavior was studied using a cylinder test. An analysis of morphometric data showed that, in comparison with control animals, experimental animals with rotenone dysfunction showed an increase in capillary diameters and a general reduction in the capillary link in SNc. Behavioral tests have shown that the animals with rotenone intoxication exhibit a form of behavior inherent in PD (freezing, immobility, apathy). Under the influence of BM, the diameter of the capillaries in the SNc approaches the norm, and the capillary link is restored. Due to the protective effect of BM in rats with rotenone intoxication, the trophism of the brain tissue increases as a result of the approach of the lumen of the vessels to the norm and the opening of new branches in the capillary network, an increase in the density of capillaries, which ensures the safety of nerve cells. Animal behavior indicators are close to normal. A comprehensive analysis of cytogenetic data of rat bone marrow was also carried out. In animals with PD, compared to controls, there is a significant increase in the amount of polyploid cells (PC) and a decrease in the level of mitotic index (MI), which usually manifests itself in inflammatory processes and is accompanied by inhibition of bone marrow hematopoiesis. Under the influence of BM, a tendency towards normalization of MI was noted and a significant decrease in the percentage of PC was obtained, which possibly indicates its beneficial effect. The data obtained suggest that BM can be used as a therapeutic agent in the treatment of PD.

Key words. Capillaries, polyploid cells, mitotic index, cylinder test, substantia nigra, bacterial melanin, Parkinson's disease.

Introduction.

Parkinson's disease (PD) is the second most common neurodegenerative disorder, often occurring in middle-aged and elderly individuals. PD is primarily characterized by the progressive loss of dopaminergic neurons in the substantia nigra pars compacta (SNc), which project into the striatum. Significant loss of dopaminergic neurons in PD leads to dysfunction of the basal ganglia and, consequently, corticostriatal imbalance, resulting in hallmark features such as bradykinesia, muscle rigidity, postural instability, gait disturbances, and hand tremors [1]. Non-motor symptoms of PD include neuropsychiatric symptoms, sleep disturbances, dysautonomia, depression, cognitive changes, and autonomic dysfunction [2]. The slow death of SNc cells in PD is largely considered to be the result of oxidative damage and neuroinflammatory processes induced by environmental toxins, likely compounded by genetic predisposition [3]. In addition to neurodegeneration, vascular degeneration plays an important role in the development of neurodegenerative conditions [4]. There is limited data on the vascular changes associated with the brain regions affected by neurodegeneration in PD. Some early studies have suggested that abnormal blood flow to the SNc may trigger metabolic cascades leading to PD. Normally, SNc neurons have a dense capillary network, but in PD, there is a reduction in neuronal-vascular density, and capillary-neuron contact is disrupted, with capillaries becoming separated from neurons by gliosis. It is possible that these microvascular problems may be the cause of abnormal metabolism [5]. One study identified small vessel disease as a primary subtype of cerebrovascular disorders and found an association with PD development [6], while another reported endothelial degeneration in cerebral capillaries in PD patients [7]. Researchers noted that the severity of small vessel disease correlates with motor impairments but not with dementia in these patients [8]. Modifiable vascular risk factors are more strongly associated with cognitive rather than motor impairments in PD. This underscores the importance of a comprehensive approach to PD treatment, including the potential long-term cognitive benefits of early and aggressive management of concomitant vascular conditions.

For a long time, the genetic component of PD was considered unlikely, as most cases appeared sporadic [9]. However, the notion that genetics plays a role in some forms of PD has been supported by various observations [10]. An increased risk of PD has been observed among first-degree relatives of patients, especially when considering the results of positron emission tomography in asymptomatic relatives, providing additional evidence of a genetic component [11,12]. According to modern views, approximately 10% of all PD cases have a monogenic inheritance basis [13,14]. There is a range of predisposing genes for PD, including genes involved in cellular detoxification and antioxidant defense systems such as GSTT1, GSTM1, GSTP1, dopamine transport and metabolism genes, and mitochondrial genome, among others, carrying unfavorable allele variants significantly increasing the risk of the disease [15,16]. The results of these studies demonstrate a high variability in the frequency of candidate gene polymorphism occurrence in PD development among different populations.

Currently, there is no clear biomarker for the early detection of PD, and the etiology of idiopathic Parkinson's disease remains unknown. In recent years, scientific research has focused on identifying blood markers in patients with Parkinsonism, which could serve as additional diagnostic tools [17]. Additionally,

studying the significance of therapeutic interventions in patients with PD using various compounds is of interest, along with the search for genetic markers in PD and the observation of potential therapeutic interventions remain relevant.

Modern strategies for treating PD are mainly symptomatic and can lead to motor disorders such as motor fluctuations and dyskinesias. Alternative medicine may offer effective treatments for PD. In this study, bacterial melanin (BM) was used as a potential protector in PD. BM is an amorphous, high-molecular-weight, water-soluble polymer of dark brown color, containing quinol, phenolic, and amino acid components [18]. It facilitates transport across the blood-brain barrier, which may contribute to the protective action of PD [19]. BM has high biological activity and a biostimulating effect; it does not induce microgliosis and has no side toxic effects [20]. Animal model studies have demonstrated the neuroprotective effects of BM, promoting motor restoration, intensive vascularization, gliosis proliferation, and inhibition of inflammatory and scar processes [21-23]. Therefore, there is a high likelihood of BM's protective influence on brain structures affected in the early stages of PD.

The aim of the study was to investigate changes in the microcirculatory bed of the SNc in rats in the rotenone-induced PD model and, in combination with BM administration, to identify its possible angioprotective properties on brain capillaries. Additionally, using a comprehensive analysis of cytogenetic data, the study aimed to examine factors determining the development or mitigation of PD.

Materials and Methods.

Several rodent studies have shown that the pesticide rotenone can induce oxidative death of nigrostriatal cells, pathological (alpha-synuclein-like) deposits, and motor changes similar to those occurring in the idiopathic form of PD, reinforcing the role of pesticides in its etiology [24]. Experiments were conducted on a rotenone-induced rat model of PD, which is considered most suitable, especially for up to 4 weeks of survival. The experiments involved three groups of 15 albino rats (220-250 g):

1. Sham-operated (5 rats, control), received injections of sterile distilled water combined with intraperitoneal injection of isotonic NaCl solution.
2. PD group (5 rats) received unilaterally injections of rotenone along with intraperitoneal injection of physiological saline every other day for 4 weeks.
3. PD+BM group (5 rats) received unilaterally injections of rotenone, similar to the PD group, combined with intraperitoneal injection of BM (6 mg/ml, at a dose of 0.17 g/kg) twice for 4 weeks (5 rats, PD+BM).

BM was provided by the "Armbiotechnology" National Academy of Sciences of Republic of Armenia. Rotenone (Sigma-Aldrich, 557368) was administered under anesthesia (pentobarbital, 40 mg/kg, intraperitoneally), at a dose of 12 μg in 0.5 μl of dimethyl sulfoxide at a rate of 1 μl/min, into the medial forebrain bundle at stereotaxic atlas coordinates (AP+0.2; L±1.8; DV+8 mm) [25]. After the rotenone injection, the animals were kept under identical conditions until the acute experiment at the of L.A. Orbeli Institute of Physiology's vivarium. The experiments were conducted between 09:00 and 12:00 h during the light period of the light–dark cycle. The animals were maintained at 25 ± 2°C with a 12-hour light–dark cycle (lights on at 07:00 h and off at 19:00 h), and they had ad libitum access to food and water.

To study motor behavior, exploratory activity, and cognitive abilities in rats with PD symptoms, the cylinder test was used [26]. Intact adult rats underwent behavioral testing in the cylinder before being unilaterally injected with rotenone and maintained for 4 weeks. Some animals received BM solution injections twice, while the rest served as the experimental PD model group. The rats were placed in a transparent cylinder (diameter 19 cm, height 30 cm) and video-recorded for 5 minutes with an assessment ranging from 0 (absence of function) to 20 (absence of deficit). Since rotenone affects motor behavior bilaterally, the number of paw touches on the cylinder wall during rearing was recorded separately for the right and left paws. Spontaneous behavior allowed for the evaluation of other features such as dynamics and interest in the environment.

To study the microcirculatory bed of the rats, the histoangiological method of Chilingaryan was used [27]. The animals were anesthetized with pentobarbital (40 mg/kg, intraperitoneally) followed by brain removal, which was fixed in a 5% neutral formalin solution for 24 hours at room temperature. Frozen brain sections in the frontal plane, 90 μm thick, were prepared and transferred to freshly prepared mixtures according to the method's requirements. Incubation was carried out at room temperature for 1.5 hours in the incubation mixture and lead mixture. Subsequently, the sections were washed in distilled water, developed in a sodium sulfate solution, and mounted in balsam. The diameter of brain capillaries in the SNc of rats was measured on prepared brain specimens using an ocular micrometer under a light optical microscope (eyepiece 15x, objective 20x). The average diameter (d) of capillaries was obtained from 100 measurements performed on brain sections of each animal. Imaging of the specimens was performed using an OPTON M-35 camera and an AmScope MU800 camera attachment through an OPTON microscope (West Germany). Student's t-test was used to evaluate the level of differences in morphometric and behavioral data, utilizing the online GraphPad tool.

Cytogenetic analysis included Giemsa staining of chromosomes. Cytogenetic parameters were studied using the Ford-Vollum method, determining the mitotic index (MI), chromosomal aberrations, and the percentage of polyploid cells (PC) in bone marrow cells of the femur (counting in 1000 cells in each specimen), according to McGregor [28] under a microscope at magnifications of 900–1400 times. The analysis of cytogenetic data was conducted using several specialized statistical packages: Statsoft and SPSS-10.0. Regression and correlation analysis methods were employed [29].

Results and Discussion.

The microscopic examination revealed a continuous vascular capillary network on thick sections: capillary, arteriole, and venule fragments, by the brown lead phosphate sediment deposited on the vessel endothelium. A dense capillary network was observed in the SNc region of the brain of control animals (Figure 1 A-B). Morphometric analysis showed that in control

rats, the average diameter of the main body of SNc capillaries was d=3.3±0.08 μm (Graph 1). In response to rotenone administration, there was a 45% increase in capillary lumen diameter (d=4.8 ±0.12 μm) compared to the control, along with a reduction in the capillary network (Figure 1B; Graph 1).

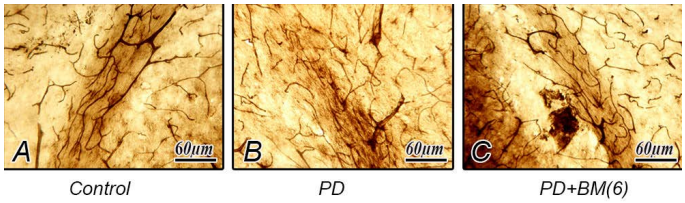
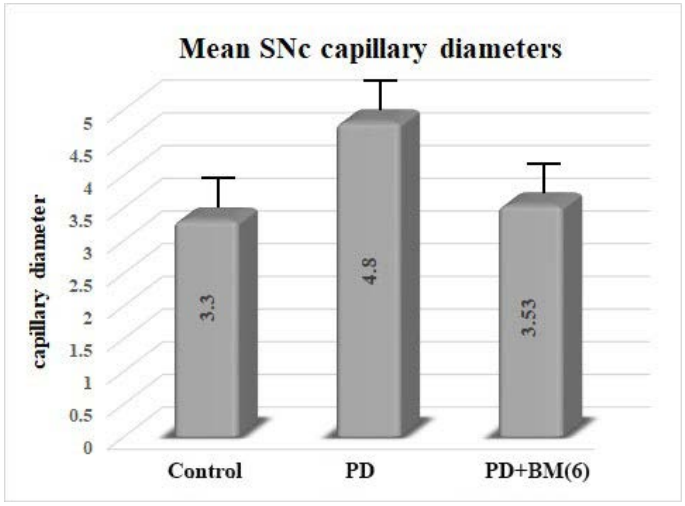


Figure 1. *Microphotographs of the capillary network of the microcirculatory bed of the substantia nigra pars compacta in rats. A - control; B - under rotenone intoxication conditions; C - under rotenone intoxication conditions in combination with BM administration. Magnification: 160 (A-C).*



Graph 1. *Changes in the lumen of capillaries in the substantia nigra pars compacta of rats: Control - control rats; PD - Parkinson's disease model; PD+BM (6) – PD model in combination with BM at a concentration of 6 mg/ml. p≤0.0001.*

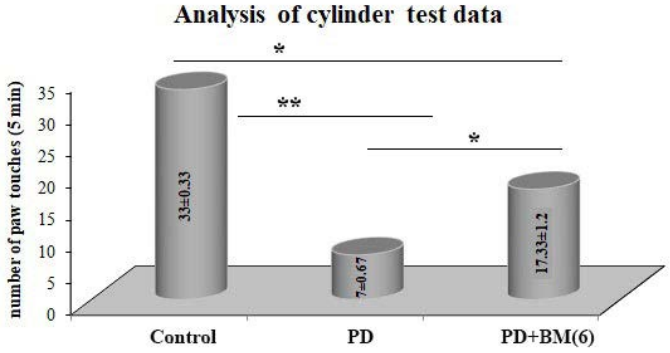
In the control group, 10 fields of view were used to count 100 capillaries. However, after rotenone exposure, the number of fields of view required for counting increased to 15, indicating a decrease in the number of functioning capillaries. In PD cases, fewer vessels were found, and they were shorter and more fragmented (Figure 1B) compared to the control group (Figure 1A). The number of vessels per mm² was measured in the microscope field. Under the influence of BM treatment, the diameter of capillaries in the SNc approached the norm (d=3.53 ±0.08 μm), and the density of the capillary network was much higher than in PD (Figure 1C; Graph 1).

Under the action of BM in conditions of rotenone intoxication, the capillary lumen in the SNc was dilated by only 7% compared to control rats and constricted by 26% compared to the PD model (Table 1). The application of BM led to the preservation of capillary network density and the number of functioning capillaries. The data were highly extremely statistically significant (p<0.0001).

Table 1. *Diameter of capillaries in the substantia nigra pars compacta in control and experimental rats. Data are presented as mean ± standard error of the mean (m±SEM). Significance of differences compared to control and PD is p<0.0001 (Student's t-test in the GraphPad online program, https://www.graphpad.com/quickcalcs/).*

Groups	**Diameter±SEM**
Control rats	**3.3±0.08**
Parkinson's disease model	**4.8±0.12** p≤0.0001 45% dilation compared to the control
Model of Parkinson's disease in combination with the administration of bacterial melanin	**3.53±0.08** p≤0.0001 7% dilation compared to the control 26% contraction compared to PD

During behavioral testing, the number of spontaneous rears performed by each animal during a 5-minute period in the cylinder test was measured. In the PD group, rats exhibited significantly fewer rears compared to control rats (7 ±0.67; p < 0.0001) (Graph 2). Four weeks after rotenone injection, animals demonstrated more rears compared to PD (number of rears with paw touch: 17.33 ±1.2; p < 0.0015). Animals receiving BM after rotenone injection showed a lower number of spontaneous rears compared to control animals (number of rears with paw touch: 33 ±0.33; p < 0.0001), but more rears than animals after rotenone intoxication (Graph 2). In addition to counting the number of rises with paws touching the walls of the cylinder, the orientation and mobility of the animals were also observed during the 5-minute experiment. Visually, animals in the PD model exhibited reduced activity, freezing in one position, apathy, and lack of interest in the surrounding space.



Graph 2. *The graph illustrates the number of rears with paws touching the walls of the cylinder for different groups of animals: control animals (Control); under rotenone intoxication PD), and under rotenone intoxication in combination with BM administration (PD+BM (6)). Data are presented as mean ± standard error of the mean (m±SEM). The significance of differences compared to control and PD is denoted by (p<0.0001) and *(p<0.0015)**, respectively. Student's t-test was used in the online GraphPad program, https://www.graphpad.com/quickcalcs/.*

The intracerebral infusion of rotenone into the medial forebrain bundle resulted in persistent feeding behaviour disturbances after 4 weeks compared to the control group and the group

receiving BM. However, rats receiving BM showed behavioral improvement after 4 weeks compared to the animals in the PD group. These data indicate that rotenone significantly affects certain aspects of motor behaviour, and that BM provides protection and can reverse the degenerative effects of rotenone.

The results of cytogenetic analysis showed that PD animals, compared to controls (PC=1.0 ± 0.16; MI=20.6 ±2.8), had a significant increase in the percentage of polyploid cells (1.5 ±0.18) and a decrease in the mitotic index (13.5 ±1.4), which is typically indicative of inflammatory processes. Under the influence of BM, a significant decrease in the percentage of polyploid cells (1.0± 0.14) was observed, possibly indicating its beneficial effect. There was also a tendency towards normalization of the mitotic index (18.2 ±1.56) (Table 2). Regarding chromosomal aberrations, the most frequently encountered chromosomal aberrations were observed as single and double fragments (Figure 2B,C).

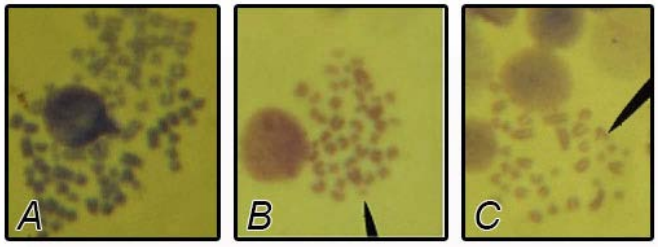


Figure 2. *Polyploid cell (A), chromosomal aberrations in the form of a double fragment (B) and deletion (C). A - polyploid cell resulting from cytokinesis blockage in bone marrow tissue; B - double fragment formed from chromosome arm breakage; C - deletion of chromosome arm, pair I. Magnification: ×390.*

Table 2. *Cytogenetic parameters of bone marrow cells in Parkinson's disease (PD) rats and under the influence of bacterial melaninat (PD+BM) a concentration of 6 mg/ml (BM (6)). Significance of differences: * p<0.05; (p1-p2) – when comparing control animals and animals with PD; (p2-p3) – when comparing animals with PD without and under action of BM.*

Indices	**Control**	**PD**	**PD+BM (6)**
Mitotic Index (%)	20,6±2,8	13,5±1,4 $^{*}(p_1\text{-}p_2)$	18,2±1,56
Chromosomal aberrations (%)	2,2±0,3	2,2±0,35	2,26±0,37
Polyploid cells (%)	1,0±0,16	1,5±0,18 $^{*}(p_1\text{-}p_2)$	1,0±0,14 $^{*}(p_2\text{-}p_3)$

It is known that the risk of polyploidy development increases when cells are exposed to physical or chemical factors that damage the spindle apparatus. Research results indicate that changes in chromosome set multiplicity suggest serious disturbances in the central nervous system [30].

Karyotype analysis revealed significant differences in almost all cytogenetic parameters between the experimental groups of animals and the karyotype data of control individuals. Based on these cytogenetic parameters, it can be noted that PD significantly suppresses hematopoiesis in the bone marrow (as evidenced by the reduced mitotic index) but does not have a significant effect on chromosome aberrations (only slight variability in the percentage of chromosomal aberrations was observed). The most frequently encountered chromosomal aberrations were observed in the form of fragments. There is an inverse correlation between the mitotic index and the number of polyploid cells. Cytogenetic abnormalities such as a decreased mitotic index and increased the polyploid cells can be considered genetic risk factors for PD development. Under the influence of BM, there is a tendency towards the normalization of mitotic index and a significant decrease in the percentage of the polyploid cells, which may indicate its beneficial effect.

In our study, we observed capillary degeneration in the SNc of PD rats. The degenerative morphology includes damage to the capillary network, possibly due to capillary fragmentation and loss of capillary connections. Compared to the control group, the diameter of the capillaries was wider in PD cases. These data suggest that vascular degeneration may be an important additional factor contributing to PD progression and may even play a role in the initial pathology leading to neuron degeneration. We hypothesized that the modulatory effect of BM on the brain of rats with rotenone dysfunction lies in the mechanism by which it improves microcirculation. Under the influence of BM, the diameter of the capillaries was similar to that of the control group, indicating the angioprotective properties of BM.

The results of behavioral test showed that rats administered rotenone exhibited significantly fewer rearing behaviors compared to control rats, indicating motor deficits associated with PD. Reduced activity, freezing, and lack of interest in the surrounding environment in animals with PD confirm the validity of the rotenone-induced PD model. In the group receiving BM after rotenone injection, there was a significant increase in the number of rearing behaviors compared to PD and fewer rearing behaviors compared to control animals. This suggests that BM partially restores motor function. As noted above, BM has a saturable transport through the blood–brain barrier and selectively affects some regions of the central nervous system. It is excreted through the liver and kidneys [19]. Such transport may contribute to the neuroprotective effects of BM due to its modulatory effect on the brain of rats with rotenone dysfunction.

Conclusion.

The data obtained demonstrate that BM's protective effect in rats with rotenone intoxication leads to increased trophism in brain tissue. This is evident through the normalization of blood vessel lumen size and the formation of new branches in the capillary network, thereby ensuring the preservation of nerve cell integrity. These findings highlight the angio- and neuroprotective effects of BM on the brain capillaries of rats with a PD model. Rats treated with BM also showed improvements in motor behavior, such as hyperactivity and increased interest in their environment. This suggests a potential therapeutic effect of BM in alleviating motor deficits and cognitive impairments associated with PD. Furthermore, under the influence of BM, cytogenetic parameters approached normal levels, indicating its positive impact on cellular function. In conclusion, BM emerges as a potential biological therapeutic product for treating PD, given its multifaceted beneficial effects on brain tissue trophism, motor behavior, and cytogenetic parameters.

Author contributions.

DMH, KKV, AZA, HAS, KAG, DAM and NKA performed the experiments and data analysis. DMH and KKV provided histological interpretation. Cytogenetic studies have been carried out KAG and DAM. DMH, KKV, AZA, HAS, KAG, DAM and NKA provided advice on data interpretation. DMH, KKV, NKA and KAG wrote the manuscript. All of the authors have contributed substantially to the manuscript.

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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).

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Морфологический и поведенческий анализ протекторного действия бактериального меланина на модели болезни Паркинсона у крыс

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Резюме

Проведено сравнительное изучение морфофункционального состояния микроциркуляторного русла компактной части черной субстанции мозга (SNc) и костного мозга крыс на ротеноновой модели болезни Паркинсона (БП) и с последующим введением бактериального меланина (БМ). Выявление микроциркуляторного русла проводилось согласно гистоангиологическому методу Чилингаряна. Поведение животных изучали с помощью цилиндрического теста. Анализ морфометрических данных показал, что в сравнении с контрольными животными у подопытных животных с ротеноновой дисфункцией выявляется увеличение диаметров капилляров, редукция капиллярного звена в SNc. Поведенческие тесты показали, что животные после ротеноновой интоксикации демонстрируют форму поведения, присущую БП у животных (фризинг, малоподвижность, апатия). Под влиянием БМ диаметр капилляров в SNc приближается к норме, капиллярное звено восстанавливается. Благодаря защитному действию БМ у крыс с ротеноновой интоксикацией повышается трофика ткани головного мозга в результате приближения просвета сосудов к норме и открытия новых ветвей в капиллярной сети, увеличение плотности капилляров, что обеспечивает сохранность нервных клеток. Показатели поведения животных близки к норме. А также проведен комплексный анализ цитогенетических данных костного мозга крыс. У животных с БП по сравнению с контрольными наблюдается достоверное повышение количества полиплоидных клеток и понижение уровня митотического индекса, что обычно проявляется при воспалительных процессах и сопровождается угнетением гемопоеза костного мозга. Понижение митотического индекса и повышение количества полиплоидных клеток можно рассматривать как факторы генетического риска развития БП. Под влиянием БМ отмечена тенденция к нормализации митотического индекса и получено достоверное снижение процента полиплоидных клеток, что возможно, указывает на его благотворное действие. Полученные данные дают основание предполагать, что БМ может быть использован в качестве терапевтического агента при лечении БП.

Ключевые слова: капилляры, полиплоидные клетки, митотический индекс, тест «цилиндр», черная субстанция, бактериальный меланин, болезнь Паркинсона