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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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ASSESSMENT OF RAT BRAIN MORPHOFUNCTIONAL STATE IN A PARKINSON'S MODEL: INFLUENCE OF THERAPEUTIC AGENTS OF ANIMAL AND SYNTHETIC ORIGINS

Danielyan M.H[1], Nebogova K.A[1]*, Avetisyan Z.A[1], Khachatryan V.P[1], Sarkissian J.S[1], Poghosyan M.V[1], Karapetyan K.V[1].

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

Abstract.

In neurodegenerative diseases, particularly in Parkinson's disease (PD), antinociceptive centers are often implicated in neurodegeneration, leading to persistent pain unresponsive to narcotic substances. This study investigated the periaqueductal gray matter (PAG) and the nucleus raphe magnus (NRM), components of the brain's antinociceptive system. In conditions of rotenone intoxication (an experimental PD model), morphological changes in intracellular structures were observed in PAG and NRM neurons, indicating metabolic disorders characteristic of PD (alterations in the shape and size of neuronal bodies and processes, disruption of acid phosphatase activity in neuron cytoplasm). Under the influence of bacterial melanin and in combination with synoestrol, positive changes in structural properties were observed in PAG and NRM neurons compared to the rotenone model of PD. This included the preservation of the morphological characteristics typical of these brain regions, with cells exhibiting shapes and sizes close to normal. Furthermore, under the influence of these therapeutic agents, an increase in phosphatase activity in cell cytoplasm was detected, indicating an acceleration of metabolic processes (metabolic activation) disrupted by rotenone intoxication. The data obtained suggests that bacterial melanin and synoestrol may act as potential neuroprotective agents against PAG and NRM neurons in the rat brain in the rotenone model of PD. Further research is needed to elucidate the mechanisms of action of therapeutic doses and propose their use in the treatment of PD, either in isolation or combination therapy.

Key words. Periaqueductal gray matter, nucleus raphe magnus, Parkinson's disease, bacterial melanin, synoestrol.

Introduction.

Neurodegenerative diseases arise from the progressive degeneration and death of neurons within specific structures of the central nervous system (CNS). This leads to the disruption of connections between CNS regions, an imbalance in the synthesis and release of corresponding neurotransmitters, and consequently, impairment of memory, movement disorders, and cognitive abilities in humans. Parkinson's disease (PD) is a chronic progressive neurodegenerative disorder of the CNS caused by the ongoing destruction and death of neurons in the substantia nigra and other CNS regions that utilize dopamine as a neurotransmitter. Clinically, PD manifests as impairments in voluntary movements. In addition to classical motor disturbances, PD is characterized by a broad spectrum of non-motor symptoms, including psychological, autonomic, sensory symptoms, and disruptions in sleep and wakefulness. Apart from the typical motor impairments, PD exhibits a wide range of non-motor manifestations, including psychological, autonomic, sensory symptoms, and sleep-wake cycle disturbances [1]. Dopamine deficiency contributes to emotional and other psychological disorders. Pain is one of the most debilitating non-motor symptoms of PD. The frequency of pain in PD patients, according to various authors [2,3], ranges from 40 to 80%, exceeding that in the general population (10-40%). Pain is often overlooked by physicians managing PD patients, yet it frequently becomes a primary symptom adversely affecting the patient's quality of life [4]. Despite the high frequency of pain, its causes, association with the disease itself, and approaches to its correction remain poorly understood. The therapeutic strategy is limited to non-opioid analgesics, opioids, antidepressants, and/or anticonvulsant medications [5].

Neuropsychiatric symptoms and pain are among the most common nonmotor symptoms of PD [6]. While nociceptive pain occurs in patients with a normal somatosensory nervous system, the origin of neuropathic pain is associated with the neurodegeneration of structures involved in pain modulation. The complex mechanisms of pain in PD are linked to pathological changes in brain structures involved in nociceptive mechanisms and are influenced by various factors such as age, gender, depression, and the severity or duration of the disease. Early involvement of structures like the nucleus raphe magnus (NRM) neurons is noted in PD [7]. One of the mechanisms of pain in PD is the loss of noradrenergic and serotonergic neurons in the locus coeruleus and raphe nucleus. These formations, together with PAG, play a pivotal role in modulating spinal nociceptive transmission pathways by inhibiting nociceptive stimuli from dorsal horn neurons [8]. As an important part of the endogenous pain inhibition system, the NRM receives projections from the PAG [9]. Damage to these areas of the brain can lead to increased pain intensity [10]. The study investigates bacterial melanin (BM) as a potential neuroprotective agent in PD. BM, obtained through biotechnological methods using Bacillus thuringiensis, demonstrates high biological activity and a biostimulating effect. Low concentrations of BM (4.5–6 mg/ml) do not induce microgliosis and have no toxic side effects [11]. BM is known for its protective and anti-inflammatory properties, suggesting a potential protective and reparative impact on early-stage PD-affected brain structures [12,13]. It is demonstrated that BM and synoestrol successfully counteract excitotoxicity (an indicator of neurodegeneration) observed in NRM and PAG in the rotenone-induced PD model in rats [14,15]. Given the above, the research aims to investigate the morphofunctional changes in NRM and periaqueductal gray matter (PAG) neurons in the rat brain in the rotenone model of PD under the influence of BM and synoestrol. The goal is to identify their potential protective effects individually and in combination on the aforementioned brain structures selectively affected in PD.

Materials and Methods.

Animals:

The work was performed using albino rats (mean weight 250 ± 30 g) from the vivarium of L.A. Orbeli Institute of Physiology NAS RA, which were kept under standard vivarium conditions.

Histochemistry study:

Morphofunctional studies were conducted on the rotenone model of PD in rats, considered the most suitable for investigating neuron damage mechanisms and assessing neurochemical, behavioral, and cognitive manifestations, especially up to 4 weeks of survival [16].

The experiments were carried out in 4 series on 20 Albino rats (220-250g): 1) sham-operated (5 rats, control), injected with sterile distilled water in combination with intravenous administration of isotonic NaCl solution; 2) unilaterally injected with rotenone (5 rats) with intravenous injection of physiological saline every other day for 4 weeks; 3) unilaterally injected with rotenone, similar to group (2), in combination with intravenous injection of bacterial melanin (6 mg/ml) twice over 4 weeks (5 rats); 4) unilaterally injected with rotenone, similar to group (2), in combination with intravenous injection of BM (6 mg/ml) twice over 4 weeks (5 rats) and intravenous injections of synoestrol (2% solution, 14 injections every other day at doses of 1 mg/kg). Rotenone (Sigma-Aldrich, 557368) was administered under anesthesia (pentobarbital, 40 mg/kg, 40 mg/kg, intraperitoneal) at a dose of 12 µg in 0.5 µl dimethyl sulfoxide at a rate of 1 µl/min, into the medial forebrain bundle at coordinates (AP+0.2; L±1.8; DV+8 mm) according to stereotaxic atlas [17]. The volume of the injected solution was calculated based on the optimally tolerated dose (0.17 g per kilogram of body weight) at the time of introducing the BM solution. The experiments were performed at the same time period of the day (09:00–12:00 h) and 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). Food and water were provided ad libitum.

To investigate the morphofunctional state of cellular structures in the PAG and NRM of rats, a histochemical method for detecting the activity of Ca^{2+}-dependent acid phosphatase (AP) was employed [18,19]. In living organisms, enzymes serve as biocatalysts that facilitate the progression of metabolic reactions due to the presence of active centers that convert a substrate specific to each enzyme [20]. The applied method adheres to all requirements of this principle. This methodological approach is based on the detection of intracellular phosphorus-containing compounds that play key roles in the energetic processes aimed at preserving and reproducing vital systems. When AP activity is tested, the phosphate ions released under the action of the enzyme can freely move in the mixture and react with different structures, regardless of their spatial arrangement, and after incubation in the solution of sodium sulfide it turns into a visible dark brown precipitate of lead sulfide. The resulting image is adequate, highly informative, and allows judgments to be made about specific links in the metabolism of the examined structures.

To obtain the brains for histochemical analysis, the animals were anesthetized with pentobarbital (40 mg/kg, intraperitoneal) followed by brain extraction. The extracted brains were fixed in a 5% solution of neutral formalin prepared in 0.1 M phosphate buffer (PBS, pH=7.4) for 48 hours at +4°C. Sections of the relevant brain regions were prepared in the frontal plane. Frozen sections, with a thickness of 50-60 µm, were transferred to freshly prepared incubation mixtures designed to detect the activity of Ca^{2+}-dependent AP. Incubation was carried out in a thermostat at 37°C for 1.5 hours. Subsequently, the sections were washed in distilled water, developed in a sodium sulfate solution, rinsed again, and mounted in balsam, followed by the description of the preparations under a light microscope. Subsequent images of the obtained preparations were captured using the OPTON M-35 camera and the AmScope MU800 camera attachment through the OPTON microscope (West Germany).

Results and Discussion.

PAG is one of the primary centers for descending regulation of pain sensitivity, representing a major component of the antinociceptive system. PAG surrounds the cerebral aqueduct of Sylvius in the midbrain and is composed of scattered neurons producing enkephalins, which reduce the perception of ascending pain impulses from the spinal cord. It contains a very delicate network of axial cylinders, with numerous small cells, primarily of triangular shape, and thin dendrites extending from their acute angles (Figure 1A). PAG also consists of large multipolar neurons, large triangular neurons, and large fusiform neurons (Figure 1A-B). The staining intensity of these cells is moderate, indicating a moderate metabolism in the rat PAG neurons. The nuclei are light, and nucleoli are visible (Figure 1B).

Data analysis revealed that under rotenone intoxication, there is a disturbance in the morphological structure of the PAG compared to the norm (Figure 1D-F). Neuronal damage occurs to varying degrees, with segmental or perinuclear chromatolysis of the basophilic substance in the cytoplasm (Figure 1D,E). Lysis of chromophilic substance granules in the nerve cell cytoplasm is observed, leading to its clarification. These neurons exhibit a noticeable decrease in AP activity, gradual disappearance of granular sediment in the cell perikaryon, resulting in a sharp clarification of the cytoplasm (Figure 1F). In some damaged cell bodies, the lead phosphate sediment is homogeneously distributed. In other affected neurons, the dark-stained coarse-granular lead phosphate sediment is unevenly distributed throughout the cell body, indicating possible complete breakdown, with thickened and shortened dendrites (Figure 1E,F). Due to the coarse-granular intracytoplasmic granulation, these neurons appear intensely stained. The characteristic shape of PAG cells is disrupted, and there is no clear distinction between cell groups. Some cells lose their characteristic shape; acquire a spherical shape due to swelling of the bodies and loss of processes (Figure 1F). Neurons of elongated or triangular shapes are encountered, but the contours of the cells are irregular, unclear, and the boundary between the body and the processes is not visible. They react with long processes, in which the lead phosphate deposit is dusty. The nucleus is swollen, occupies an eccentric position, and against its light background, a dark nucleolus is revealed (Figure 1E,F).

Thus, with rotenone intoxication of the brain, metabolic and

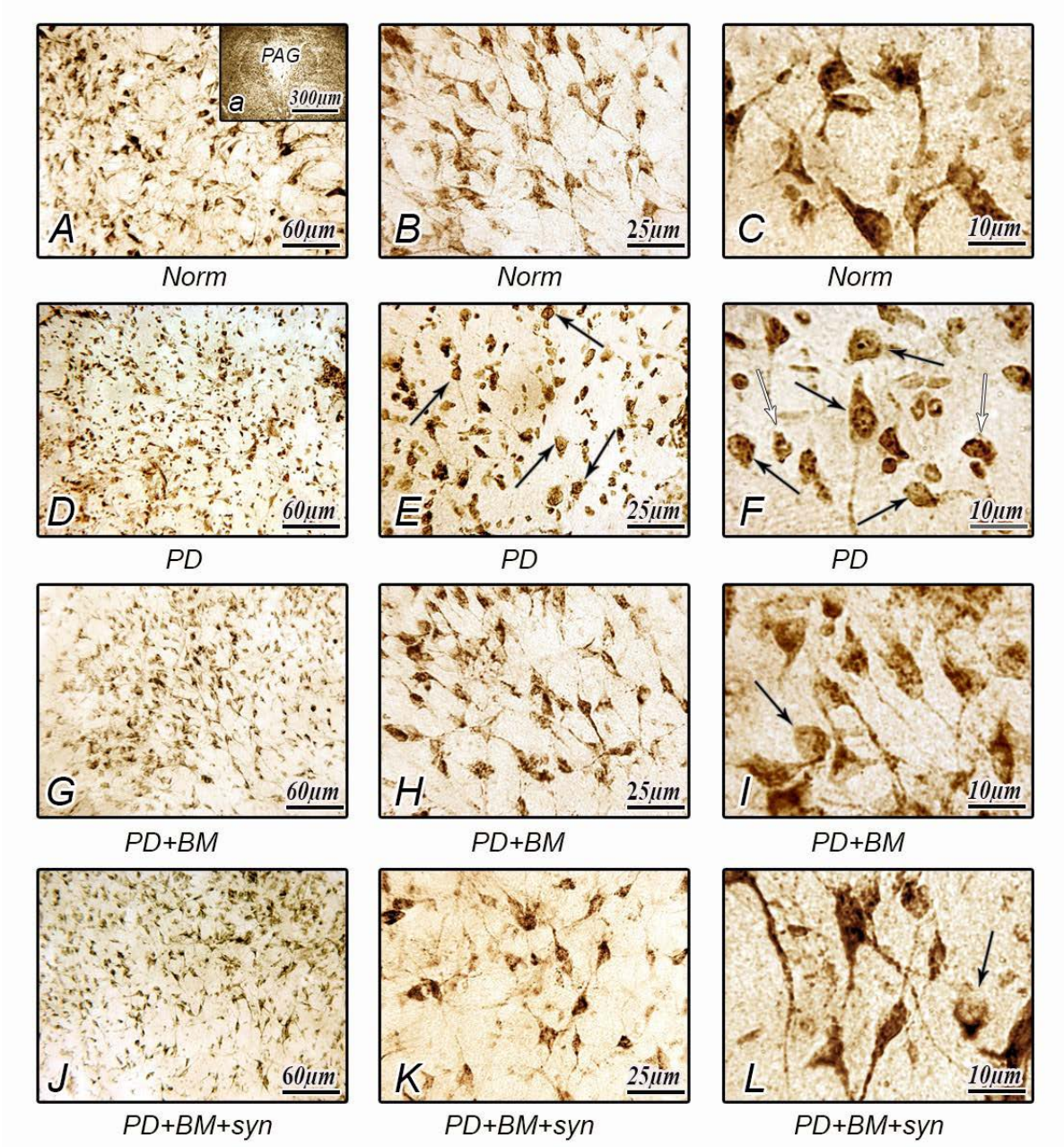

Figure 1. *Microphotographs of cellular structures in the PAG of intact (A-B, Norm) and experimental (D-L) rats (D-F - under rotenone intoxication (PD); G-I - under rotenone intoxication in combination with bacterial melanin (PD+BM); J-L - under rotenone intoxication in combination with simultaneous administration of bacterial melanin and synoestrol (PD+BM+syn); D-F - deformation of shape, degenerated cells with unclear contours, clarified cytoplasm; G-L - preservation of the morphological picture of neurons, high AP activity in the cytoplasm and processes, clear contours, centrally located nuclei (black arrow - chromatolysis; white arrow - eccentrically located nucleus). Detection of Ca^{2+}-dependent AP activity. Magnification: ×25(a); ×160 (A, D, G, J); ×400(B, E, H, K); ×1000 (C, F, I, L).*

morphological disturbances are observed in PAG neurons, accompanied by morphological changes in intracellular structures. With the damage to nerve cells, a disturbance in the morphological picture is noted. Neurons with clarified cytoplasm are found, indicating a reduced level of metabolic processes. In the cytoplasm of some PAG neurons, high phosphatase activity is detected, indicating the activation of metabolic processes aimed at maintaining the homeostasis of the body disrupted by rotenone intoxication. In other words, the cells are seeking optimal conditions for cell survival. The increase in metabolism apparently reflects the mobilization of the cell's defensive capabilities and the initiation of adaptive-compensatory mechanisms.

In animals that received rotenone injection in combination with BM injections, the characteristic shapes of PAG cells are more often preserved, and the sizes are close to the norm. For most neurons, against a moderately stained cytoplasm, centrally located nuclei are visible, and thin, long processes are observed, indicating the preservation of their connections with neighboring cells and other brain areas (Figure 1G-I).

Intracellular granulation is fine-grained (Figure 1H, I). Among the cells that have preserved their shape and sizes, occasionally, damaged neurons are revealed, with weak phosphatase activity, unclear contours, and no processes, but with noticeable nuclei with a dark nucleolus (Figure 1H).

In animals that received rotenone injection in combination with simultaneous injections of BM and synoestrol, the shapes of PAG cells are preserved, and there is a tendency to preserve the characteristic sizes of neurons (Figure 1J-L). The cytoplasm is intensely stained, and a centrally located nucleus is visible. The lead phosphate deposit in the cytoplasm of cells is either fine-grained or coarse-grained. Neurons have thin, long processes (Figure 1K,L). In PAG, along with cells that have preserved their shape and sizes, occasionally, damaged neurons are revealed, with weak phosphatase activity, distorted contours, no processes, but with centrally located nuclei (Figure 1K,L). Thus, under the influence of BM and synoestrol, a morphological picture of PAG neurons close to normal is observed. Occasionally, damaged neurons can be encountered. Compared to the effect of BM alone, the intensity of cytoplasm staining in the bodies and processes of PAG neurons is higher with the simultaneous administration of BM and synoestrol, indicating an increased level of metabolic processes and the preservation of contacts between PAG neurons and other brain regions with which they are anatomically connected.

The nucleus raphe magnus (NRM) is a cluster of neurons located in the midline of the medulla oblongata. NRM is a serotonergic nucleus located in the rostroventromedial part of the brainstem. The axons of NRM project to the spinal cord, predominantly ending in the spinal cord's dorsal horn. They regulate the release of enkephalins that suppress pain sensations [21].

Mostly, NRM neurons are triangular or somewhat elongated (Figure 2A-B). In the cytoplasm of these neurons, chromophilic substance is diffusely distributed, granulation is observed in the cytoplasm and processes of neurons, while the nuclei appear light (Figure 2B,C). All cells have long axons and several moderately or weakly branching dendrites. The lead phosphate deposit in the form of granules is clearly visible in the processes (Figure 2B,C).

Analysis of data of rotenone-intoxicated rats showed damage to nervous tissue in NRM, primarily affecting nerve cells and their processes (Figure 2D-F). The shape and sizes of neurons are disrupted. Elongated spindle-shaped cells with clarified cytoplasm are observed. Some cells undergo karyocytolysis, and neurons are in a state of severe alteration. Individual neurons shrink, reducing in size and acquiring an elongated curved shape. Processes thin out and shorten (Figure 2E). In the cytoplasm of most NRM neurons, there is dispersion of tigroid substance – lightening of the cytoplasm compared to the norm. The level of AP metabolism is reduced in the cytoplasm, and fine sparse granular sediment is visible in the perikaryons (Figure 2E,F). Many neurons exhibit long processes, but the phosphatase activity in them is greatly reduced. Among neurons undergoing chromatolysis, there are also neurons with activation of AP in the cytoplasm and nucleus. In such damaged neurons, the darkly stained coarse-grained sediment of lead phosphate is unevenly distributed throughout the cell body, obscuring the boundary between the body and the processes. Neurons lack clear contours, their processes are shortened, and the nucleus is ectopic and deformed (Figure 2F). The observed intranuclear granules are new structures induced by rotenone, providing evidence that, under rotenone intoxication conditions, the nucleus of nerve cells undergoes dynamic reorganization [22]. Thus, under conditions of rotenone intoxication, damage to most neurons in NRM is accompanied by lysing of chromophilic substance in the cytoplasm and processes, a decrease in the level of metabolism. Various types of cellular atrophy are revealed, and neurodegenerative changes characteristic of PD develop in NRM.

The morphological integrity of NRM cells is significantly maintained, and their sizes closely resemble the normal state in rats injected with rotenone and administered BM (Figure 2G-I). In neurons that preserved their shape and sizes, centrally located light-colored nuclei are observed, distinctly contrasting with the moderately stained cytoplasm. The lead phosphate deposit in the cell cytoplasm is fine-grained, localized evenly, and there is a slight decrease in phosphatase activity compared to the norm. Thin, long processes with high AP activity are identified, indicating the preservation of connections with neighboring cells and other brain regions. Among the neurons with preserved shape and sizes, there are instances where AP activity is very weak in the cytoplasm and processes, but the shape and sizes are preserved, and the nuclei are centrally located (Figure 2H, I). Occasionally, light-colored affected degenerated neurons are identified, losing shape and sizes, with no apparent processes (Figure 2I). Thus, under the influence of BM, the morphological picture of NRM neurons is close to normal, the level of metabolic processes is slightly reduced compared to the norm, and neurons with low AP activity can be found in some places.

In conditions of rotenone intoxication with simultaneous administration of BM and synoestrol in NRM, neurons with preserved shape and sizes, characteristic of this brain area, are observed (Figure 2J-L). For most of them, against the hyperchromic cytoplasm, centrally located light-colored nuclei appear, and long processes with high AP activity are identified, which is typical for the norm. The lead phosphate deposit is present inside the cytoplasm in the form of coarse-grained formations (Figure 2L), indicating an increased level of AP activity compared to intact animals.

Thus, in the rotenone model of PD, changes in PAG and NRM neurons are characterized by morphological changes in intracellular structures, indicating metabolic disorders. Neurodegenerative damage to important antinociceptive structures of NRM and PAG contributes to the development of persistent chronic pain in PD. Under the protection of BM in the PD model, positive data were obtained on the enhancement of metabolism, preservation and normalization of structures, the strengthening of Ca^{2+}-dependent AP, which characterizes cell survival and prevents neurodegeneration. The therapeutic effect of BM is likely associated with its favorable modulation of the secondary inflammatory process, microglial inhibition, and improvement of brain tissue trophism [23]. The biological compensatory action of BM, along with its

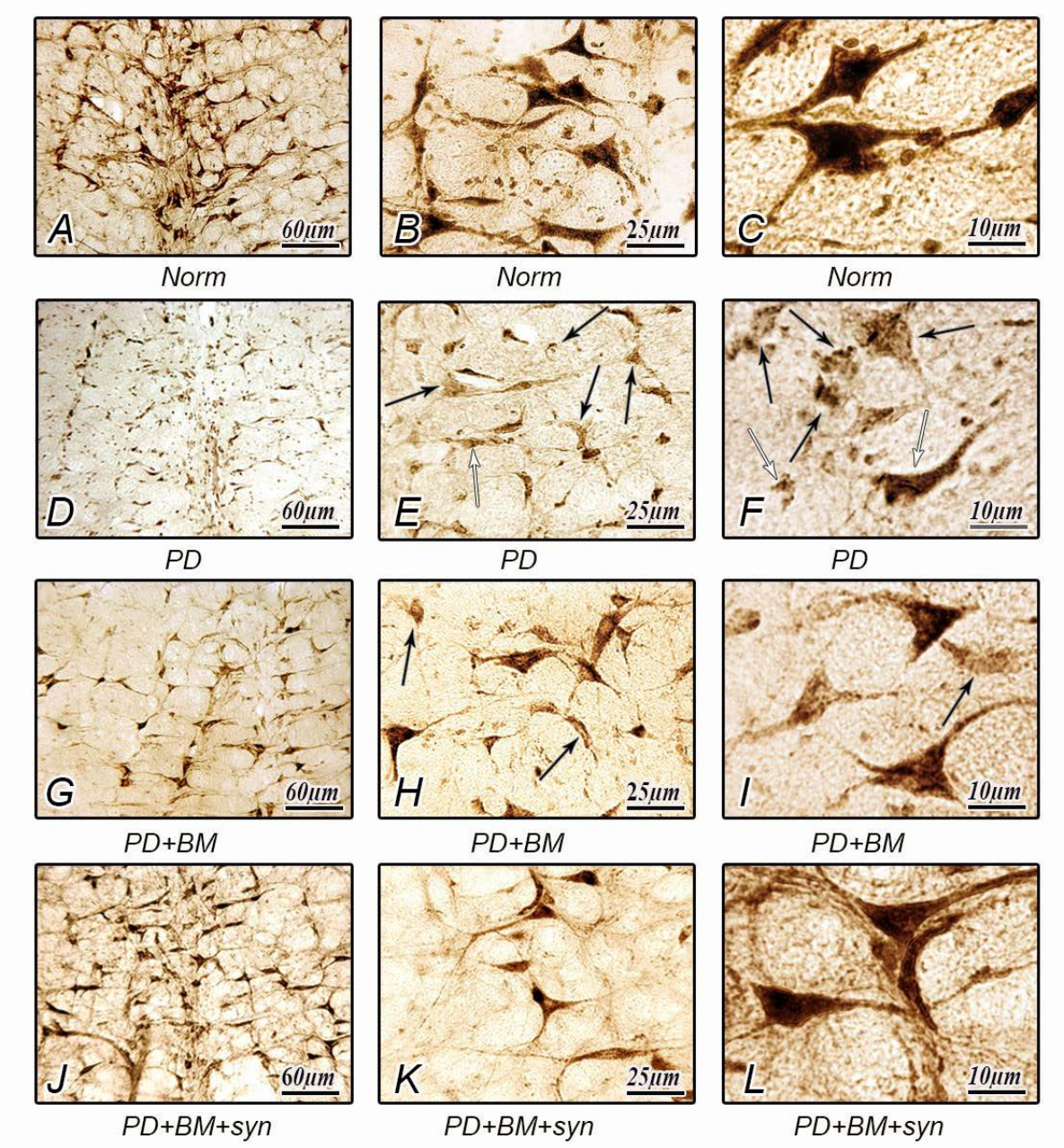

Figure 2. *Microphotographs of cellular structures of the NRM in intact (A-B, Norm) and experimental (G-M) rats (D-F - under conditions of rotenone intoxication (PD); G-I - under conditions of rotenone intoxication in combination with bacterial melanin (PD+BM); J-L - under conditions of rotenone intoxication in combination with simultaneous administration of bacterial melanin and synoestrol (PD+BM+syn); G-F - disruption of shape and sizes, cytoplasm clarification, nucleus ectopia, and thickening of processes; G-I, J-L - preservation of structural properties of neurons, high AP activity in the cytoplasm and processes, nuclei centrally located (black arrow - chromatolysis; white arrow - eccentrically located nucleus). Detection of Ca^{2+}-dependent AP activity. Magnification: ×160 (A, D, G, J); ×400 (B, E, H, K); ×1000 (C, F, I, L).*

immunomodulatory effect, allows alleviating the manifestations of neurodegenerative disorders. Pharmacokinetic studies confirmed the ability of BM to cross the blood-brain barrier [24]. Moreover, BM is excreted through the liver and kidneys, indicating a favorable pharmacokinetic profile for its use as a therapeutic and neuroprotective agent.

Conclusion.

Thus, with the simultaneous administration of BM and synoestrol, a more effective neuroprotective effect is noted, probably due to the enhanced protective properties of both components when administered together, activating the mechanisms of their therapeutic doses' action on the structures affected in PD. The obtained data provide a basis to consider BM and synoestrol as neuroprotective agents, suggesting the possibility of recommending them for the therapy of PD either individually or in combination.

Author contributions.

DMH, NKA, AZA, KhVP, SJS, PMV and KKV performed the experiments and data analysis. DMH and KKV provided histological interpretation. DMH, NKA, AZA, KhVP, SJS,

PMV and KKV provided advice on data interpretation. DMH, KKV and NKA 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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Оценка морфофункционального состояния головного мозга крыс на модели болезни Паркинсона: влияние терапевтических средств животного и синтетического происхождения

Даниелян М.А[1], Небогова К.А[1]*, Аветисян З.А[1], Хачатрян В.П[1], Саркисян Дж.С[1], Погосян М.В[1], Карапетян К.В[1].

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

Резюме

При нейродегенеративных заболеваниях, особенно при болезни Паркинсона (БП), в нейродегенерацию

часто вовлекаются антиноцицептивные центры, что приводит к стойкой боли, не поддающейся лечению наркотическими веществами. В этом исследовании изучались околоводопроводное серое вещество (PAG) и большое ядро шва (NRM), которые являются частями антиноцицептивной системы мозга. В условиях ротеноновой интоксикации (экспериментальная модель БП) в нейронах PAG и NRM наблюдались морфологические изменения внутриклеточных структур, свидетельствующие о метаболических нарушениях, характерных для БП (изменения формы и размеров тел и отростков нейронов, нарушение активности кислой фосфатазы в цитоплазма нейрона). Под влиянием бактериального меланина и в сочетании с синэстролом отмечались положительные изменения структурных свойств в нейронах PAG и NRM по сравнению с ротеноновой моделью БП. Наблюдалось сохранение морфологических характеристик, типичных для этих областей мозга, при этом клетки имели форму и размеры, близкие к нормальным. Кроме того, под влиянием этих терапевтических средств выявлялось повышение активности кислой фосфатазы в цитоплазме клеток, что свидетельствует об ускорении метаболических процессов (метаболической активации), которые были нарушены при ротеноновой интоксикацией. Полученные данные позволяют предположить, что бактериальный меланин и синэстрол могут выступать в качестве потенциальных нейропротекторов в отношении нейронов головного мозга крыс PAG и NRM в ротеноновой модели БП. Необходимы дальнейшие исследования для выяснения механизмов действия терапевтических доз и предложения их использования при лечении БП как в изолированной, так и в комбинированной терапии.

Ключевые слова: околоводопроводное серое вещество, большое ядро шва, болезнь Паркинсона, бактериальный меланин, синэстрол

ვირთხის ტვინის მორფოფუნქციური მდგომარეობის შეფასება პარკინსონის მოდელში: ცხოველური და სინთეზური წარმოშობის თერაპიული აგენტების გავლენა

დანიელიან მ.ჰ[1], ნებოგოვა კ.ა[1*], ავეტისიან ზ.ა[1], ხაჩატრიან ვ.პ[1], სარქისიან ჯ.ს[1], პოღოსიან მ.ვ[1], კარაპეტიან კ.ვ[1]

[1]ორბელის ფიზიოლოგიის ინსტიტუტი NAS RA, ერევანი 0028, სომხეთი

ბსტრაქტული

ნეიროდეგენერაციულ დაავადებებში, განსაკუთრებით პარკინსონის დაავადებისას (PD), ანტინოციცეპტიური ცენტრები ხშირად მონაწილეობენ ნეიროდეგენერაციაში, რაც იწვევს მუდმივ ტკივილს, რომელიც არ რეაგირებს ნარკოტიკულ ნივთიერებებზე. ამ კვლევამ გამოიკვლია პერიაკვედუქტური ნაცრისფერი ნივთიერება (PAG) და ბირთვი რაფე მაგნუს (NRM), ტვინის ანტინოციცეპტური სისტემის კომპონენტები. როტენონით ინტოქსიკაციის პირობებში (ექსპერიმენტული PD მოდელი), უჯრედშიდა სტრუქტურებში მორფოლოგიური ცვლილებები დაფიქსირდა PAG და NRM ნეირონებში, რაც მიუთითებს PD-სთვის დამახასიათებელ მეტაბოლურ დარღვევებზე (ნეირონების სხეულებისა და პროცესების ფორმისა და ზომის შეცვლა, მჟავა ფოსფატაზას აქტივობის დარღვევა ნეირონის ციტოპლაზმა). ბაქტერიული მელანინის გავლენის ქვეშ და სინოესტროლთან ერთად, სტრუქტურულ თვისებებში დადებითი ცვლილებები დაფიქსირდა PAG და NRM ნეირონებში PD-ს როტენონის მოდელთან შედარებით. ეს მოიცავდა თავის ტვინის ამ რეგიონებისთვის დამახასიათებელი მორფოლოგიური მახასიათებლების შენარჩუნებას, უჯრედები ავლენენ ფორმებს და ზომებს ნორმასთან ახლოს. გარდა ამისა, ამ თერაპიული აგენტების გავლენით, გამოვლინდა ფოსფატაზას აქტივობის მომატება უჯრედის ციტოპლაზმაში, რაც მიუთითებს მეტაბოლური პროცესების დაჩქარებაზე (მეტაბოლური აქტივაცია) დარღვეული როტენონის ინტოქსიკაციით. მიღებული მონაცემები ვარაუდობს, რომ ბაქტერიული მელანინი და სინოესტროლი შეიძლება იმოქმედონ, როგორც პოტენციური ნეიროპროტექტორული აგენტები ვირთხების ტვინში PAG და NRM ნეირონების წინააღმდეგ PD-ს როტენონის მოდელში. საჭიროა შემდგომი კვლევა თერაპიული დოზების მოქმედების მექანიზმების გასარკვევად და PD-ის სამკურნალოდ მათი გამოყენების შეთავაზებისთვის, იზოლირებულ ან კომბინირებულ თერაპიაში.

საკვანძო სიტყვები: პერიაქვედუქტალური ნაცრისფერი ნივთიერება, რაფე მაგნუსის ბირთვი, პარკინსონის დაავადება, ბაქტერიული მელანინი, სინოესტროლი