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NEUROPROTECTIVE EFFECTS OF DEPROTEINIZED CALF BLOOD HAEMODIALYSATE IN CASE OF INTRANASALLY ADMINISTRATION UNDER CHRONIC CEREBRAL ISCHEMIA

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Statistical data on acute cerebrovascular accident rates in our country highlight the progressive increase in new stroke cases. Such an extremely unsatisfactory situation has arisen due to some reasons, one of which is the ineffective pharmacotherapy of strokes, which is a sequence of our incomplete understanding of chronic cerebral ischemia pathophysiological mechanisms. It seems interesting and expedient to clarify the efficacy of intranasal administration of deproteinized haemoderivative of young calves' blood throughout the post-ischemic period from the point of view of the potential restoration of impaired mnestic functions.

The aim of the work was to determine the efficacy of endonasal administration of deproteinized calf blood dialysate for restoring memory impairment in rats throughout the post-ischemic period dynamics.

Materials and methods. A model of chronic brain ischemia was reproduced by carotid arteries bilateral isolation and ligation. Rats with chronic brain ischemia were intranasally administered deproteinized calf blood dialysate. The rats were observed for 7 days, during which the animals' mnestic functions were examined in the forms of active avoidance conditioned reflex and a conditioned food reflex development.

Results and their discussion. The data obtained indicate the formation of mnestic dysfunctions during the postischemic period, which are characterized by learning process decline as well as short– and long-term memory marked suppression under active avoidance conditioned reflex, which is combined with conditioned food reflex learning, preservation and extinction processes disturbances. The registered amnestic effects are persistent, noted 24 hrs after the carotid arteries occlusion and continued throughout the whole trial. Considering the mechanisms for achieving neuroprotective effect of deproteinized calf blood dialysate and its intranasal route of administration, the authors suppose it appropriate and pathogenetically justified to include deproteinized calf blood dialysate in a comprehensive scheme of pharmacological correction of cognitive disorders or their prevention under cerebrovascular pathology.

Keywords: chronic cerebral ischemia, cognitive disorders, deproteinized calf blood dialysate, pathogenetic mechanisms, pharmacological correction.

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НЕЙРОПРОТЕКТИВНІ ЕФЕКТИ ДЕПРОТЕЇНОВАНОГО ГЕМОДІАЛІЗАТУ ТЕЛЯЧОЇ КРОВІ ПРИ ІНТРАНАЗАЛЬНОМУ ВВЕДЕННІ ЗА УМОВ ХРОНІЧНОЇ ІШЕМІЇ МОЗКУ

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Мета дослідження – визначення ефективності ендоназального введення депротеїнованого гемодіалізату телячої крові у відновленні мнестичних порушень у щурів в динаміці постішемічного періоду. Отримані дані свідчать про формування мнестичних дисфункцій в динаміці постішемічного періоду, які характеризуються погіршенням процесу навчання, а також пригніченням коротко– та довгострокової пам'яті за умов умовного рефлексу активного уникнення та харчового умовного рефлексу. Відзначені амнестичні ефекти є стійкими, реєструються у щурів вже через 24 год після білатеральної оклюзії сонних артерій та тривають протягом всього досліду. Зважаючи на механізми реалізації нейропротективного ефекту депротеїнованого гемодіалізату телячої крові та ендоназального шляху його введення, автори вражають доцільним та патогенетично обґрунтованим включення депротеїнованого гемодіалізату телячої крові до комплексної схеми фармакологічної корекції когнітивних розладів або запобігання їх розвитку при цереброваскулярій патології.

Ключові слова: хронічна ішемія мозку, когнітивні порушення, депротеінований діалізат телячої крові, патогенетичні механізми, фармакологічна корекція.

Introduction. The statistical indicators of acute cerebrovascular disease incidence in our country unfortunately demonstrate a negative trend, reflecting a progressive increase in new stroke cases [1, 1]. According to the statistics, our country ranks among the leading nations in Europe, highlighting a global trend of the so-called "cerebrovascular catastrophe," as declared by the World Health Organization at the end of the last century.

Currently, patients who have suffered a stroke due to acute cerebrovascular events constitute the majority of individuals with cerebrovascular pathology [2, 3]. Consequently, from

social, economic, and medical perspectives, the treatment of such patients, their comprehensive rehabilitation – including the restoration of motor, sensory, cognitive, and other physiological functions – and the prevention of stroke-related complications are of great importance [2, 4].

This quite unsatisfactory situation has arisen due to a number of factors, among which, if not the most critical, are the inefficacy of pharmacotherapy for stroke patients and the incomplete understanding of the pathophysiological mechanisms underlying chronic cerebral ischemia (CCI), and the progressively developing vascular catastrophe [5].

From a fundamental perspective, it is essential to consider the complete disruption of regulatory activity and the loss of central control over descending projections due to cascading, multi-chain positive feedback mechanisms in CCI [5]. It complicates the rationale for using specific pharmacological agents for treating relevant patients, as well as for secondary neuroprotection. The effectiveness of such neuroprotection must be directed toward restoring lost motor, sensory, and other physiological functions, as well as emotional expression and the modulation of stereotypic, coordinative, exploratory, and investigative behaviours, among others [1, 6].

We conducted a series of experimental studies aimed at restoring lost or impaired behavioural responses in the post-ischemic period [7]. When designing these studies, it is crucial to consider the challenges associated with drug penetration through the blood-brain barrier to achieve a direct therapeutic effect. This limitation prompted us to explore alternative drug administration routes to the affected area.

Current positive experimental and clinical findings on the intranasal administration of pharmacological agents [8, 9] captured our interest regarding their potential efficacy in the context of CCI. Therefore, given the aforementioned considerations, we find it both relevant and promising to investigate the effectiveness of intranasal administration of deproteinized haemoderivative obtained from young calves' blood during the post-ischemic period, particularly in terms of its potential to restore impaired cognitive functions through the activation of sanogenetic mechanisms in this complex pathophysiological condition.

The aim of this study was to evaluate the efficacy of intranasal administration of deproteinized calf blood dialysate in restoring rats' memory impairments during the post-ischemic period.

Materials and methods. The experiments were conducted under chronic conditions on 62 male Wistar rats weighing 180–250 g, housed in a vivarium. The housing, handling, and experimental procedures were carried out in compliance with the General Ethical Principles of Animal Experiments, approved by the Fifth National Congress on Bioethics (Kyiv, 2013). Additionally, the study adhered to the recommendations of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1985), the methodological guidelines of the State Pharmacological Centre of the Ministry of Health of Ukraine (Preclinical Drug Studies, 2001), and the principles of humane treatment of laboratory animals.

The CCI model was induced by skin incision, isolation, and bilateral ligation of the carotid arteries [8]. The animals were divided into the following groups:

Group 1 (Control): Intact rats that underwent skin incision without carotid artery ligation (n = 7).

Group 2 (Experimental): Rats with carotid artery ligation and CCI induction (n = 12).

Group 3 (Experimental + deproteinated calf blood dialysate*)*: Rats with CCI that received intranasal administration of deproteinated calf blood dialysate (DCBD; 10 μL; "Valeant Pharmaceuticals Switzerland GmbH", Switzerland; n = 12).

Following carotid artery ligation, the rats were observed for seven days. During this post-ischemic period, memory functions were assessed using two behavioural tests: the conditioned active avoidance reflex (CAAR) test and the conditioned food reflex test [10]. The experiments began with the assessment of locomotor activity by counting the number of squares crossed in the open field test and the number of vertical rearing episodes demonstrated by the rats over a 2-min period [7]. The obtained results were statistically analysed using the Bonferroni parametric criterion. The minimum statistical significance threshold was set at $p<0.05$.

Results and discussion. In rats with CCI, immobilisation was observed starting from the first day of the experiment, as evidenced by a significantly lower number of squares crossed in the open field test compared to the control group (a 4.4-fold decrease; $p<0.01$; Fig. 1A). Over the following seven days of observation, CCI rats were only able to cross, on average, a single square in the open field, typically remaining in close proximity to their initial position on the horizontal plane.

Similar trends were observed for vertical rearing activity, which showed a significant reduction during the post-ischemic period (Fig. 1B). Both horizontal and vertical motor activity measures were significantly less in CCI group compared to control values ($p<0.01$).

In rats during the post-ischemic period, an expressed impairment of learning processes, as well as short-term and long-term memory, was observed. This was evident from a significant increase in the number of pairings of the conditioned and unconditioned stimuli required for the formation of the CAAR (Fig. 2A), its retrieval in 24 hrs (Fig. 2B), and its retention after seven days (Fig. 2C) from the moment of induction.

Indeed, on the first day of CCI, the measured parameter necessary for the formation of CCI (the learning process) was 43.6% higher than the corresponding value in the control observations ($p<0.05$; Fig. 2A). The number of pairings of conditioned and unconditioned stimuli required for CAAR development after 24 hrs and 7 days from the acquisition of the conditioned reflex was found to be 92.4% and 94.8% greater, respectively, than in the control group (in both cases, $p<0.05$; Fig. 2B and 2C).

Starting from the 3rd day of the trial, the values of learning, short-term memory, and long-term memory in CCI rats that received intranasal DCBD were comparable to those in the control group and were significantly lower when compared to the corresponding values in the CCI group without pharmacological correction ($p<0.05$; Fig. 2).

When examining cognitive dysfunctions in CCI rats using the conditioned food reflex test, it was found that attempts to enter the maze and successfully locate food during training were effective only on the third day of the

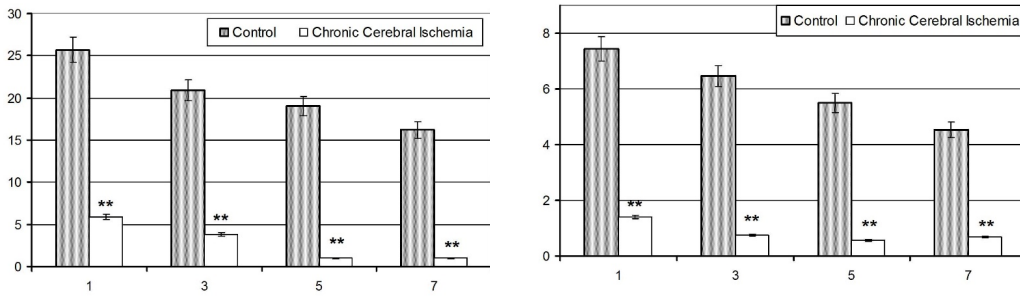

Fig. 1. Distribution of Horizontal (A) and Vertical (B) Motor Activity in the Open Field Test in Rats During the Post-Ischemic Period
Legend: The x-axis represents the observation days (duration of the experiment). The y-axis indicates the number of squares crossed (Figure A) and the number of vertical rearing episodes (Figure B) in the open field test

Notes: *** – p<0.01 – statistically significant differences of the investigated parameters compared with corresponding control values.*

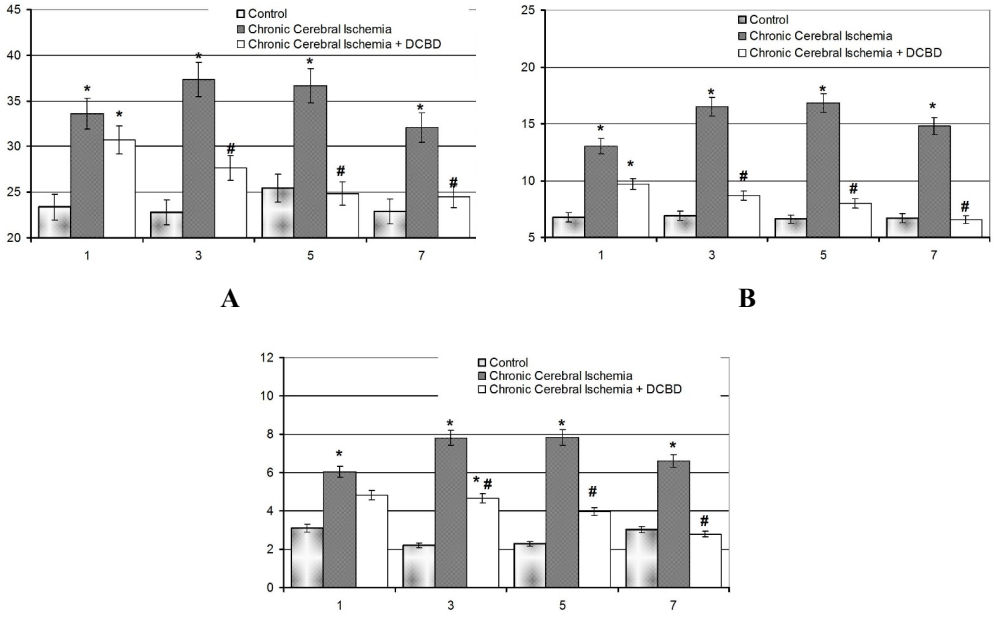

Fig. 2. Effect of Intranasal DCBD Administration on Learning Processes (A), Short-Term Memory (B), and Long-Term Memory (C) in Rats During the Post-Ischemic Period
Legend: The x-axis represents the observation days (experiment duration). The y-axis indicates the number of pairings of conditioned and unconditioned stimuli

Notes: * – p<0.05 – statistically significant differences of the investigated parameters compared with corresponding control values; # – p<0.05 – statistically significant differences of the investigated parameters compared with the same values in CCI rats without pharmacological correction.*

post-ischemic period (Fig. 3A). In contrast, the assessment of retention and extinction of the conditioned reflex showed significant results only on the 5th day of the experiment (Fig. 3B and 3C, respectively). This delay can be attributed to the animals' akinesia during the initial days of the post-ischemic period.

During the training period, the number of attempts to enter the maze leading to successful food localisation began to appear only on the 5th day of the postischemic period. This index in rats with CCI receiving intranasal DCBD was 2.3±0.3, which on 91.7% prevailed the corresponding value in rats with CCI without pharmacological correction (p<0.05; Fig. 3A). This expression of DCBD intranasal administration efficacy in learning process improving was maintained until the end of the trial.

The intranasal DCBD administration in rats with CCI significantly improved the conditioned food reflex retention (Fig. 3B) and extinction (Fig. 3C) when compared to

corresponding values in CCI rats without pharmacological correction (in all cases, $p<0.05$). This similar pattern of mnestic functions recovery, assessed by the criteria of the conditioned food reflex under the influence of intranasal DCBD, was consistently observed until the end of the experiment.

Thus, the data obtained characterises a sufficiently prolonged postischemic period during which rats exhibit mnestic dysfunctions. The cognitive impairments formation is confirmed by the establishment and subsequent persistence of conditioned active avoidance and conditioned food reflexes. A clear deterioration in the learning process is evident, along with pronounced suppression of both short-term and long-term memory during CAAR, which is associated with disruptions in learning, retention, and extinction of the conditioned food reflex. These mnestic effects are stable, being recorded in rats just 24 hrs after carotid arteries bilateral occlusion and lasting throughout the experiment.

From a discussion perspective, we would like to emphasise the following four aspects. Firstly, from a methodological standpoint, the relevance of the applied CCI model to the corresponding clinical condition has been confirmed, as similar mnestic impairments, disorders of higher nervous activity, and psychological manifestations are observed in patients with ischaemic stroke and dominate the clinical picture of cerebrovascular diseases [4, 11–13].

Secondly, we note that the cognitive impairments formed in rats clearly reflect the neuropathophysiological mechanisms that persist in the body during CCI, characterised by nearly complete disorganisation and/or dysregulatory effects [5, 12]. In our opinion, such a dysregulatory effect also contributes to the disruption of the learning process during the formation of conditioned food reflexes due to an additional mechanism of motor deficit alongside ischaemic neuronal damage. This combination prevents rats from forming a memory engram in the 8-arm radial maze test during the post-ischaemic period. This is consistent with similar data on reduced motor activity, development of neurological deficits, and emotional disturbances in experimental CCI under conditions of vertebral blood supply insufficiency [14]. We believe that the akinesia observed in animals with CCI determines the severity of learning processes, retention of memory engrams, and resistance to their extinction.

Thirdly, we consider it an important finding that cognitive impairments can be restored during the development and retention of conditioned reflexes throughout the post-ischaemic period due to DCBD administration. Interestingly, the DCBD anti-ischaemic efficacy was achieved through its endonasal administration, and the process of mnestic impairments restoring was sustained, starting from the 3rd day of the experiment and continuing until its conclusion.

The obtained data regarding the effectiveness of DCBD endonasal route of in CCI aligns with corresponding results highlighting the effectiveness of similar routes for administering pharmacological compounds in cases of ischaemic and traumatic brain injuries [8]. Through endonasal admin-

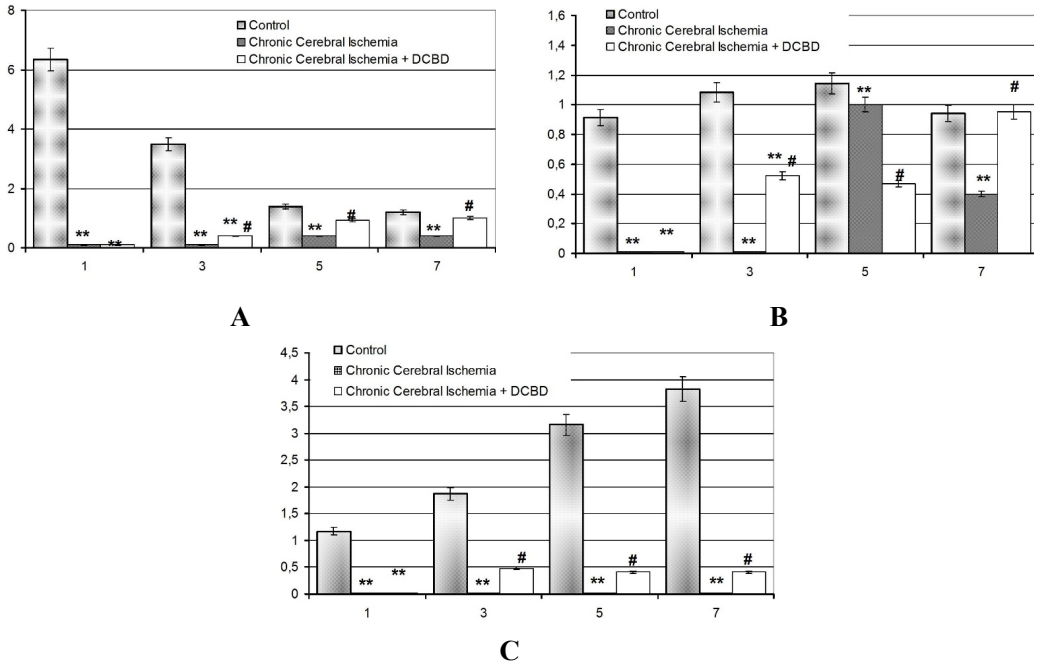

Fig. 3. Effect of Intranasal DCBD Administration on Learning Processes (A), Retention (B) and Extinction (C) of the Conditioned Food Reflex in the 8-Arm Radial Maze Test in Rats During the Post-Ischemic Period

Legend: The x-axis represents the observation days (experiment duration). The y-axis indicates the number of attempts to enter the maze beams leading to successful food localization

Notes: *** – $p<0.01$ – statistically significant differences of the investigated parameters compared with corresponding control values; # – $p<0.05$ – statistically significant differences of the investigated parameters compared with the same values in CCI rats without pharmacological correction.*

istration, pharmacological agents can immediately penetrate the brain without the need to cross the blood-brain barrier, which generally enhances the effective therapeutic concentration and facilitates a quick and efficient response [8, 9, 15].

It is well known that, in clinical conditions of vascular injury, the speed of the implementation of the neuroprotective effect and its effectiveness determine the success of secondary neuroprotection. Therefore, we consider the obtained data as experimental justification for the appropriateness of endonasal administration of pharmacological agents with neuroprotective mechanisms in clinical settings for acute cerebrovascular circulation disorders.

In the fourth point, it is interesting to examine the neuropathophysiological mechanisms of post-ischaemic memory disorders, detailing that the chosen models for forming conditional reflexes involve clarifying the mechanisms of various types of memory, specifically: visual, spatial, reference, working, etc. [11]. It was proved that, with DCBD endonasal administration, the most effective recovery was observed in the processes of learning and short-term memory. Our attempts to alleviate the deficit in long-term memory were somewhat less effective, as its neurophysiological mechanisms are more complex and are more significantly affected by CCI. Additionally, it is important to recall the substantial contribution of the reduced "motor component" to the expression of long-term memory deficits during the postischemic period [7].

Finally, we supposed important to follow the mechanism of DCBD neuroprotective efficacy in the used CCI model. DCBD is a compound of natural origin with reparative and regenerative effects in damaged tissues by increasing both cellular proliferation stimulation and their migration directly to focus of injury [16, 17]. DCBD is also shown to facilitate oxygen utilization and stimulate glucose transport by cells in conditions of hypoxia and metabolic resources reduction [17]. Taking into account the above-mentioned effects DCBD contributed to neurological status normalization and blood pressure stabilization in patients during the post-traumatic period [5] and was also effective in brain bioelectrical activity normalization in experimental brain trauma [18].

Thus, considering the mechanisms of DCBD neuroprotective effect and its route of administration, we find it appropriate and pathogenetically justified to include it in a comprehensive pharmacological correction scheme for cognitive disorders or to prevent their development in cerebrovascular pathologies.

Consequently, the demonstrated effectiveness of this pathogenetically justified approach to restoring memory impairments during the post-ischaemic period indicates the development of a neuroprotective effect and the potential to enhance treatment efficacy for patients with chronic cerebral ischaemia through the intranasal DCBD administration. Therefore, we consider the obtained data as experimental grounds for the clinical testing of DCBD effects as part of secondary neuroprotection, which could facilitate the recovery of cognitive dysfunctions in patients with acute cerebrovascular disorders.

Conclusions. The data obtained indicate the formation of pronounced post-ischaemic memory dysfunctions, characterised by a deterioration in the learning process and significant suppression of both short– and long-term memory in the context of the active avoidance conditional reflex, which is associated with disruptions in the processes of learning, retention, and extinction of the food conditional reflex.

The noted amnestic effects are stable, being recorded in rats 24 hrs after carotid arteries bilateral occlusion and lasting throughout the entire experiment. The neuroprotective efficacy of DCBD was achieved with the intranasal administration of the drug, and the process of restoring memory impairments was consistent, starting from the third day of the experiment until its completion.

Considering the mechanisms of DCBD neuroprotective effect realization and the endonasal route of its administration, we consider it appropriate and pathogenetically justified to include it in a cognitive disorders complex scheme of pharmacological correction or their development prevention in cerebrovascular pathology.

BIBLIOGRAPHY

1. Karger G. 30 years of Cerebrovascular Diseases. *Cerebrovasc Dis*. 2021; 50(1): 1. doi: 10.1159/000514372.
2. Khramtsov DN, Stoyanov ON, Muratova TN, Pulyk OR. Neurorehabilitation in the Early Recovery Period of Ischemic Stroke. Pharmacology Support. *Acta Balneologica*. 2021; 63(1): 22-25. doi: 10.36740/ABAL202101103
3. Cherniy T.V., Cherniy V.I., Svitlytska D.V. Chronic cerebral ischemia. modern view of the problem. Clinical and Preventive Medicine. 2023; 3(25):100-112. https://doi.org/10.31612/2616-4868.3(25).2023.14
4. Khramtsov DM, Stoyanov OM, Gruzevskiy OA, Shevchuk GYu. Comprehensive neurorehabilitation of post-stroke patients. *Medical Science of Ukraine*. 2022; 18(3): 52-59. (In Ukrainian). doi: https://doi.org/10.32345/2664-4738.3.2022.08
5. Béjot Y, Bailly H, Durier J, Giroud M. Epidemiology of stroke in Europe and trends for the 21st century. *Press Medical*. 2016; 45: 391–398. doi: 10.1016/j.lpm.2016.10.003
6. Chyniak O. Features of neurocognitive impairments in patients with mild cognitive disorder of different etiology. *Eastern Ukrainian Medical Journal.* 2022;10(4):351-359. https://doi.org/10.21272/eumj.2022;10(4):351-359
7. Kirchev VV. Muscle activity and emotional behavior changes in rats with chronic brain ischemia via comprehensive pathogenetic correction. *Journal of Education, Health and Sport*. 2023; 13(3): 442-257. doi: 10.12775/JEHS.2023.13.03.054
8. Stoyanov OM, Vastyanov RS, Mirdzhurayev YeM, Son AS, Volokhova GA, Kalashnikov VI. Possibilities of intranasal therapeutic influence on the autonomic system in rehabilitation neurology. *International Neurological Journal (Ukraine)*. 2024; 20(3): 156-165. (In Ukrainian). doi: 10.22141/2224-0713.20.3.2024.1070
9. Belenichev I, Aliyeva O, Burlaka B, Burlaka K, Kuchkovskyi O, Savchenko D. et al. Development and Optimization of Nasal Composition of a Neuroprotective Agent for Use in Neonatology after Prenatal Hypoxia. *Pharmaceuticals*. 2024; 17(8): 990. https:// doi.org/10.3390/ph17080990.

10. Kirchev V.V. Cognitive function restoration in rats with chronic brain ischemia using Semax and hopantenic acid comprehensive administration. Journal of Education, Health and Sport. 2023; 13(4): 404-422. doi: 10.12775/JEHS.2023.13.04.046
11. Elendu C, Amaechi DC, Elendu TC, Ibhiedu JO, Egbunu EO, Ndam AR. et al. Stroke and cognitive impairment: understanding the connection and managing symptoms. *Ann Med Surg (Lond)*. 2023; 85(12): 6057–6066. Doi: 10.1097/MS9.0000000000001441
12. Huang YY, Chen SD, Leng XY, Kuo K, Wang ZT, Cui M. et al. Post-Stroke Cognitive Impairment: Epidemiology, Risk Factors, and Management. *J Alzheimers Dis*. 2022; 86(3): 983-999. Doi: 10.3233/JAD-215644.
13. Yelyzaveta Y, Oleksandr K, Svitlana T. Biological effects of schisandra chinensis medicines and prospects of their use in clinical medicine (literature review). *Journal of V. N. Karazin Kharkiv National University. Series 'Medicine'*. 2022;44:127-139. DOI: https://doi.org/10.26565/2313-6693-2022-44-10
14. Andreyeva T.O., Stoyanov O.M., Chebotaryova G.M., Vastyanov R.S., Kalashnikov V.I., Stoyanov A.O. Comparative clinical and morphometric investigations of cervical stenosis of the spinal canal in humans and dogs. *Regulatory Mechanisms in Biosystems,* 2022; 13(3): 301–307. doi:10.15421/022239
15. Rajeev V, Fann DY, Dinh QN, Kim HA, De Silva TM, Lai MKP. et al. Pathophysiology of blood brain barrier dysfunction during chronic cerebral hypoperfusion in vascular cognitive impairment. *Theranostics*. 2022; 12(4): 1639-1658. Doi: 10.7150/thno.68304.
16. Konturek SJ, Drozdowicz D, Pytko-Polonczyk J, Brzozowski T, Bielański W. Solcoseryl in prevention of stress-induced gastric lesions and healing of chronic ulcers. *J Physiol Pharmacol*. 1991; 42(1): 73-84.
17. Song MS, Ku YA, Kim S, Chung MH, Kim YH, Kim DH. Comparison of Corneal Epithelial Wound Healing between Topical RCI001, Solcoseryl, and Polydeoxyribonucleotide in the Murine Ocular Alkali Burn Model. *Korean J Ophthalmol*. 2023; 37(3): 236-244. doi: 10.3341/kjo.2023.0019.
18. Sysoev Yu.I., Prikhodko V.A., Chernyakov R.T., Idiyatullin R.D., Musienko P.E., Okovityi S.V. Effects of Alpha-2 Adrenergic Agonist Mafedine on Brain Electrical Activity in Rats after Traumatic Brain Injury. *Brain Sci.* 2021; 11(8): 981. doi: 10.3390/brainsci11080981

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