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THE EFFECT OF TABLETS CONTAINING DRY EXTRACT OF PEONY ROOTS, L-TRYPTOPHAN, AND GLYCINE ON THE CONDITION OF THE BRAIN IN THE CONTEXT OF EXPERIMENTAL CRANIO-CEREBRAL TRAUMA

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The aim of this work *was to experimentally substantiate the neuroprotective potential of new combined tablets containing dry extract of peony roots, L-tryptophan, and glycine to eliminate the existing gap in the pharmacotherapy of traumatic brain injury (TBI) associated with the insufficient effectiveness of current monotherapeutic approaches.*

Material and methods. *The study was conducted on 40 male white rats weighing 200–250 g. The TBI model was induced by the free fall of a weight onto a fixed head of the animal. The test preparation was administered orally for 7 days. The effectiveness was assessed using morphological, morphometric, and biochemical methods, including the determination of neuron-specific enolase (NSE) and S100 protein levels in blood serum, as well as analysis of the condition of neurons in the sensorimotor cortex and hippocampus.*

Results. *Rats with TBI demonstrated a significant increase in NSE and S100 levels, a decrease in the number of normochromic neurons, activation of glial cells, and cerebral tissue oedema. Administration of the combined drug contributed to a reduction in neuronal damage markers, a decrease in the glio-neuronal index, and normalization of brain microstructures. Morphological examination revealed preservation of the neuronal layer and a reduction in destructive changes, indicating a pronounced neuroprotective effect of the drug.*

Conclusions*. The developed combined drug demonstrated an effective protective effect on brain tissue under conditions of experimental TBI. The obtained results substantiate the feasibility of further preclinical studies to investigate its mechanisms of action and potential clinical application*

Keywords*: traumatic brain injury, neuroprotector, glycine, tryptophan, peony, neurons, enolase, S100 protein, rats, histology*

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1. Introduction

Traumatic brain injury (TBI) continues to be one of the leading causes of mortality and long-term disability both in the world and in Ukraine. Millions of cases of TBI are registered annually, a significant part of which are severe lesions that cause persistent impairment of cognitive, motor, emotional and sensory functions. It is alarming that brain injuries remain the leading cause of mortality among people under the age of 45 [1].

Of particular note is the significant increase in the number of TBI cases among the population of Ukraine, caused by full-scale aggression. The armed conflict has led to a sharp increase in cases of open, penetrating and explosive brain injuries among both military and civilians, including children. In combat conditions, traumatic brain injuries account for up to 78% of all cases of central nervous system damage [2].

Despite advances in neurosurgery and intensive care, effective pharmacological support for the restoration of damaged brain structures remains limited. This creates an urgent need for the development of new drugs with a complex effect - neuroprotective, nootropic, antioxidant and regenerative. This problem becomes especially relevant in conditions of high social and economic pressure associated with long-term treatment and rehabilitation of patients.

Today, various approaches to pharmacological support for TBI have been developed in international practice. In the EU countries (in particular, in Germany) and Japan, drugs with proven neuroprotective effects are actively used, in particular citicoline, cerebrolysin, magnesium sulfate, as well as herbal preparations based on peony (*Paeonia officinalis*), ginkgo (*Ginkgo biloba*), and valerian (*Valeriana officinalis*), mainly during the recovery phase [3]. In the USA, the main emphasis is on standardized treatment protocols, while supplements (glycine, tryptophan) are used as dietary supplements for self-administration [4]. China and Japan demonstrate the successful integration of traditional herbal medicine into modern medicine, using complex drugs based on paeoniflorin, L-tryptophan and other bioactive substances with neuroprotective potential [5].

In Ukrainian practice, insufficient effectiveness of monotherapy - nootropics, antioxidants or neuroprotectors, which is not always confirmed in clinical protocols, is often observed. This causes polypharmacy and reduc-

es adherence to treatment, which, in turn, reduces its effectiveness. That is why there is a need to create combined drugs that would combine the positive properties of several active substances, ensuring synergism of action and minimizing side effects [6].

In this context, the development of a formulation that includes the following components appears promising: L-tryptophan, an essential amino acid involved in serotonin synthesis, modulation of inflammation, and reduction of oxidative stress; glycine, an amino acid with nootropic and antioxidant properties that normalizes neuronal activity and reduces glutamate toxicity; and peony root extract (*Paeonia anomala*), which represents a complex multicomponent phytoconcentrate whose pharmacological activity is determined by the presence of a range of biologically active substances (BAS). The main BAS identified in the root of *Paeonia anomala* include: paeoniflorin (a monoterpene glycoside) – exhibits neuroprotective, anti-inflammatory, antioxidant, and sedative effects; flavonoids (kaempferol, quercetin) – possess antioxidant and anti-inflammatory properties; phenolic compounds – contribute to the reduction of oxidative stress; tannins and organic acids, which potentiate the overall biological activity of the extract. The neuroprotective and anti-inflammatory effects of paeoniflorin and peony extracts have been confirmed by experimental studies, particularly regarding the reduction of neuroinflammation, modulation of glutamatergic transmission, and inhibition of oxidative neuronal damage [7].

Based on the above, it can be concluded that the development and study of the psychotropic and neuroprotective properties of a combined drug based on L-tryptophan, glycine, and dry extract of peony roots is a relevant scientific task that meets both the internal needs of Ukrainian medicine and global pharmaceutical trends.

The aim of the research. The aim of the study was to experimentally substantiate the neuroprotective potential of new combination tablets containing dry extract of peony roots, L-tryptophan, and glycine, in order to eliminate the existing gap in the pharmacotherapy of TBI associated with the insufficient effectiveness of modern monotherapeutic approaches.

2. Research planning

Step 1. Analysis of publications on the prevalence of TBI in Ukraine and the relevance of the development of domestic combined drugs with psychotropic and neuroprotective properties.

Step 2. Modelling TBI in rats by freely falling a load onto the fixed head of the animal and administering the test agents for 7 days.

Step 3. Determination of the level of neuron-specific enolase and S100 protein in rat blood serum, obtaining and preparing the brain for histological examination.

Step 4. Identification of neurons in the sensorimotor cortex of the cerebral hemispheres after staining sections with hematoxylin and eosin and thionin using the Nissl method.

Step 5. Morphometric analysis of the state of neurons in the sensorimotor cortex of the cerebral hemispheres.

Step 6. Processing and analysis of the results obtained.

Step 7. Identifying promising areas for further research.

3. Materials and methods

The study was conducted at the Educational and Scientific Training Laboratory of Medical and Biological Research of the National University of Pharmacy during the period from February 1, 2024 to March 1, 2025 (Certificate of compliance of the measurement system with the requirements of DSTU ISO 10012:2005 No. 01-0084/2021 dated August 6, 2021).

At the National University of Pharmacy, at the Department of Industrial Technology of Medicines, under the supervision of the Head of the Department, Professor O. A. Ruban, a new combined product containing dry peony root extract, L-tryptophan, and glycine was developed. The formulation includes lactose monohydrate (Lactose GranuLac 200) and microcrystalline cellulose (MCC 102) as fillers, polyvinylpyrrolidone (Plasdone K-25) as a binder, HPMC (HPMC Methocel K4M CR Premium) as a mucoadhesive agent, crospovidone (Crospovidone XL-10) as a disintegrant, flavor and odor correctors aspartame and Mint Chloroph FLV PDR, anti-caking agents magnesium aluminometasilicate (Neusilin) and calcium stearate, and as active ingredients – L-tryptophan, glycine, and dry peony extract in the following mass ratio (g) (Table 1).

Table 1

Composition of the new combined product containing dry peony root extract, L-tryptophan, and glycine

Glycine	0.0983–0.1013
L-tryptophan	0.0983–0.1013
Dry peony extract	0.0738–0.0751
Lactose monohydrate (Lactose GranuLac 200)	0.0530–0.0552
Microcrystalline cellulose (MCC 102)	0.0775–0.0807
HPMC (Methocel K4M CR Premium)	0.0109–0.0111
Aspartame	0.0274–0.0278
Mint cloroph FLV PDR	0.0054–0.0056
Polyvinylpyrrolidone (Plasdone K-25)	0.0322–0.0329
Crospovidone (Crospovidone XL-10)	0.0347–0.0354
Magnesium aluminometasilicate (Neusilin)	0.0249–0.0251
Calcium stearate	0.0054–0.0056
Tablet weight	0.5418–0.5571

The experiment was carried out on 40 white non-linear male rats weighing 200–250 g, which were kept under standard vivarium conditions at the Educational and Scientific Institute of Applied Pharmacy of the National University of Pharmacy. All experimental procedures were performed in accordance with the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986), the Law of Ukraine "On the Protection of Animals from Cruel Treatment" (dated 15.12.2009 No. 1759-VI), as well as Directive 2010/63/EU of the European Parliament and of

the Council of 22 September 2010 [8–10]. The conduct of the experiment was approved by the Bioethics Commission of the National University of Pharmacy (Protocol of the Bioethics Commission No. 8 dated 15.02.2023).

Traumatic brain injury (TBI) was modelled using the standard weight-drop injury method applied to the fixed head of the animal. A metal rod weighing 50 g was used as the load, which moved freely inside a vertically fixed metal tube 65 cm in length. Under ether anesthesia, the rat's head was fixed beneath the tube so that its outlet was positioned along the sagittal suture, symmetrically, 5 mm anterior to the interauricular line. The load, freely falling through the tube, delivered an instantaneous impact to the cranial vault, inducing a standardized moderate-severity TBI [11, 12]. Traumatic brain injury (TBI) was modelled using the standard weight-drop injury method applied to the fixed head of the animal. A metal rod weighing 50 g was used as the load, which moved freely inside a vertically fixed metal tube 65 cm in length. Under ether anesthesia, the rat's head was fixed beneath the tube so that its outlet was positioned along the sagittal suture, symmetrically, 5 mm anterior to the interauricular line. The load, freely falling through the tube, delivered an instantaneous impact to the cranial vault, inducing a standardized moderate-severity TBI [11, 12].

Laboratory studies within the framework of the experiment were performed on the 2nd (after 24 hours) and 8th day. The level of neuron-specific enolase (NSE) in the blood serum was determined by enzyme-linked immunosorbent assay using the "Rat NSE ELISA Kit" ("Elabscience Biotechnology Inc., USA) according to the manufacturer's instructions on the STAT FAX 303/PLUS automatic analyzer [13].

25 µl of standard solutions with known concentrations (0; 7.5; 22.9; 68.4 and 138.0 µg/l) and blood serum samples were added to the wells of the plates, the inner walls of which were coated with antibodies to NSE. After that, 100 µl of a solution of specific antibodies was added, incubated for 2 h at room temperature with constant stirring on a shaker. After incubation, six washing cycles were performed, 100 µl of TMB substrate was added and left for 30 min to form a color reaction. The reaction was terminated by adding 100 µl of stop reagent. Optical density measurements were performed on a STAT FAX 303/PLUS analyzer at a wavelength of 405 nm.

The level of S100 protein in blood serum was determined by enzyme-linked immunosorbent assay using the "Rat S100 ELISA Kit" (Elabscience Biotechnology Inc., USA) according to the manufacturer's instructions. 50 µl of standards and serum samples were added to the wells of the plate coated with antibodies to the S100 protein. Then 100 µl of biotinylated antibodies were added, followed by a two-hour incubation at room temperature with constant stirring. After the incubation, the wells were washed, 100 µl of TMB substrate was added and incubated for another 30 min. The reaction was stopped by adding stop reagent (100 µl), after which spectrophotometric reading was performed at 405 nm using a STAT FAX 303/PLUS analyzer [14].

Euthanasia of laboratory animals was performed by decapitation after prior intraperitoneal administration of propofol (60 mg/kg) to induce general anesthesia. The morphofunctional state of structures in the sensorimotor cortex (SMC) of the cerebral hemispheres was studied – a neural center whose activity ensures regulation of various body functions and complex forms of behaviour and receives signals from all major sensory systems; the cerebellar cortex (CC) – the center of balance, maintenance of muscle tone, coordination of movements, and control of complex acts performed automatically; and the functional zones CA_1 and CA_3 of the ventral hippocampus (VH) – which ensure the implementation of memory mechanisms, behaviour-related responses, and perform the function of fixation of emotionally significant events in rats.

The rat brains were completely fixed in 96% ethanol for 2 days immediately after removal from the cranial cavity, after which three frontal sections were made: at the level of the anterior margins of the temporal lobes (for examination of the SMC), at the level of the medial temporal region of the cerebral hemispheres (for examination of the VH), and at the level of the midline of the cerebellar hemispheres (for examination of the CC) [15]. The samples were additionally kept in 96% ethanol for 2 days and then embedded in paraffin. From the paraffin blocks, sections 2–4 µm thick were obtained using a rotary semi-automatic microtome and stained with hematoxylin and eosin as well as thionine according to the Nissl method [16, 17]. The obtained histological preparations were analyzed under a Granum light microscope, and microphotography of microscopic images was performed using a Granum DCM 310 digital video camera. The photographs were processed on a Pentium 2.4 GHz computer using Levenhuk 310 Toup View software.

In the histological preparations, in addition to studying the histoarchitectonics of the selected brain regions (hematoxylin and eosin staining), a quantitative assessment of the functional state of pyramidal neurons was performed (thionine staining according to the Nissl method): neurons of layers III and V of the neocortex—these layers are the most functionally active and are most frequently studied in various experiments; neurons of the pyramidal layer of the CA_1 and CA_3 zones of the ventral hippocampus (VH). In the hippocampus of vertebrates, including rats, the pyramidal layer is the most densely populated with neuronal cell bodies among neuronal layers. Based on the density and pattern of neuronal arrangement within this layer, several functionally distinct zones are identified: CA_1–CA_4. The most numerous cellular elements are found in the CA_1 and CA_3 zones, which are directly involved in learning and memory processes; Purkinje cells of the ganglionic layer of the cerebellar cortex (CC), which exhibit the most pronounced changes under various pathological conditions.

The morphofunctional state of neurons was assessed based on their distribution into the main structural and functional types according to the classification based on the state of tigroid (Nissl substance) [16]. Normochromic neurons were identified, in which the tigroid in the neuroplasm exhibited moderate basophilia, corre-

sponding to a moderate level of functional activity of the cells; hyperchromic neurons with intensely basophilic Nissl substance, which were regarded as a pool of functionally inactive cells that may serve as a reserve for the restoration of brain functions after various influences; as well as hypochromic neurons, characterized by weak basophilia of the chromatophilic substance, indicating prolonged maintenance of cells at the peak of functional activity followed by depletion of certain ultrastructural components and a decrease in functional activity. Hyperchromic pyknotic neurons were identified separately; in these cells the dye uniformly stained the neuroplasm and nucleus without clear differentiation between them, as well as shadow cells, characterized by pale cell contours and the absence of tigroid substance and nucleus. The presence of these cellular forms was regarded as a morphological sign of irreversible degenerative changes in neurons [16, 17].

The relative proportion of each neuron type was determined (calculated per 100 cells), and the alteration index was calculated (the ratio of destructive neurons to unchanged ones). In the SMC, the glial–neuronal index (the ratio of the number of glial elements to pyramidal neurons) and the perineuronal satellite index (the number of satellite glial cells per one pyramidal neuron) were additionally determined [16]. The analysis was performed in the 3rd and 5th layers of the neocortex. All measurements were carried out within a single microscopic field of view (eyepiece 10×, objective 40×).

As a reference drug, citicoline (Ceraxon, "Ferrer Internacional S.A.", Spain), batch D003U1, was used at a dose of 500 mg/kg. Citicoline is a natural endogenous compound, an intermediate metabolite in the biosynthesis of phosphatidylcholine – the main phospholipid of cell membranes, especially neuronal ones. Its use is mainly justified by its membrane-stabilizing action, antioxidant effect, and antiglutamatergic activity: citicoline reduces excessive glutamate release, a key neurotransmitter which, at toxic concentrations, induces excitotoxic neuronal apoptosis, among other effects [18]. Thus, the choice of citicoline as a reference drug in this study is based on its ability to exert a complex (multifaceted) influence on the key pathogenetic mechanisms of traumatic brain injury – oxidative stress, membrane damage, and glutamate-induced neuronal apoptosis. This allows an objective comparison of the effectiveness of the investigated agent under conditions of experimental TBI.

Based on the goal, all animals were divided into the following groups ($n = 10$):

1. Control group – animals that received intragastric administration of 0.9% isotonic sodium chloride solution in a volume equivalent to that of the administered test substances.

2. Experimental TBI without treatment.

3. Experimental TBI with transbuccal administration of the test substance (TS) at a conditionally therapeutic dose of 35 mg/kg (TBI + TS).

4. Experimental TBI with transbuccal administration of the reference drug, citicoline, at a dose of 500 mg/kg (TBI + citicoline).

The advantages of transbuccal administration include avoidance of the first-pass hepatic metabolism (first-pass effect); lower stress for animals compared with injections; easier administration in rats compared with the sublingual route; and a rapid onset of drug action [19]. The preparations were dissolved in physiological saline with a total volume of 0.2 mL. The rat was wrapped in a towel with the head left free; the oral cavity was opened using a spatula, and the solution was administered into the buccal pouch (the inner space between the cheek and the gums) using a micropipette. The rat was then held for several minutes to allow absorption [20].

The investigated agents were administered for 7 days. Animals were withdrawn from the experiment on day 8 after TBI.

Statistical processing of the numerical data was performed using methods of variation statistics with the standard statistical software packages IBM SPSS Statistics 23 (USA) and Microsoft Office Excel 2010. Intergroup differences were considered statistically significant at $p < 0.05$.

4. Research results

The activity of neurodestruction was assessed based on the study of serum levels of neuron-specific enolase (NSE) against the background of the applied therapy (Table 2).

Table 2

The effect of tablets with dry extract of peony roots, L-tryptophan and glycine on serum levels of NSE in rats under conditions of TBI ($n = 10$; $M \pm m$)

Group of animals	NSE, ng/ml	
	Study period	
	24 hours	8 days
Control	0.450 ± 0.021	0.458 ± 0.032
TBI without treatment	4.23 ± 0.14*	2.86 ± 0.15*
TBI+TS	2.07 ± 0.10**	1.19 ± 0.069**
TBI+citicoline	2.26 ± 0.12**	1.34 ± 0.05**

*Note: * – $p \leq 0.05$ relative to control; ** – $p \leq 0.05$ relative to TBI without correction.*

The use of the studied agent caused a decrease in neurodestruction in the early stages, as well as a decrease in the activity of neuroglioproliferation in the later stages after modelling TBI in rats. Thus, the use of the studied agent in rats against the background of TBI caused a significant decrease in the serum content of the S100 protein by 1.8 times after 24 hours and on day 8 compared with the corresponding indicators in animals with TBI without correction. The administration of citicoline caused a significant decrease in the serum content of the S100 protein by 1.7 times after 24 hours and on day 8 compared with the corresponding indicators in animals with TBI without correction.

We further assessed the changes in the content of S100 protein in the blood serum of rats after TBI (Table 3). Modelling TBI in rats was accompanied by a significant increase in the level of S100 protein in the blood serum already 24 h after the start of the experiment, which is

evidence of neurodestruction, and continued to increase until day 8 due to the activation of neuroglioproliferation processes. After 24 h, the serum content of S100 protein increased by 8.6 times ($p < 0.05$), compared with the control group. As of day 8 after TBI, the content of S100 protein in the blood serum was significantly higher by 1.6 times compared with the previous period of the study.

Table 3

Effect of tablets with dry peony root extract, L-tryptophan and glycine on serum levels of S100 protein in rats with TBI ($n = 10$; $M \pm m$)

Group of animals	S100 protein, ng/ml	
	Study period	
	24 hours	8 days
Control	0.636 ± 0.031	0.644 ± 0.020
TBI without treatment	5.44 ± 0.21*	8.45 ± 0.25*
TBI+TS	2.98 ± 0.13**	4.69 ± 0.23**
TBI+citicoline	3.25 ± 0.13**	5.04 ± 0.21**

*Note: * – $p \leq 0.05$ relative to control; ** – $p \leq 0.05$ relative to TBI without correction.*

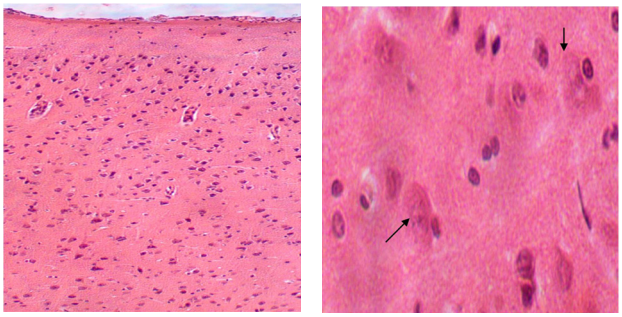
The use of the studied agent caused a decrease in neurodestruction in the early stages, as well as a decrease in the activity of neuroglioproliferation in the later stages after modeling TBI in rats. Thus, the use of the studied agent in rats against the background of TBI caused a significant decrease in the serum content of the S100 protein by 1.8 times after 24 hours and on day 8 compared with the corresponding indicators in animals with TBI without correction. The administration of citicoline caused a significant decrease in the serum content of the S100 protein by 1.7 times after 24 hours and on day 8 compared with the corresponding indicators in animals with TBI without correction.

In animals of the control group, the histological organization of the gray cerebral cortex of the hemispheres corresponded to the typical morphological characteristics described in the scientific literature. The pia mater had a usual structure, was covered externally with a continuous layer of squamous epithelial cells. Its numerous blood vessels were moderately filled with blood.

In the structure of the brain matter, all obligate layers with unchanged architectonics were clearly visualized. The clarity of the boundaries and the vertical organization of neurons in the corresponding layers were preserved. The vast majority of neurocytes were characterized by perikaryons with smooth contours, centrally located nuclei and diffuse (non-condensed) chromatin. One or two nucleoli were localized in the center of the nucleus.

The neuropil around the cells had a homogeneous, structureless appearance. Capillaries and other vessels passing through the neuropil mostly had well-defined vascular walls. Glial cells were observed both singly and in the form of small clusters (up to 2–3 cells), and in some places perineuronal in the form of single satellite cells (Fig. 1).

The calculations showed that the glio-neuronal and perineuronal indices in control rats were 0.72 and 1.32, respectively (Table 4), which fall within the normal range for this animal species.



a *b*

Fig. 1. SMC of the cerebral hemisphere of control rats: *a* – cytoarchitectonics of the layers, neuropil density is unchanged (×150); *b* – single satellite cells (arrows), normal ratio of neurons to glial cells in the neuropil; Hematoxylin and eosin (×400)

Thionine staining according to the Nissl method revealed that in the sensorimotor cortex most pyramidal neurons were normochromic. Hyperchromic and hypochromic cells were observed only occasionally. In addition, pyramidal neurons with destructive changes–hyperchromic pyknomorphic neurons and shadow cells–were detected very rarely. The alteration index was 0 (0;0) (Fig. 2, Table 5).

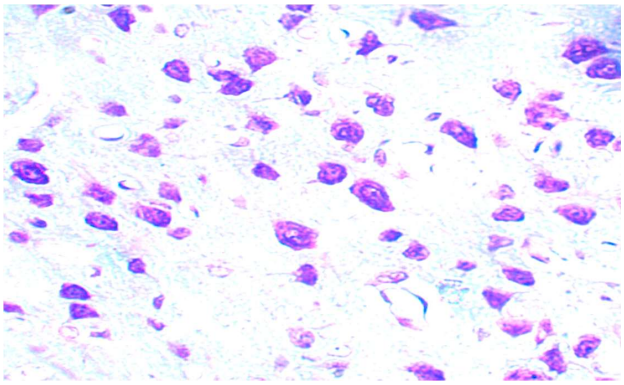


Fig. 2. SMC of the cerebral hemisphere of a control rat. The vast majority of neurons are of the normochromic type. Thionine staining by the Nissl method; ×400

The histological structure of the cerebellum in all vertebrates, according to the literature, is also uniform. The cerebellum is dissected by deep fissures that form large primary (first-order) folia, which include smaller secondary (second-order) folia and even smaller tertiary (third-order) folia. Each tertiary folium contains a thin layer of white matter formed by nerve fibers and the cortex. The latter consists of an outer molecular layer, a middle ganglionic layer, and an inner granular layer. Each of these layers is represented by strictly defined neuronal populations. In the CC of control rats, the histological structure of the tissue was unchanged and corresponded to that described in the literature. The folia were clearly visible. Blood vessels of the pia mater had a normal appearance and blood filling. All three cortical layers were distinctly identified. The ganglionic layer consisted of Purkinje cells arranged in a single row at approximately equal distances from each other and oriented vertically relative to the surface of the cerebellar

cortex. The neuronal perikarya had a pear-shaped form and large euchromatic nuclei. Visually, the number of Purkinje cells and the density of their arrangement varied somewhat on the exposed surface of the folium and in the depth of the fissure. A moderate number of Bergmann glial cells were observed between Purkinje cells (Fig. 3).

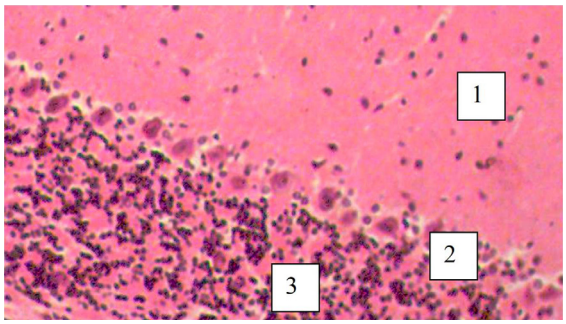


Fig. 3. CC of a control rat. Unchanged histoarchitectonics of the tissue; the molecular (1), ganglionic (2) layer with a dense arrangement of Purkinje cells, and the granular layer (3) are clearly visible. Hematoxylin and eosin, ×250

Most Purkinje cells were normochromic, although a fairly noticeable proportion exhibited a hyperchromic appearance (Fig. 4). A small number of neurons were hypochromic; hyperchromic pyknomorphic neurons and shadow cells were rare. The alteration index of Purkinje cells was 0.1 (0; 0.02) (Table 4).

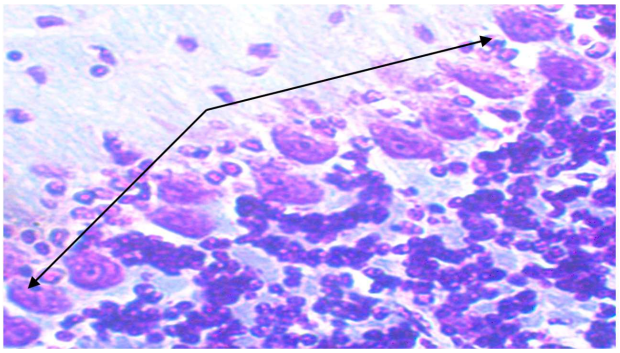
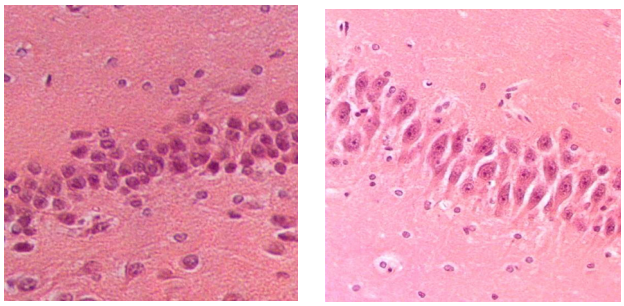


Fig. 4. CC of a control rat. Normochromic Purkinje cells. Thionine by Nissl method; ×400

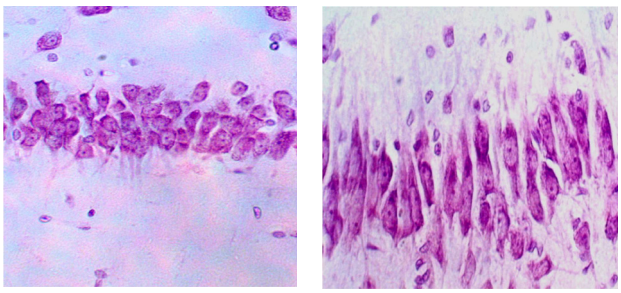
During the examination of the VH, the morphological patterns of the CA_1 and CA_3 zones were clearly identifiable both topographically and by the characteristic structure of the pyramidal layer, corresponding to the described cytoarchitectonic features of these hippocampal zones in rats. In the micropreparations of intact rats, the pyramidal layer of the CA_1 zone of the VH was represented as a band with neurons arranged densely in 3–6 rows. The cells were of medium size, with round nuclei, well-defined nucleoli, and perikarya of roughly circular shape. In the CA_3 zone of the hippocampus, pyramidal layer neurons had perikarya of spherical, oval, or triangular shapes, with clearly defined nuclear membranes and noticeable nucleoli. Apical dendrites were distinctly outlined. The cells were larger, less densely packed, and formed several rows (Fig. 5).



a *b*

Fig. 5. Zone CA_1 (*a*) and CA_3 (*b*) of the VH of control rats: *a* – rows of neurons of a rounded shape; *b* – rows of large polygonal neurons; Hematoxylin-eosin, ×400

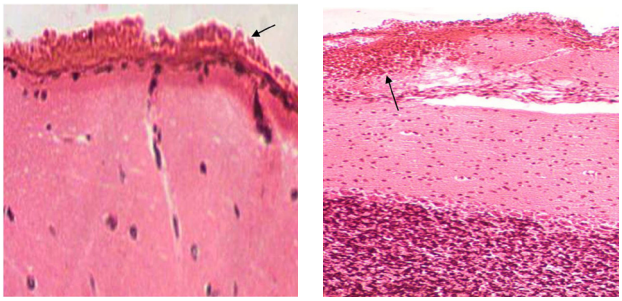
Nissl's substance is evenly distributed in the neuroplasm of most pyramidal neurons of the CA_1 and CA_3 zones. Hyperchromic and hypochromic cells are few, hyperchromic pyknomorphic neurons and shadow cells are single. The alteration index of pyramidal neurons in the CA_1 and CA_3 zones was 0.03 (0.02; 0.08) and 0.06 (0.03; 0.17), respectively (Fig. 6, Table 6).



a *b*

Fig. 6. Zone of VH control rats: the state of chromatophilic substance in the neuroplasm of most neurons corresponds to normal: *a* – CA_1; *b* – CA_3. Thionine according to the Nissl method; ×400

On day 8 post-TBI, rats exhibited pronounced congestive hyperemia of the blood vessels in the meningeal layer of the neocortex of the cerebral hemispheres and the cerebellum. Focal subarachnoid hemorrhages (erythrocytes on the surface of the meninges) and submeningeal hemorrhages were observed in some areas (Fig. 7).



a *b*

Fig. 7. SMC of the cerebral hemisphere of rats on day 8 post-TBI: *a* – subarachnoid hemorrhage, ×400; *b* – submeningeal hemorrhage, ×200; Hematoxylin-eosin

In the SMC, single foci of damage were detected - the layered structure of neurons was not visualized, the neuropil was sparse, and most nerve cells were dead - similar to foci of contusion (Fig. 8).

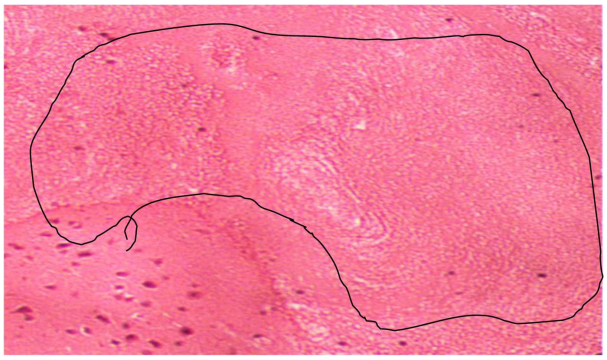
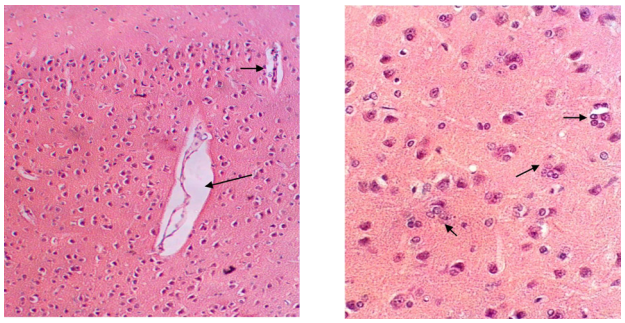


Fig. 8. SMC of the rat cerebral hemisphere on day 8 of TBI. Area of damage to the brain substance; Hematoxylin-eosin; ×250

In the areas of the SMC with preserved histological structure, thin-walled blood vessels were observed, partly in a state of spasm, partly with congestive full blood. Pericapillary and pericellular edema were detected. In some places, small focal accumulations of glial cells were recorded, sometimes with a number of more than 4–5 elements. Satellitetosis was often recorded, which was manifested by an increased number of satellite cells around one neuron (Fig. 9).



a *b*

Fig. 9. SMC of the cerebral hemisphere of rats on the 8th day of TBI: *a* – spasm of thin-walled capillaries, pericapillary and pericellular edema (×200); *b* – increase in small focal glial clusters and satellites (×250); Hematoxylin-eosin

Quite noticeable cytophagic activity of microglia cells was observed – neuronaphagy – glial cells surrounded and destroyed the neuron (Fig. 10).

Morphometric analysis showed an increase in the glio-neuronal and perineuronal satellite index in these rats by 22.2% and 1.95 times, respectively, compared with the control (Table 4). The increase in these indices is associated with the loss of plastic and bioenergetic reserves by neurons (the replenishment of which is carried out at the expense of and through contacts with glial satellite cells) and, as a result, an increase in the level of neuronal voltage. The increase in these indices is morphologically regarded as a compensatory nonspecific glial reaction.

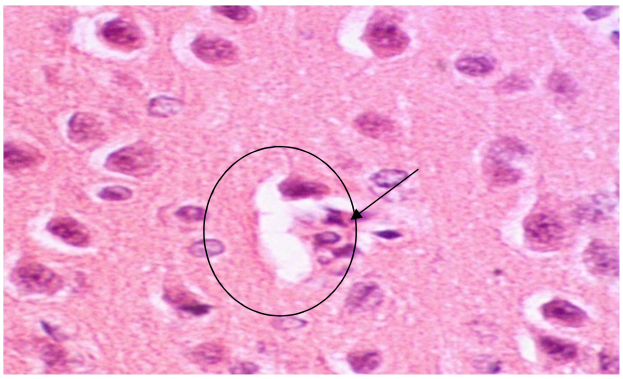


Fig. 10. SMC of the rat cerebral hemisphere on day 8 of TBI. Glial cells surround and destroy the neuron. Hematoxylin-eosin; ×400

The functional state of neurons also changed: the presence of hyperchromic and hypochromic neurons increased, the number of normochromic cells decreased. In some neurocytes, signs of chromatolysis (tigeroid lysis) of varying degrees of severity are visible, which morphologically indicates a decrease in the reserve energy and plastic resources of these cells and vacuolization of neuroplasm (Fig. 11, 12).

Quantitative calculations confirmed visual changes in the functional state of neurons: normochromic neurocytes significantly decreased by 24.36%, the relative number of irreversibly changed neurons (hyperchromic pyknomorphic and shadow cells) significantly increased by 12.3 and 9.4 times. An increase in the number of cells with manifestations of metabolic disorders was observed: hyperchromic – by 2.16 times, and hypochromic – by 2.55 times. At the same time, the alteration index significantly increased by 3 times (Table 5).

In CC, certain visual signs of destruction were found among Purkinje cells – zones of "falling out, disappearance" of Purkinje cells from the row were noted. Sometimes, a violation of the clarity of the single-row arrangement of these cells is visible – some cells penetrated the molecular and/or granular layers (Fig. 13, 14). Purkinje cells themselves are quite often degeneratively changed – the shape of the perikaryon from pear-shaped becomes spherical, the nuclei are pyknotic, some Purkinje cells are in a state of acute edema – signs of neurotoxic action.

Table 4

Effect of tablets with dry extract of peony roots, L-tryptophan and glycine on the severity of the glial reaction of the SMK region of the cerebral hemispheres of rats under conditions of TBI

Group of animals	Indicators	
	Glio-neuronal index	Perineural satellite index
Control	0.72 (0.69; 0.73)	1.32 (1.12; 1.5)
TBI without treatment	0.88 (0.79; 0.9)*	2.58 (2; 3)*
TBI+TS	0.75 (0.75; 0.79)*/**	1.42 (1.33; 2)*/**
TBI+citicoline	0.78 (0.74; 0.81)*/**	1.55 (1; 2)**

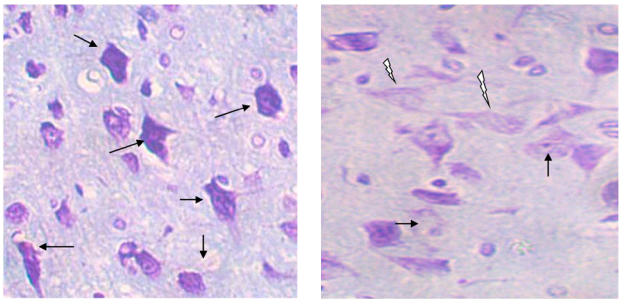
*Note: * – $p \leq 0.05$ relative to control; ** – $p \leq 0.05$ relative to TBI without correction.*

Table 5

Effect of tablets with dry extract of peony roots, L-tryptophan and glycine on the functional state and alteration index of pyramidal neurons of the SMC region of the cerebral hemispheres of rats under conditions of TBI, %

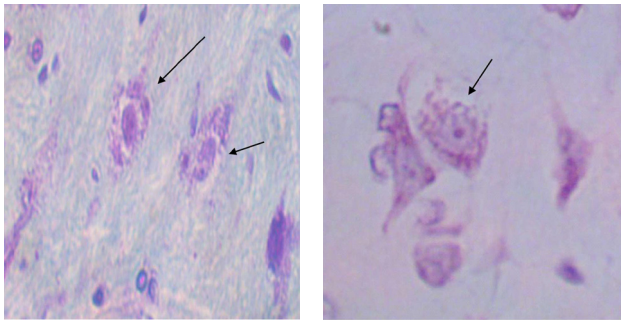
Group of animals	Main structural and functional types of neurons, %					Alteration index
	Normochromic	Hyperchromic	Hyperchromic pyknomorphic	Hypochromic	Shadow cells	
Control	82.1 (80; 86.2)	9.7 (6.5; 10)	0 (0; 3.1)	6.7 (6.5; 9.7)	0 (0; 3)	0 (0; 0)
TBI without treatment	62.1 (59.3; 66.7)*	21 (19.7; 24.5)*	12.3 (8.3; 13.6)*	17.1 (15.0; 19)*	9.4 (8.3; 12.7)*	0.3 (0.3; 0.4)*
TBI + TS	71 (66.7; 75)*/**	11.3 (10.3; 13.7)*/**	8.8 (6.7; 9)*/**	7.1 (6.3; 9.6)*/**	4.5 (3.7; 6.5)*/**	0.2 (0.2; 0.3)*/**
TBI + citicoline	69.5 (65.4; 75)*/**	13.5 (72.4; 75)*/**	9.5 (72.4; 75)*/**	13.5 (72.4; 75)*/**	5.5 (72.4; 75)*/**	0.2 (02; 0.3)*/**

Note: * – $p \leq 0.05$ *relative to control;* ** – $p \leq 0.05$ *relative to TBI without correction.*



a *b*

Fig. 11. SMC of the rat cerebral hemisphere on the 8th day of TBI: *a* – increase in hyperchromic neurons; *b* – increase in hypochromic (black arrow) neurocytes and shadow cells (white arrow); Thionin by Nissl method; ×400



a *b*

Fig. 12. SMC of the rat cerebral hemisphere on the 8th day of TBI: *a* – chromatolysis; *b* – vacuolization of the cell's neuroplasm; Thionin by Nissl method; ×400

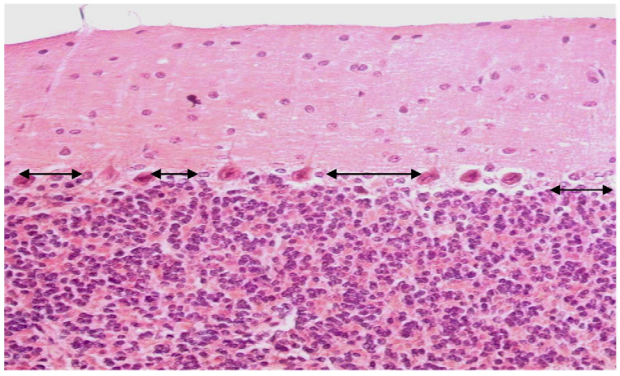
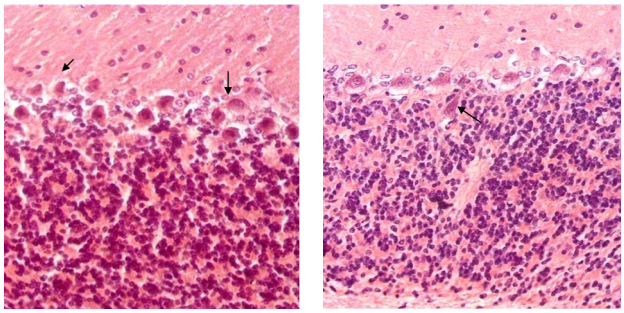


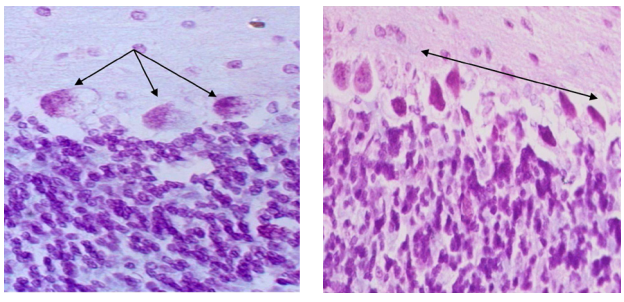
Fig. 13. BC of a rat on the 8th day of TBI. Zones of "loss, disappearance" of Purkinje cells in the ganglionic layer; Hematoxylin-eosin; ×200



a *b*

Fig. 14. CC of rats on day 8 of TBI. Shift of Purkinje cells: *a* – molecular layers; *b* – granular layers; Hematoxylin-eosin; ×200

Nissl staining showed a significant visual increase in the number of hyperchromic pycnomorphic cells, hypochromic neurons with varying degrees of chromatolysis, and shadow cells (Fig. 15). Morphometric calculations revealed a significant decrease in normochromic Purkinje cells (by 24.4%), an increase in hyperchromic pycnomorphic neurons – more than 28 times. The increase in hyperchromic and hypochromic cells, shadow cells was not so pronounced – 1.45, 2.9 and 2.5 times, respectively, relative to the CP. The Purkinje cell alteration index significantly increased and was equal to 1 (0.7;2) (Table 6, Fig. 15).



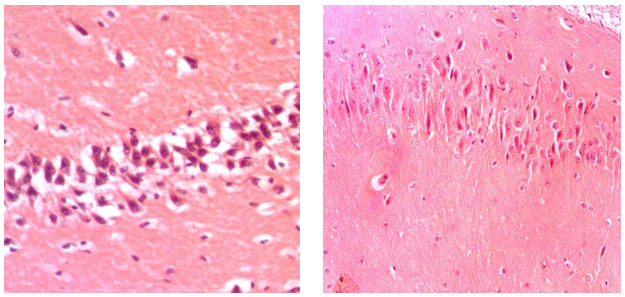
a *b*

Fig. 15. CC of rats on the 8th day of TBI. Purkinje cells: *a* – with signs of chromatolysis; *b* – different degrees of hyperchromic state; Thionin by Nissl method; ×400

In the pyramidal layer of the CA_1 and CA_3 zones of the VH of rats on the 8th day of TBI, a decrease in the density of the location of neurons and indistinct rows of cells were visualized in some places. In addition, disorganization of the cellular location within the cortical

layers was noted, with focal "loss" of neurons. The morphology of the cell bodies underwent changes, pericellular edema was observed (Fig. 16).

Nissl staining showed noticeable polymorphic changes in the chromatophilic substance in neurocytes: from focal



a *b*

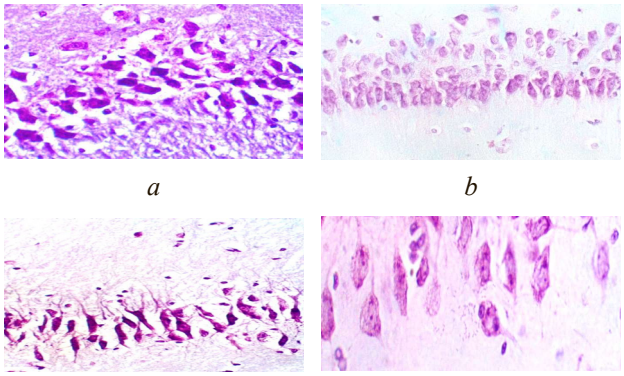
Fig. 16. Zones of the VH of rats on the 8th day of TBI. Disorganization, decrease in the density of neurons, "falling out" of cells from the layers, pericellular edema, change in the shape and size of neurocytes: *a* – CA_1, ×250; *b* – CA_3, ×200; Hematoxylin-eosin; ×200

chromatolysis to pronounced hypochromia, the appearance of shadow cells; hyperchromatosis, including hyperchromatosis with pyknomorphic features. When dividing neurons by their functional state, it was found that in the CA_1 zone, unchanged pyramidal neurons significantly decreased in number by 17.14% compared to the intact control. No significant changes in the number of hyperchromic and hypochromic neurons were detected. The number of shadow cells significantly increased by 6.5 times, hyperchromic pyknomorphic cells increased by 6.33 times.

In the CA_3 zone, the number of hypochromic neurons increased by 52.87% against the background of a decrease in normochromic cells by 26.46%. Shadow cells increased by 10.6 times. The number of cells with hyperchromatosis did not significantly change, unlike hyperchromic pyknomorphic cells, which increased by 2.53 times (Table 7, Fig. 17).

On the 8th day of drug administration, vasoconstriction of the pia mater, gray matter of the cerebral cortex and CC was detected. However, the intensity of this phenomenon was visually less pronounced compared to the control group (Fig. 18).

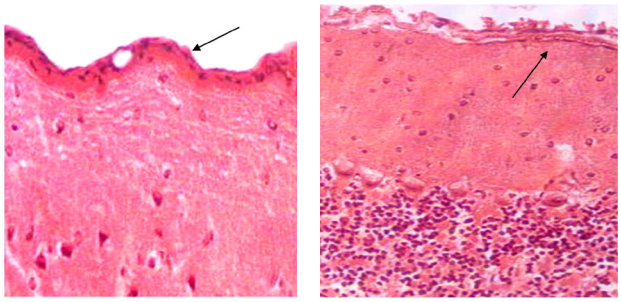
Spasm of thin-walled blood vessels, pericapillary and pericellular edema were significantly less pronounced (Fig. 19).



a *b*

c *d*

Fig. 17. CA_1 (*a*, *c*) and CA_3 (*b*, *d*) zones of the VH of rats on the 8th day of TBI. Predominance of hyperchromic and hyperchromic pyknomorphic (*a*, *c*), or hypochromic (*b*) neurons; chromatolysis (*d*, black arrows), neuronophagy (d, white arrow); Thionin by the Nissl method; *a*–*c* – 250, *d* – ×400



a *b*

Fig. 18. SMC of rats treated with TS on day 8 of TBI: pleural vascularization. Hematoxylin-eosin: *a* – cerebral hemisphere; *b* – the cerebral cortex. ×400

There is no glial reaction and foci of damage to the structure of brain tissue. Morphometrically, the glio-neuronal index decreased by 14.8% compared to CP. The perineuronal satellite index decreased by 1.8 times (Table 4).

An improvement in the functional state of neurons was noted: the number of normochromic cells visually increased, while the number of hypochromic neurocytes decreased. There were even more hyperchromic neurons, but no clear signs of pathological changes were observed in most of them, which gives reason to consider this fact as an increase in reserve cells. Signs of chromatolysis were found in single neurons (Fig. 20).

Table 6

Effect of tablets with dry extract of peony roots, L-tryptophan and glycine on the functional state and alteration index of Purkinje cells of the CC of rats under conditions of TBI, %

Group of animals	Main structural and functional types of Purkinje cells,%					Alteration index
	Normochromic	Hyperchromic	Hyperchromic pyknomorphic	Hypochromic	Shadow cells	
Control	82.1 (80; 86.2)	12.1 (10; 16.2)	1.1 (0.6; 1.6)	2.1 (1; 2.6)	1 (0; 1.5)	0.1 (0; 0.2)
TBI without treatment	62.1 (59.3; 66.7)*	17.6 (10; 19.3)*	28.6 (22.2; 42.9). *	6.1 (5.3; 10.7)*	2.5 (1; 3.5) *	1 (0.7; 2)) *
TBI+TS	71 (66.7; 75)*/**	15.1 (10.7; 20)*/*	17.5 (10.7; 21.5)*/**	5.9 (4.7; 7.5)*	2.2 (1.0; 3.5)*	0.5 (0; 1.0)*/**
TBI+citicoline	69.5 (72.4; 75)*/**	17.4 (10; 19)*	18.2 (12.4; 21)*/**	6.1 (5.3; 10.7)*	2.5 (1; 3.5) *	0.3 (0.1; 0.4)*/**

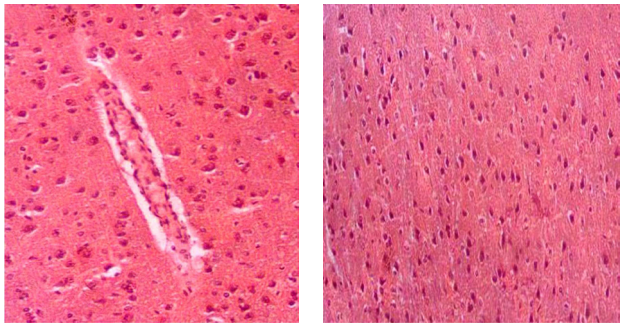
Note: * – $p \le 0.05$ *relative to control;* ** – $p \le 0.05$ *relative to TBI without correction.*

Table 7

Effect of tablets with dry extract of peony roots, L-tryptophan and glycine on the functional state of neurons of the pyramidal layer CA_1 and CA_3 of the VH zones of rats under conditions of TBI (%)

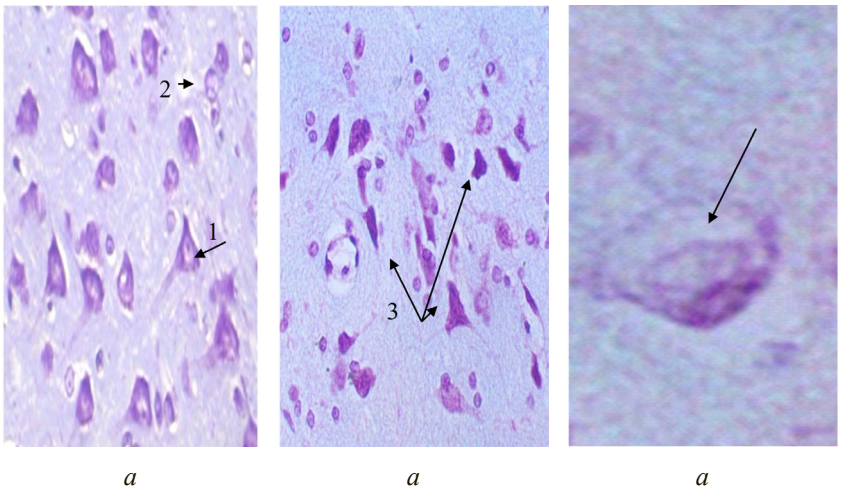
Hippocampal areas	Main structural and functional types of neurons, %	Group of rats			
		Control	TBI without correction	TBI + TS	TBI + citicoline
CA_1	Normochromic	80.5 (77.8; 83.9)	66.7 (61.8; 70.6)*	77.2 (71.5; 82.9)**	73.5 (72.4; 75)*/**
	Hyperchromic	6.6 (5.8; 11.1)	7.6 (5.6; 8.8)	6.6 (4.9; 9.5)**	7.7 (6.3; 10.7)
	Hyperchromic pyknomorphic	1.2 (0; 2.4)	7.6 (4.8; 12.5)*	1.3 (0; 4.8)**	3.7 (2.9; 6.7)*/**
	Hypochromic	9.4 (7.1; 11.4)	9.1 (7.6; 16.7)	8.8 (6.6; 11)	9.4 (6.9; 10)
	Shadow cells	1.2 (0; 3.4)	7.8 (6.6; 11)*	2.7 (0; 3.7)**	5.9 (0; 6.9)*/**
	Alteration index	0.03 (0.02; 0.08)	0.24 (0.19; 0.29)*	0.04 (0.02; 0.08)**	0.1 (0.1; 0.2)*/**
CA_3	Normochromic	76.7 (72.9; 80.5)	56.4 (50; 66.7)*	77.8 (77; 79.8)**	57.5 (54.1; 57.89)*
	Hyperchromic	6.5 (4.4; 8.7)	6.8 (2.6; 11.1)	6.3 (4.4; 13.2)	8.1 (5.5; 11.1)*/**
	Hyperchromic pyknomorphic	4.5 (2.2; 6.8)	11.4 (5.6; 15.2)*	1.2 (1.1; 2.7)*/**	9.1 (7.5; 14.7)*/**
	Hypochromic	8.7 (6.7; 9.8)	13.3 (9.1; 19)*	6.7 (5.6; 9.9)**	14 (12.1; 19.4)*
	Shadow cells	0 (0; 5)	10.6 (4.5; 16.7)*	2.2 (1.1; 3.4)*/**	9.3 (5.6; 14.3)*
	Alteration index	0.06 (0.03; 0.17)	0.41 (0.21; 0.5)*	0.05 (0.03; 0.07)**	0.36 (0.26; 0.47)*/**

*Note: * – $p \le 0.05$ relative to control; ** – $p \le 0.05$ relative to TBI without correction.*



a *b*

Fig. 19. SMC of the cerebral hemisphere of rats treated with TS on the 8th day of TBI: *a* – absence of capillary spasm, marked reduction of pericapillary edema (×250); *b* – reduction of pericellular edema; Hematoxylin-eosin (×200)



a *a* *a*

Fig. 20. SMC of the cerebral hemisphere of a rat receiving TS on the 8th day of TBI: *a* – increased normochromic cells (1), reduced presence of hypochromic neurons (2); *b* – hyperchromic neurons (3); *c* – chromatolysis in the neuroplasm of a single neuron. Thionin by the Nissl method; *a, b* – ×250, *c* – ×400

Morphometric measurements confirmed the visual picture. Probably normochromic neurons increased by 14.33%. There were 2.34 times fewer hypochromic pyknomorphic neurocytes, 2.1 times fewer shadow cells compared to CP. The number of hyperchromic neurons decreased by 1.85 times, and hypochromic neurocytes by 2.4 times. The alteration index of pyramidal neurons in this area of the brain was significantly reduced by 1.5 times compared to CP (Table 5).

The state of Purkinje cells in the ganglionic layer of CC against the background of "treatment" varied. There were still noticeable foci with expanded gaps between ganglionic cells, sometimes a violation of the single-row arrangement of neurons was visible (Fig. 21). The functional state of Purkinje cells also varied. In some places, there was a visually significant increase in normochromic cells. In others, hyperchromic, hyperchromic pycnomorphic cells, and shadow cells predominated (Fig. 22).

According to morphometric analysis in rats receiving TS, the relative proportion of normochromic Purkinje cells, compared with rats in the CC group, on average for the group significantly increased by 16.55%. Hyperchromic pyknomorphic neurocytes significantly decreased by 30%, although their level still significantly exceeded that in intact rats. The number of shadow cells decreased, but not significantly (by 8.35%), and the alteration index of ganglion cells became 2 times lower compared to CP (Table 6).

The study of the VH zones of rats after the TS treatment course showed that in the CA_1 zone, the density of neurons in the layer was visually significantly increased in most areas, and the order of the layers themselves was restored. Most neurons were characterized by a characteristic shape that was identical to that in control rats (Fig. 23). Pericellular edema persisted in some areas.

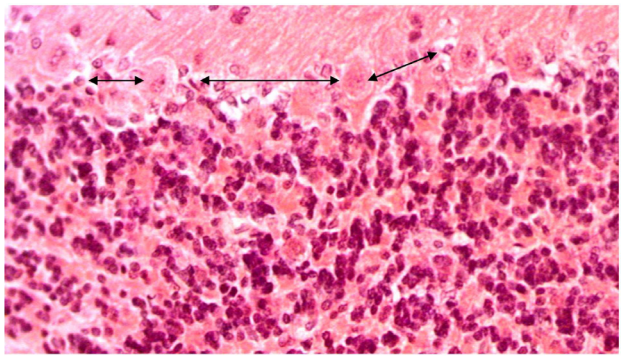
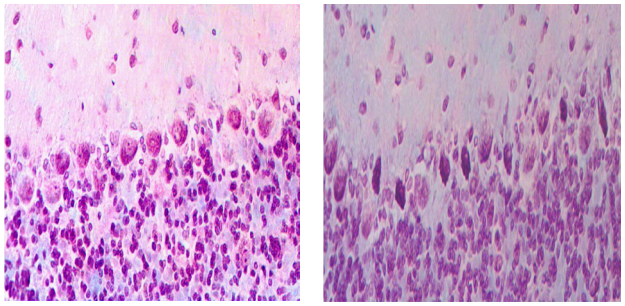


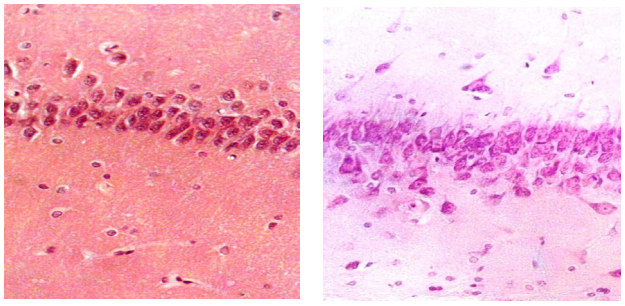
Fig. 21. CC of a rat treated with TS on day 8 of TBI. Increased Purkinje cell spacing; Hematoxylin-eosin; ×400.



a *b*

Fig. 22. CC of rats treated with TS on day 8 of TBI. Different functional states of Purkinje cells: *a* – normochromic cells; *b* – hyperchromic and hyperchromic pyknomorphic cells; Thionin by Nissl method; ×400

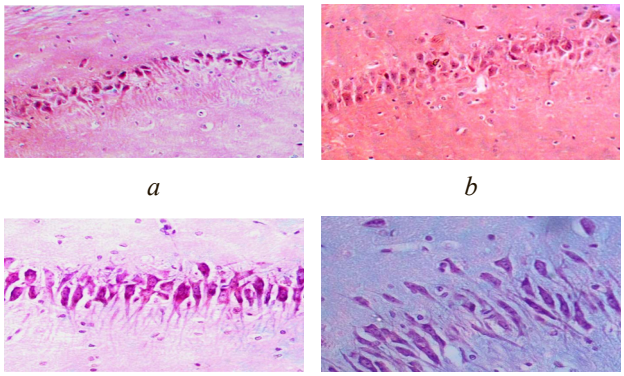
When qualifying the pyramidal neurons of this zone by functional state, it turned out that the number of normochromic neurons approached the intact control. The number of hyperchromic cells also reached the level of intact rats, hypochromic neurocytes did not significantly change in number compared to the control. Hyperchromic pyknomorphic neurons and shadow cells became significantly less by 5.8 and 2.9 times compared to the control. The alteration index decreased by 6 times (Table 5).



a *b*

Fig. 23. CA_1 zone of VH of rats receiving TS on the 8th day of TBI: *a* – restoration of the architectonics of the layers, the density of the arrangement of neurons in rows (hematoxylin-eosin. ×250); *b* – the state of the tigroid in the neuroplasm of most cells is normal (thionin according to the Nissl method. ×400)

In the CA_3 zone of the VH, the state of pyramidal neurons often resembled the control pathology – areas containing neurons with signs of dystrophic changes and cell loss were detected. When staining with thionin, almost the entire spectrum of functional states of pyramidal neurons was revealed (Fig. 24).



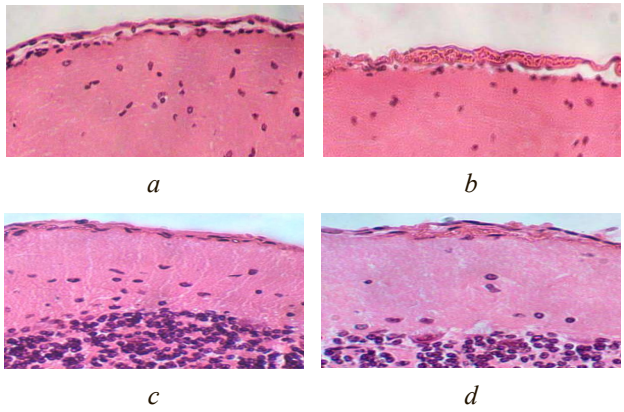
a *b*

c *d*

Fig. 24. CA_3 zone of the VH of rats receiving TS on the 8th day of TBI. Areas with greater (*a*) or lesser (*b*) severity of neuronal loss from the pyramidal layer; predominance of hyperchromic pyknomorphic and hypochromic neurons (*c*), normochromic cells (*d*); *a, b* – hematoxylin-eosinl ×250; *c, d* – thionin according to the Nissl method; ×400

The relative number of normochromic cells increased by 38%, hypochromic cells did not change; hyperchromic neurons were not significantly reduced (by 7.3%). Hyperchromic pyknomorphic cells were 9.5 times less, shadow cells were 4.82 times less than in the control. The alteration index was reduced by 8 times (Table 4).

After administration of citicoline to rats with TBI, polymorphism of the state of blood vessels of the pia mater of the gray matter of the cerebral cortex and cerebellum was observed: in different animals – from physiological norm to congestive plethysmosis (Fig. 25)



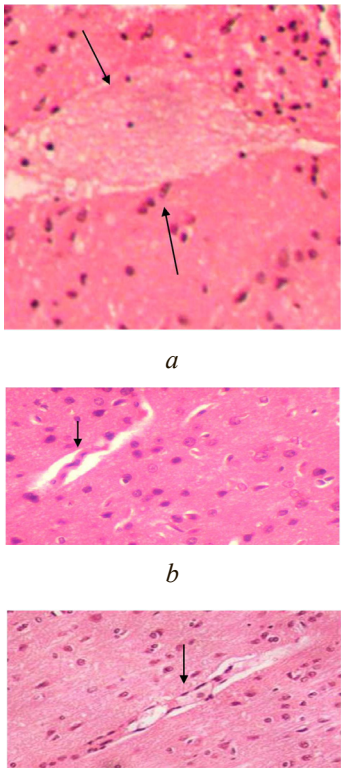
a *b*

c *d*

Fig. 25. SMC of the cerebral hemisphere (*a, b*) and CC (*c, d*) of rats treated with citicoline on day 8 of TBI: *a, c* – normal state of the vessels of the pia mater; *b, d* – full blood supply of the vessels of the pia mater; Hematoxylin-eosin; ×400

No structural abnormalities were found in the studied area of the SMC of the cerebral hemisphere, except for

one rat with small foci of rarefaction of the neuropil and neurons in the deep layers of the SMC. There was also spasm of some capillaries, although overall pericapillary edema was reduced, as was pericellular edema (Fig. 26).



a

b

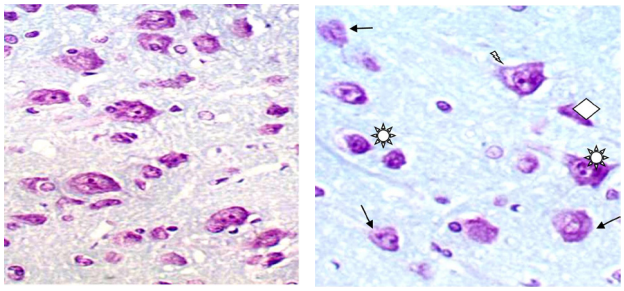
c

Fig. 26. SMC of the cerebral hemisphere of rats treated with citicoline on the 8th day of TBI: *a* – focus of liquefaction of neuropil and neurons; *b* – capillary spasm, pericapillary edema; *c* – normal state of the blood capillary; Hematoxylin-eosin; ×250

The glial reaction in the form of small clusters of glial cells and the perineuronal glial environment are visually reduced. Manifestations of necrophagy are rare. The glio-neuronal and perineuronal satellite index became 11.4% and 1.66 times lower than the control (Table 4). Thionin staining showed that many SMC neurons were characterized by a normochromic state, hyperchromic and hyperchromic pyknomorphic neurocytes were visually reduced, as were significantly hypochromic cells. Neuroplasm vacuolation, chromatolysis of part of neurons against the background of the normochromic state of the tigroid (Fig. 27). That is, in general, the presence of neurons with certain metabolic disorders decreased.

According to morphometric calculations, the relative proportion of normochromic neurons significantly increased by 12%, the proportion of hyperchromic and hypochromic cells decreased by 1.55 and 1.26 times, hyperchromic pycnomorphic neurons and shadow cells decreased by 1.3 and 1.7 times compared to the control. The alteration index decreased by 1.5 times (Table 5).

In the gyri of the CC of rats that received citicoline against the background of TBI, foci with widespread gaps between ganglion cells were observed, some of them migrated into the granular layer. Nissl staining with thionin showed that the functional state of Purkinje cells varied in different areas of the gyri from normochromic to irreversibly changed, while the ratio of cells of different tigroid status in them fluctuated within quite wide limits. In general, hypochromia, chromatolysis, and destructive changes prevailed in Purkinje cells (Fig. 28).



a *b*

Fig. 27. SMC of the cerebral hemisphere of rats treated with citicoline on day 8 of TBI: *a* – normal state of pyramidal neurons; *b* – hypochromic cells (black arrows), chromatolysis (white arrow), hyperchromic neurons (asterisk), hyperchromic pyknomorphic neuron (rectangle); Thionin by Nissl method; ×400

According to the results of the average total quantitative analysis, it was found that the number of normochromic cells increased by 12%, while the number of hyperchromic pyknomorphic neurons decreased by 1.57 times. At the same time, the level of hypo- and hyperchromic neurocytes, as well as shadow cells, remained at the level of the control group. The Purkinje cell alteration index decreased 3.33 times compared to the control (Table 6).

In the CA_1 zone of the VH after the administration of citicoline, there are no visual changes in the pyramidal layer in terms of the order and density of neurons located in it. The state of the tigroid in the neuroplasm is normochromic or moderately hyperchromic (Fig. 29). The percentage distribution of cells by their functional state showed: the percentage of normochromic neurons increased by 10.2%, hyperchromic and hypochromic cells did not change. The number of hyperchromic pyknomorphic and shadow cells decreased by 2 and 1.3 times, respectively, the alteration index decreased by 2.4 times (Table 7).

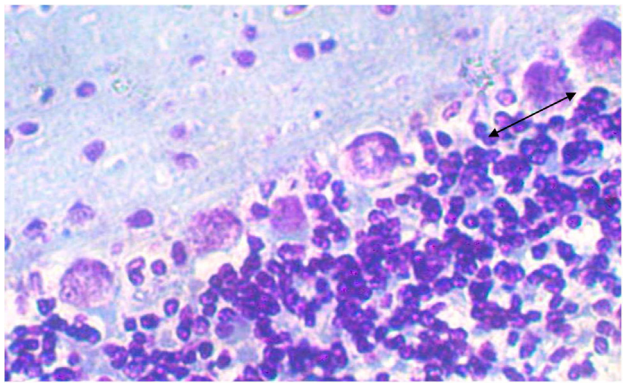
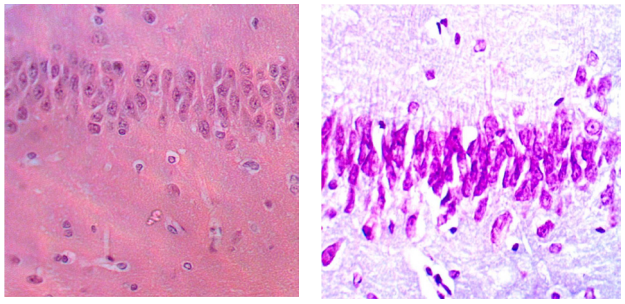


Fig. 28. CC of a rat treated with citicoline on day 8 of TBI. Hypochromia, chromatolysis, destructive changes, thinning of the Purkinje cell arrangement; Thionine by Nissl method; ×400

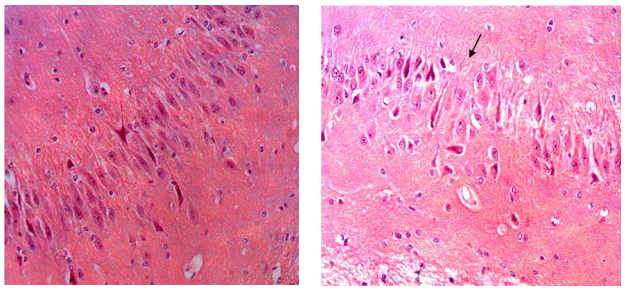
As for the CA_3 zone of the VH, in it, along with areas that were practically unchanged in terms of histological structure, areas were found in which loosening and loss of cells, changes in their shape, and pronounced eosinophilic staining of the neuroplasm were observed (Fig. 30).

Staining with thionin revealed that in the cytostructurally unchanged areas of this VH zone, the state of the tigroid in most neurons was hypochromic. In row disorders, hyperchromic and hyperchromic pyknomorphic cells predominated among neurons (Fig. 31).



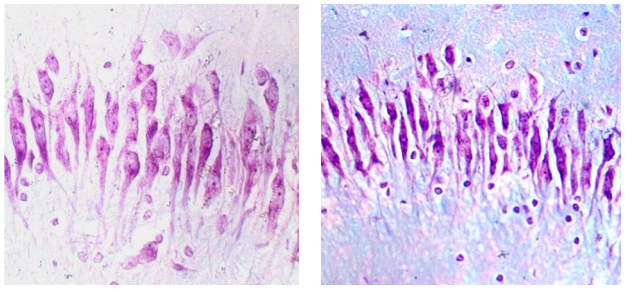
a *b*

Fig. 29. CA_1 zone of the VH of rats treated with citicoline on the 8th day of TBI: *a* – normal cytoarchitecture of the pyramidal layer, morphostructure of neurons (hematoxylin-eosin; ×250); *b* – normochromic and hyperchromic neurons (thionin according to the Nissl method; ×400)



a *b*

Fig. 30. CA_3 zone of the VH of rats treated with citicoline on day 8 of TBI: *a* – normal area of rows of pyramidal neurons; *b* – disruption of the cytoarchitectonics of the layer, "falling out" of neurons from the rows (arrow)l Hematoxylin-eosinl ×250



a *b*

Fig. 31. CA_3 zone of the VH of rats treated with citicoline on day 8 of TBI: *a* – hypochromic neurons in the area with unchanged histoarchitecture; *b* – hyperchromic and hyperchromic pyknomorphic neurons in the area with "dropout" of cellsl Thionin by the Nissl methodl ×400

A certain number of cells with pronounced chromatolysis were observed (Fig. 32).

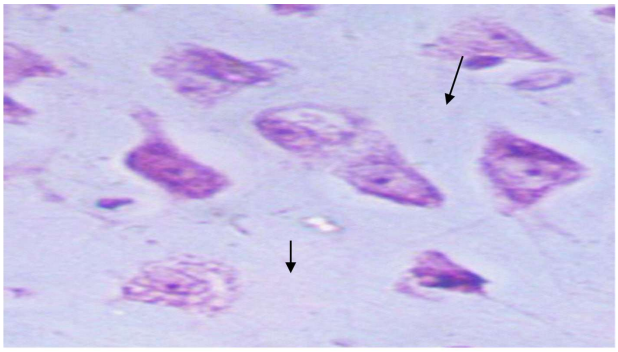


Fig. 32. CA_3 zone of the VH of a rat treated with citicoline on day 8 of TBI. Neurons with pronounced chromatolysis. Thionine staining by the Nissl methodl ×400

Morphometric measurements showed that only the relative number of hyperchromic and hyperchromic pycnomorphic neurons was significantly reduced compared to controls. The alteration index decreased by 1.14 times (Table 5).

4. Discussion of research results

The obtained results of the study confirmed the pronounced neuroprotective potential of the new combined agent containing dry extract of peony roots, L-tryptophan and glycine. In a model of TBI that simulates moderate brain damage, the agent demonstrated the ability to significantly reduce the biochemical and morphological manifestations of traumatic injury. At the biochemical level, a decrease in the concentrations of NSE and S100 protein in the blood serum of rats treated with the combined agent was found compared to the untreated group. Both proteins are recognized as sensitive markers of nerve tissue damage, and their increased level is associated with the severity of the injury, the risk of brain edema and a worse prognosis. A decrease in these indicators indicates a limitation of the volume of neurodestruction and, probably, a decrease in secondary neuroinflammation.

Morphological analysis of brain tissue showed that rats treated with the drug had improved microstructural organization of the sensorimotor cortex, reduced edema, preserved normochromic neurons, decreased neuronal alteration index and glio-neuronal ratio. In addition, the number of dark (degeneratively altered) neurons in the hippocampal areas, especially in the CA1 area, decreased, indicating the drug's potential ability to prevent necrotic changes.

The obtained data are consistent with the literature sources, which indicate the separate neuroprotective properties of each of the components of the drug. L-tryptophan, as a precursor of serotonin, is involved in the regulation of neurotransmitter activity, sleep, mood, as well as in the reduction of oxidative stress through the kynurenine pathway [21–23]. Glycine acts as an inhibitory neurotransmitter, capable of stabilizing the neuronal membrane, reducing the toxic effects of glutamate, and stimulates the synthesis of glutathione – one of the main antioxidants in brain tis-

sue [24]. Peony root extract has sedative, anti-inflammatory and antioxidant activity due to a complex of phytochemicals, including glycosides, flavonoids and alkaloids [25, 26].

Thus, the synergistic effect of the components allows for the simultaneous influence on several pathogenetic links of TBI: hemomicrocirculation disorders, hypoxia, glutamate-induced toxicity and neuroinflammation.

Comparison with the drug citicoline, which is used in clinical practice as a neuroprotector, showed that the new agent is not inferior to it in effectiveness, and in several indicators (morphological preservation of neurons, biochemical markers) even surpasses it. This is especially relevant given the need to create an effective domestic drug with an affordable price and broad therapeutic capabilities.

Practical significance. The proven effectiveness of the drug in reducing neuroinflammation, glial reaction and biochemical markers of neuronal damage (NSE, S100) opens up the possibility of its use in the complex treatment of patients with TBI and forms the basis for the creation of a domestic drug with a combined effect, which can reduce the dependence of the Ukrainian medical system on imported drugs and strengthen the pharmaceutical safety of the country, especially in wartime conditions.

Study limitations. The duration of the experiment was 7 days, which allows assessing only the acute and subacute phase of TBI, not covering long-term consequences and possible remote effects of the combined drug. A detailed study of the mechanisms of action of the drug was not conducted, in particular the effect on oxidative stress, cytokine response, apoptosis or neurogenesis, which requires further study using immunohistochemical and molecular biological methods.

Prospects for further research. In-depth study of the mechanisms of action of the developed combined drug at the molecular level using immunohistochemical, biochemical and genetic methods (assessment of the level of pro-inflammatory cytokines, oxidative stress, apoptosis markers, etc.). Study of the chronic phase of TBI with extended periods of observation of the drug's effect – to determine its effectiveness in restoring cognitive functions, memory and neuroplasticity.

5. Conclusions

1. Traumatic brain injury in rats leads to significant morphological and biochemical changes in brain tissues, including the development of congestive hyperemia, edema, subarachnoid hemorrhages, a decrease in the number of morphologically and functionally unchanged neurons, an increase in the content of markers of neurodestruction – S100 and NSE proteins in blood serum, and activation of the glial reaction (increase in glio-neuronal and perineuronal satellite indices).

2. The combined agent, which includes dry extract of peony roots, L-tryptophan and glycine, significantly reduced the level of NSE and S100 protein at 24 hours and 8 days after TBI, compared to animals without treatment, improved cerebral blood circulation, reducing capillary spasm and edema, reduced the severity of neurodestructive changes, maintained the number of normochromic neurons and reduced the alteration index, and demonstrated a more pronounced neuroprotective effect than the comparator drug citicoline.

3. Morphometric and histological data indicate the cerebroprotective properties of the drug, especially in the sensorimotor cortex of the cerebral hemispheres and the CA1 area of the hippocampus.

Conflict of interest

The authors declare that they have no conflict of interest regarding this study, including financial, personal, authorship or other, that could influence the study and its results presented in this article.

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Data availability

The manuscript has no linked data.

Use of artificial intelligence technologies

The authors confirm that they did not use artificial intelligence technologies when creating the presented work.

Authors' contributions

Nadiia Kononenko: Conceptualization, Methodology, Writing – reviewing and editing, Management, Project administration; **Ruslan Mirzaliiev:** Research, Resources, Writing – initial draft, Formal analysis, Visualization.

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