Original Article

Phytopharmacotherapy of neurodegenerative disorders of the rat brain with an aqueous extract of *Chlorophytum comosum*

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ABSTRACT

Basic pharmacotherapy of cerebral ischemia includes the use of vasoactive, antiplatelet, nootropic, neuroprotective drugs, antihypoxants, and antioxidants. However, despite their sufficiently high efficiency, their combined use often leads to the development of undesirable reactions. The work aimed to conduct a morphometric study of neurons of the frontal lobe of the brain and the CA1 region of the hippocampus of rats with simulated total ischemia of the brain and correction with an aqueous extract of *Chlorophytum comosum*. In a statistical study, it was found that ischemic damage to neurons of both the frontal lobe and the hippocampus was significantly more pronounced in the group with a 4-vessel model of brain ischemia without correction with an aqueous extract of *Chlorophytum comosum*. The minimum diameter, perimeter, and area of neurons, diameter, perimeter, and area of nuclei were significantly different ($p<0.05$). The number of bicellular neurons was significantly greater when corrected with an aqueous extract of *Chlorophytum comosum* ($p<0.05$), which reflects the functional state of neurons, their greater activity, and their ability to regenerate and reparative processes. No significant differences were found when measuring the maximum diameter of the neuronal nuclei ($p>0.05$). Correction of ischemic damage with an aqueous extract of *Chlorophytum comosum* showed a statistically significant increase in the resistance of neurons of the frontal lobe and CA1 region of the hippocampus to hypoxia and ischemia, a significant decrease in the number of dead neurons and greater activity of reparative processes.

Keywords: Rats, *Chlorophytum comosum,* Extract, Phytopharmacotherapy, Neuroprotective effect

Introduction

Disorders of cerebral circulation of various etiologies are one of the most significant nosologies and occupy second place in the structure of total mortality [1]. The development of methods of prevention and treatment is still one of the urgent problems of both clinical pharmacology and neurology. The search and use of a large number of experimental models of cerebral ischemia are due to various variants of cerebral circulatory disorders [2]. To simulate total cerebral ischemia in an experiment, a 4-vessel model of cerebral ischemia is used. In this model, an occlusion of two vertebral and two common carotid arteries is created [3]. This variant of pathology is one of the most technically difficult to perform and allows to obtain total brain ischemia in more than 90% of animals [4, 5]. The assessment of the consequences of cerebral ischemia can be carried out using a morphometric examination of its sections [6]. Neurons of the frontal lobe, cerebellum, and hippocampus are the most sensitive to hypoxia

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and ischemia [7]. The hippocampus provides the implementation of memory mechanisms, and behavioral reactions, and performs the function of fixing emotionally significant events [8]. Functional, biochemical, and morphological signs of ischemia and neuron damage are recorded after 2-minute ischemia [9].

In a study it was shown that the administration of hydroalcoholic extract of saffron petal decreased serum levels of TG, cholesterol, and LDL-c and increased serum levels of HDL-c in treated rat, it is also known that Glucose level significantly decreased in DM+MT rats.

Basic pharmacotherapy of cerebral ischemia includes the use of vasoactive, antiplatelet, nootropic, neuroprotective drugs, antihypoxants, and antioxidants [10]. However, despite their rather high efficiency, their combined use often leads to the development of undesirable reactions [11]. In this regard, an urgent problem is a search for new safe pharmacological agents that have a complex effect on the factors of stroke development, trigger mechanisms, and basic pathological reactions, as well as stimulating recovery processes. The use of herbal raw materials with several advantages over synthetic drugs is promising: a wide range of pharmacological properties, simultaneous effect on several pathogenetic targets due to a complex of biologically active substances, as well as the absence of undesirable side reactions with prolonged use [12].

One of the plants contributing to the increase of adaptive reserves of the brain is *Chlorophytum comosum*, used in folk medicine for the treatment of diseases of the nervous system, and also used as a coronary dilator, anticoagulant and antispasmodic agent [13]. According to several authors, it has been established that remedies from the plant have a sedative, vasodilating, and hypolipidemic effect [14, 15]. The pharmacological properties of this plant are due to a wide range of biologically active substances, the main of which are coumarins contained in its rhizomes [16]. According to the literature, coumarins exhibit antiplatelet, anticoagulant, antispasmodic, antioxidant, and neuroprotective properties [17]. The presence of a group of coumarin compounds in the plant makes it possible to consider it a promising cerebroprotective agent for the treatment of cerebral ischemic disorders and stroke prevention.

The effects of Cichorium powder on organ weight in obese rats shows a substantial variation in the liver weight between the positive.

The aim of the study

To conduct a morphometric study of neurons of the frontal lobe of the brain and the CA1 region of the hippocampus of rats with simulated total ischemia of the brain and correction with an aqueous extract of *Chlorophytum comosum*.

Materials and Methods

The study was performed on 100 mature male rats of the Wistar line weighing 230-260 g. The experimental animals were divided into 4 groups:

Group 1 – a control group (n=25);
Group 2 – a group with 2-vessel pathology (n=25);
Group 3 – a group with 4-vessel pathology (n=25);
Group 4 – a group with pathology and correction with an aqueous extract of *Chlorophytum comosum* (40 mL/kg, *per os*, n=25).

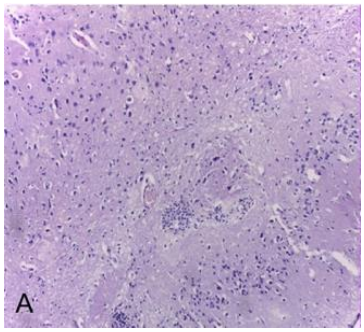
The animals were kept in standard vivarium conditions with free access to food and water. The animals were kept and the experiment was conducted in accordance with the Guide for the Care of Laboratory Animals [18]. 2-vessel local ischemia was simulated in Group 2: blood flow in the common carotid arteries was blocked for 4 minutes. Total 4-vessel cerebral ischemia with an ischemic period of 4 minutes was simulated in Group 3 [19]. Group 4 was intragastrically injected with a phosphodiesterase-5 (PDE-5) inhibitor, an aqueous extract of *Chlorophytum comosum*, at a dose of 40 mL/kg 60 minutes before the simulation of total ischemia. The animals were anesthetized with 60 mg/ml Zoletil 100 (Virbac Sante Animale, France) and 150 mg/ ml chloral hydrate. The animals were removed from the experiment 72 hours after its start by overdosing on anesthetic drugs.

The brain was extracted from the cranial cavity, cut into 3 fragments, and fixed in a 10% solution of neutral formalin for 2-3 days. To obtain histological preparations, pieces of the brain were poured into paraffin according to a standard procedure, after which serial sections with a thickness of 4 μm were made on a certified rotary microtome Leica RM2125 RTS (Leica, Germany). For histological and morphometric examination, the sections were stained with hematoxylin and eosin in the Autostainer XL ST5010 (Leica, Germany) histological sections and smears staining machine, as well as with thionin according to the Nissl method. Morphometry was performed after microscopic examination using a Leica DM 4000 B microscope (Leica, Germany) and the creation of a digital image on a semi-automatic scanner Mirax Desk (Carl Zeiss Microimaging GmbH, Germany). To do this, we used the Pannoramic Viewer 1.15 software (3DHISTECH Ltd., Hungary). The unaltered and hyperchromic neurons of the pyramidal and polymorphic layers of the CA1 region of the hippocampus and neurons of the frontal lobe were counted, the larger and smaller diameters of the precarious, their perimeter and area, the diameter of the nuclei and nucleoli were measured, the relative number of neurons in several fields of vision was determined, followed with recalculation by 0.01 mm^2. For each animal, 30 measurements of each planimetric and quantitative indicator were performed with data entry in MS Excel (Microsoft, USA) and Statistica 12.0 (Statsoft, USA) software. Descriptive statistics were applied to all the data: the data were checked for the normality of the distribution. The type of distribution was determined by the Shapiro-Wilk criterion. In the case of a normal distribution, the mean (M) and the standard error of the mean (m) were calculated. In the cases of an abnormal distribution, the median (Me) and quartile span (QR) were calculated. The intergroup differences were analyzed by parametric (Student's t-test) or nonparametric (Mann-Whitney test) methods depending on the type of distribution. The differences were determined at a 0.05

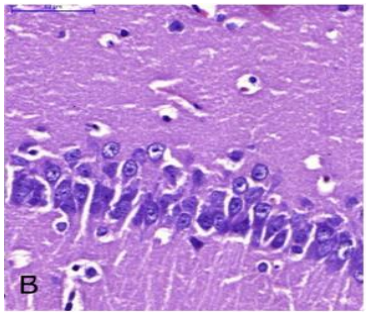
significance level. Statistical analysis was performed using the Statistica 12.0 software (Statsoft, USA).

Results and Discussion

During survey microscopy in intact animals, our results corresponded to the descriptions of cytoarchitectonic features of the frontal lobe and hippocampus [20]. The neurons were predominantly pyramidal, rounded, or polygonal in shape, with large rounded nuclei and fine-grained cytoplasm. In many neurons, the basophilic substance of the cytoplasm had the appearance of large lumps located peripherally. In some neurons, one or two centrally located nucleoli were clearly defined **(Figure 1)**.



a)



b)

Figure 1. Histological sections of brains of intact rats (Group 1): a) frontal lobe, ×100, hematoxylin+eosin; b) the hippocampus, ×400, hematoxylin+eosin

The frontal lobe region was characterized by a low density of location, and the CA1 region of the hippocampus was characterized by a high density of medium-sized neurons. Morphometric changes of neurons in the 2-vessel ischemia model had a general tendency to increase and were characterized by an increase in the maximum and minimum diameters of the perikaryon, their perimeter, and area. The diameter, area, and perimeter of the nuclei of neurons also increased. The total number of hyperchromic neurons was 36.7%, and the number of bicellular neurons was 16.7% **(Table 1)**. Changes in similar indicators in the CA1 region of the hippocampus were less significant, but there was a general trend toward their increase **(Table 2)**.

Table 1. Morphometric characteristics of neurons of the frontal lobes of the rat brain

Index	Intact	2-vessel CI	4-vessel CI	PRE5 + 4- vessel CI
Max. diameter	12.51±0.29	13.23±0.42#	13.54±0.63#	8.57±0.35#
Min. diameter	8.46±0.28	10.41±0.40*	6.58±0.21*	5.62±1.51*
Cell perimeter	35.87±0.90	41.58±1.27*	38.60±1.38*	23.83±4.46*
Cell square	83.33±3.25	120.48±5.81*	75.45±3.62*	40.67±1.28*
Nuclear perimeter	21.98±0.89	29.68±1.01*	23.58±0.65*	16.38±1.89*
Nuclear square	35.08±2.21	58.61±4.21*	38.90±2.02*	20.56±0.39*
Nuclear diameter	6.28±0.24	9.15±0.42*	7.37±0.24*	5.64±0.11*
Nucleus	1.88±0.07	Hypochromic neurons (46.7%): 2.65±0.50 Hyperchromic (36.7%) Two-nucleated (16.7%): 2.40±0.24	Hypochromic neurons (10%): 1.67±0.18 Hyperchromic (90%) Two-nucleated (14.5%): 2.08±0.17	Hypochromic neurons (43.4%): 1.63±0.16 Hyperchromic (43.4%) Two-nucleated (13.2%): 1.26±0.10

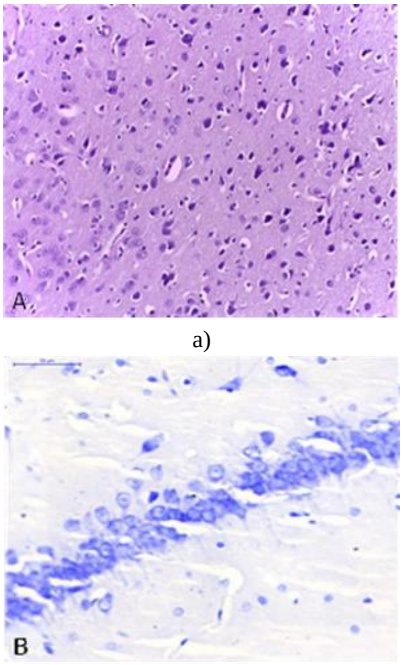
*– $p<0.05$, # – $p>0.05$.

Table 2. Morphometric characteristics of rat hippocampal neurons

Index	Intact	2-vessel CI	4-vessel CI	PRE5 + 4- vessel CI
Max. diameter	11.85±0.29	13.64±0.16*	11.38±0.16#	8.49±0.21*
Min. diameter	8.03±0.28	6.90±0.20*	6.34±0.20*	5.70±0.78*
Cell perimeter	33.01±0.80	37.22±0.34*	30.40±0.34*	24.30±2.30*
Cell square	68.90±2.63	77.14±2.63*	57.71±1.76*	38.94±0.81*
Nuclear perimeter	22.66±0.72	27.11±0.47*	20.86±0.47*	17.31±1.28*
Nuclear square	38.06±2.50	50.41±1.25*	29.96±1.25*	20.98±0.26*
Nuclear diameter	6.23±0.26	6.47±0.25#	7.09±0.25*	5.56±0.07*
Nucleus	2.38±0.07	Hypochromic neurons – Hyperchromic (86.4%) Two-nucleated (15.6%): 2.02±0.11	Hypochromic neurons – Hyperchromic (90%) Two-nucleated (10%): 1.83±0.13	Hypochromic neurons – Hyperchromic (91%) Two-nucleated (9%): 1.65±0.14

*– $p<0.05$, # – $p>0.05$.

Qualitative changes were characterized by an increase in the number of hyperchromic neurons, chromatolysis, and moderate disorganization of layers in the CA1 region. Cell bodies lost the clarity of contours, deformed. Nuclear changes were polymorphic and manifested both by swelling and pyknosis of individual nuclei. In some neurons, there was a displacement of the nucleus at the periphery of the perikaryon. An increase in the number of bicellular neurons in combination with a general increase in the area of the nucleus and perikaryon is a morphological manifestation of regenerative processes and an increase in their functional activity arising in response to local cerebral ischemia **(Figure 2)**.

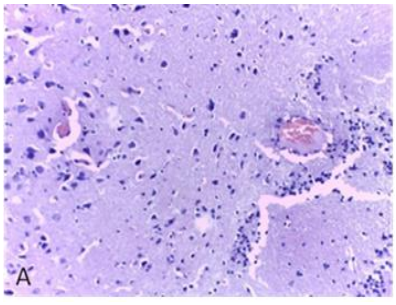


a)

b)

Figure 2. 2-vessel model of cerebral ischemia: a) frontal lobe, ×400, hematoxylin+eosin; b) CA1 hippocampal region, ×400, thionin according to Nissl.

In the 4-vessel ischemia model, pronounced hyperchromasia of frontal lobe neurons with perivessel and pericellular edema was noted. The shape of the cells was mainly polygonal, and elongated, the nuclei in many of them were not determined. Capillaries are partially expanded, and full-blooded. Disorganization of neuronal layers, chromatolysis, swelling, and pycnotic changes of nuclei were observed in the CA1 region of the hippocampus, biconuclear neurons were not detected **(Figure 3)**.



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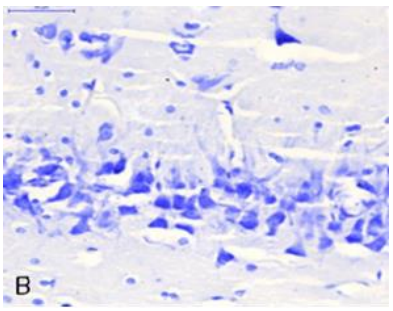
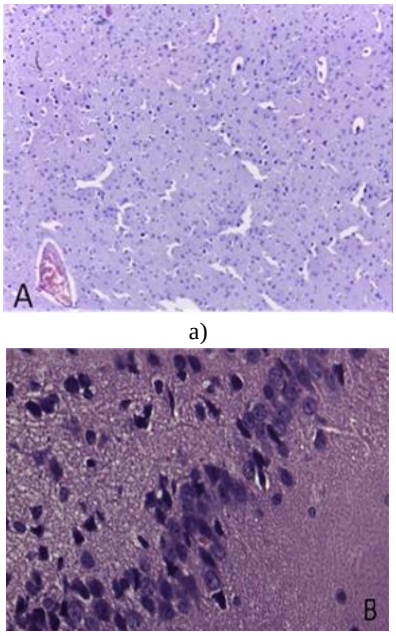


b)

Figure 3. 4-vessel model of cerebral ischemia: a) frontal lobe, ×400, hematoxylin+eosin; b) CA1 hippocampal region, ×400, thionin according to Nissl

When an aqueous extract of *Chlorophytum comosum* was administered at a dose of 40 mL/kg a 60 min. before the modeling of 4-vessel pathologies, morphological changes in neurons had a predominantly necrobiotic nature. The number of dead neurons was significantly lower in comparison with other groups. In the hippocampus, the violation of stratification of layers was moderate **(Figure 4)**.



a)

b)

Figure 4. 4-vessel model of cerebral ischemia under correction with aqueous extract of *Chlorophytum comosum*: a) frontal lobe, ×100, hematoxylin + eosin; b) the hippocampus, ×400, hematoxylin+eosin

Currently, several scientific studies have been carried out, which show the effectiveness of using PDE-5 inhibitors for prevention, as well as reducing the volume of damage in ischemic organ injuries [21, 22]. The hypothetical mechanism of cerebroprotective action of an aqueous extract of *Chlorophytum comosum*, as PDE-5, is the effect on the pathway of NO/cGMP protein kinase G (PC-G). When PC-G is activated, K^+ channels are phosphorylated, the sensitivity of the membrane to nerve impulses decreases, and hyperpolarization occurs [23-25]. Then there is a decrease in the concentration of free Ca^{2+} in the cytoplasm [26]. These mechanisms are the basis of the

vasodilating effects of nitric oxide [27]. PDE-5 plays a major role in this mechanism. It reduces the accumulation of cGMP [28]. Thus, based on the results obtained, an aqueous extract of *Chlorophytum comosum* with PDE-5 has a protective effect on cerebral ischemia.

Conclusion

In a statistical study, it was found that ischemic damage to neurons of both the frontal lobe and the hippocampus was significantly more pronounced in the group with a 4-vessel model of brain ischemia without correction with an aqueous extract of *Chlorophytum comosum*. The minimum diameter, perimeter, and area of neurons, diameter, perimeter, and area of nuclei were significantly different ($p<0.05$). The number of bicellular neurons was significantly greater when corrected with an aqueous extract of *Chlorophytum comosum* ($p<0.05$), which reflects the functional state of neurons, their greater activity, and their ability to regenerate and reparative processes. No significant differences were found when measuring the maximum diameter of the nuclei of neurons ($p>0.05$).

Thus, moderate necrobiotic changes with signs of activation of regenerative processes in both frontal lobe and hippocampal neurons were observed in the 2-vessel model of cerebral ischemia. In the 4-vessel ischemia model, damage to most neurons was irreversible, with no signs of activation of reparative processes. Correction of ischemic damage with an aqueous extract of *Chlorophytum comosum* showed a statistically significant increase in the resistance of neurons of the frontal lobe and CA1 region of the hippocampus to hypoxia and ischemia, a significant decrease in the number of dead neurons and greater activity of reparative processes. The results obtained open new prospects in sturdy of the neuroprotective effect of the aqueous extract of *Chlorophytum comosum* and its application in phytopharmacotherapy at neurodegenerative diseases.

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Conflict of interest: None

Financial support: None

Ethics statement: The protocol for experiments with laboratory animals complied with the requirements of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes.

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