Original Article

Evaluation of the neuroprotective effect of root and leaf extracts of Chlorophytum comosum

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ABSTRACT

The aim of this study was a comparative study of the neuroprotective efficacy of root and leaf extracts of Chlorophytum comosum, as well as their combined use in glutamate excitotoxicity in a culture of rat cerebellar neurons. The effect of Chlorophytum comosum extracts on glutamate excitotoxicity was studied on 7–8 day old cultures of cerebellar neurons obtained from 7-9 day old rats by enzyme mechanical dissociation. We found that the addition of extracts of Chlorophytum comosum to the culture medium in the post-glutamate period led to a significant reduction in the death of cerebellar neurons in culture. The percentage of surviving neurons, when Chlorophytum comosum was introduced at a concentration of 60 μg/mL, ranged from 32.7 to 36.5%, and at a concentration of 90 μg/mL, from 37.8 to 42.4%. As a result of the experiment, it was concluded that root and leaf extracts of Chlorophytum comosum have a pronounced and almost equal neuroprotective effect in the case of glutamate excitotoxicity.

Keywords: Chlorophytum comosum, Neuroprotective action, Glutamate excitotoxicity, Biological active supplements

Introduction

Cerebral circulation disorders and ischemic damage to brain tissue are important medical and social problems [1]. According to the World Health Organization, 15-20 million new cases of stroke are registered annually in the world, while more than 80% of survivors remain disabled [2]. In Russia, about 450-500 thousand people fall ill with stroke. per year, over the past 10 years, the number of patients in this category has increased by more than 30%, which causes significant economic damage to society [3]. Therefore, the search for effective ways to protect neurons in various pathological conditions is one of the most important tasks of fundamental and applied neurobiology today. Currently, along with well-known synthetic neuroprotective agents (Mexidol, Emoxypin, etc.), considerable attention is paid to natural ones, incl. herbal preparations with a neuroprotective effect [4, 5]. Chlorophytum comosum is of great interest and importance in this field.

Chlorophytum comosum is a perennial herbaceous plant of the family, a traditional remedy in China, Mongolia, and Tibet. Most often there it is used as a tonic. In Chinese traditional medicine, the root of Chlorophytum comosum is used as a key component with anti-inflammatory, antioxidant, cardioprotective, and immunostimulatory effects [6]. It is known from literary sources that Chlorophytum comosum root has an antiradical effect, inhibits lipid peroxidation, and increases the activity of antioxidant enzymes [7, 8].

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For experimental and clinical neuropharmacology, Chlorophytum comosum is of significant interest because, in Chinese traditional medicine, its root has been used for about 300 years to treat post-stroke patients [9]. The results of scientific studies obtained over the past 10 years indicate that the Chlorophytum comosum root extract and its components have a neuroprotective effect in modeling ischemia *in vitro* and *in vivo*. It is a potential neuroprotector in the treatment of Parkinson's disease, stimulates the growth of peripheral nerves, and prevents excitotoxicity [10-13]. At the same time, there is no data on the use or study of the aerial part of Chlorophytum comosum as a neuroprotective agent.

In this regard, the purpose of this study was a comparative study of the neuroprotective efficacy of root and leaf extracts of Chlorophytum comosum, as well as their complex use in glutamate excitotoxicity in a culture of rat cerebellar neurons.

It is known that the toxic effect of glutamate on brain neurons is one of the main factors leading to cell death in stroke [14]. The accumulation of glutamate in the extracellular space and its toxic effect on neurons is an important link in the chain of pathological processes leading to cell death during strokes and injuries [15]. Hyperstimulation of glutamate receptors leads to disruption of calcium homeostasis, energy deficiency, activation of free radical oxidation, and lipid peroxidation of neuronal membranes [16]. Therefore, the results obtained in this model are an important indicator of the neuroprotective efficacy of the studied substances.

Materials and Methods

The roots and leaf of Chlorophytum comosum were collected in the vicinity of Krasnodar, Russia (grass - in July, roots - in September). The raw materials were dried, crushed, and extracts were prepared [8]. Extraction of biologically active compounds from the root and leaf of the plant was carried out with 70% ethanol solution for 21 days at 37 °C. The resulting extract was filtered, freed from alcohol on a rotary evaporator, and sublimated at –45°C. Aqueous solutions of extracts were used in the work.

The effect of Chlorophytum comosum extracts on glutamate excitotoxicity was studied on 7–8 day old cultures of cerebellar neurons obtained from 7–9 day old rats by enzyme mechanical dissociation. Cells were grown in 96-well plates, and KCl was added to the culture medium at the last pipetting. Glutamate exposure was carried out in a saline solution with the following composition: 0.35 mM of Na_2HPO_4, 2.3 Mm of $CaCl_2$, 136.7 Mm of NaCl, 5.6 Mm of KCl, 11.9 Mm of $NaHCO_3$, and 11.1 of mM glucose (pH 7.5). The exposure time was 10 minutes. Control cultures were placed in saline without glutamate for 10 minutes. Cultures were then returned to the original nutrient medium and placed in a CO2 incubator for 4.5 hours. The extracts were added to the cultures immediately after they were returned to the original nutrient medium at final concentrations of 60 and 90 μg/ml. To study the complex of extracts, the root and leaf extracts were added in equal amounts (30+30 and 45+45 μg/ml). After 4.5 h, cultures were fixed with FUS and stained with trypan blue. Morphological analysis was performed on a Zeiss Inverted Microscope ID-03 (Carl Zeiss Gmbh., Germany), and the number of living and dead neurons was taken into account. The results were presented as a percentage of intact neurons. For each data point, the results of at least three independent experiments were used. Statistical data processing was performed using Student's t-test.

Results and Discussion

The analysis of the results showed that the proportion of living neurons in the control cultures was 91.4±1.2%. Exposure to glutamate led to a sharp reduction in the number of surviving neurons to 17.6±1.6%. The addition of Chlorophytum comosum extracts to the culture medium in the post glutamate period led to a significant reduction in the death of cerebellar neurons in culture (ture 1).

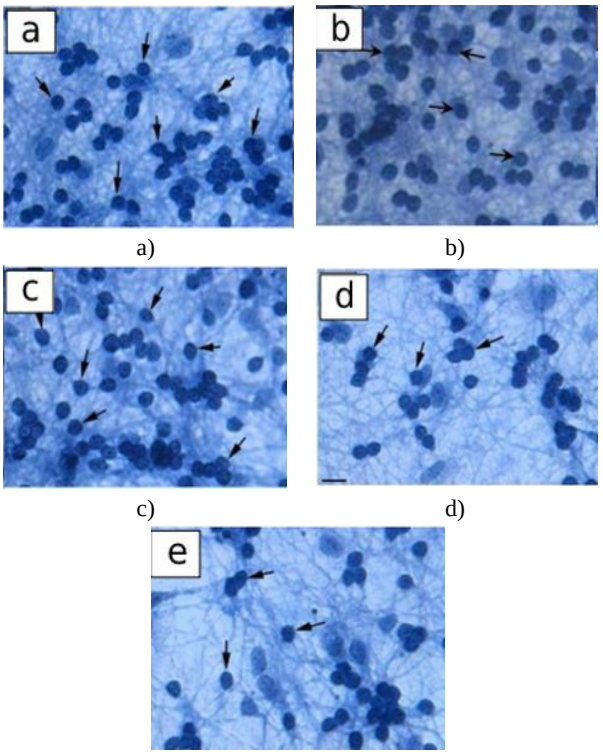

Figure 1. Granular neurons of the rat cerebellum in a control sample (a), a sample with glutamate (b), a sample with glutamate and Chlorophytum comosum root extract (c), a sample with glutamate and a complex of 2 extracts (d), a sample with glutamates and a leaf extract of Chlorophytum comosum (e). Scale 50 microns. Stained with trypan blue.

According to **Figure 1**, in the sample with glutamate (b), along with intact cells, the pycnotic nuclei of dead neurons are visible. In the control sample (a) and samples with extracts of Chlorophytum comosum, many preserved cells are visible grains of the cerebellum of a rounded shape, with native sprouts, without signs of destruction.

The percentage of surviving neurons with the introduction of leaf, root, and complex extracts of Chlorophytum comosum at a concentration of 60 μg /ml was: 32,7±1,3%, 36,5±2,1%, 34,3±2,1%, at a concentration of 90 μg/ml: 37,8±2,4%, 42,4±1,2%, 37,1±2,3% respectively **(Figure 2)**. The use of leaf and root extracts, as well as their complex extract in the absence of glutamate, did not affect the cultures of cerebellar neurons, the number of living cells remained at the control level.

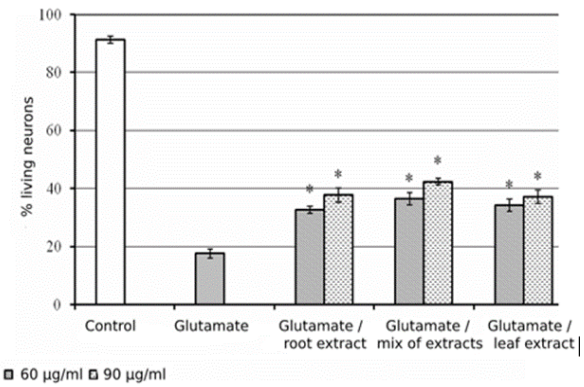

Figure 2. The effect of root and leaf extracts of Chlorophytum comosum on the excitotoxicity of glutamate in cultures of rat cerebellar neurons with their complex (mixture) and separate use.
* – p<0.001 compared to glutamate

Conclusion

Scientific researches report on the use in the traditional medicine of both the root and leaf part of Chlorophytum comosum in the native state. However, attention is drawn to the fact that it is the root of Chlorophytum comosum that is used for the treatment of strokes, and among the modern publications devoted to the study of the neuroprotective properties of this plant, we could not find works in which the leaf extract would be investigated. Meanwhile, the data obtained by us indicate that with respect to the excitotoxicity of glutamate, the leaf extract of Chlorophytum comosum, as well as the root extract of Chlorophytum comosum and their mix, have high and almost equal effectiveness.

The property we have identified in the root and leaf extracts of Chlorophytum comosum to increase the resistance of neurons to glutamate excitotoxicity *in vitro* will serve as the basis for deeper studies of this plant to effectively use it in the treatment of cerebrovascular pathologies.

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Conflict of interest: None

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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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