



Research Article

Correction of Mitochondrial Dysfunction by 4-Hydroxy-3,5-Ditretbutyl Cinnamic Acid in Experimental Alzheimer's Disease Induced by A β Injection in Rats

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Abstract

Background: Alzheimer's disease is the main form of dementia, which affects more than 46 million people every year. In the pathogenesis of Alzheimer's disease, a significant role played mitochondrial dysfunction, which is a promising pharmacotherapeutic target of neuroprotective therapy. In this regard, this study aimed to evaluate the effect of the 4-hydroxy-3,5-ditretbutyl cinnamic acid on changes of mitochondrial function in experimental Alzheimer's disease induced by A β injection in rats.

Methods: Alzheimer's disease was modeled on Wistar rats by injecting a fragment of β -amyloid (A β_{1-42}) into the CA1 part of the hippocampus. The test-compound (4-hydroxy-3,5-ditretbutyl cinnamic acid, 100 mg/kg, *per os*) and the reference drugs (resveratrol, 20 mg/kg, *per os* and EGB671, 100 mg/kg, *per os*) were administered for 60 days after surgery. The restoration of a memorable trace in animals was evaluated in the Morris water maze test. The concentration of β -amyloid, Tau-protein, and changes in parameters characterizing mitochondrial function (cellular respiration, concentration of mitochondrial ROS, activity of apoptosis reactions (caspase-3 and apoptosis induced factor) were also determined.

Results: This study showed that the administration of 4-hydroxy-3,5-ditretbutyl cinnamic acid at a dose of 100 mg/kg (*per os*) in rats with reproduced Alzheimer's disease contributed to the normalization of mitochondrial respiratory function. It was expressed in the normalization of aerobic metabolism, increased activity of respiratory complexes and stabilization of mitochondrial membrane potential. Also, when animals were treated with 4-hydroxy-3,5-ditretbutyl cinnamic acid, there was a decrease in the concentration of intracellular calcium (by 39.7% ($p < 0.05$)), the intensity of apoptosis reactions, and an increase of the latent time of the mitochondrial permeability transition pore opening (by 3.8 times ($p < 0.05$)), and decreases H₂O₂ concentration (by 21.2% ($p < 0.05$)).

Conclusion: In the course of this study, it was found that 4-hydroxy-3,5-ditretbutyl cinnamic acid exceeds the value of neuroprotective action in compared to the reference agents – resveratrol (20 mg/kg) and *Ginkgo biloba* extract (EGB671, 100 mg/kg).

Introduction

Alzheimer's disease (AD) is a chronic neurodegenerative disease that is the main cause of dementia and accounts for about 80% of all cases of diagnosed dementia.¹ To date, about 46.8 million people suffer from AD, and by 2030, the number of cases of AD will increase to 131.5 million. Between 2000 and 2014, AD-related deaths increased by 89% in the United States alone. Besides, according to various estimates, the direct and indirect costs of the world's 18 largest economies for the treatment and maintenance of a proper standard of living for patients with AD amount to more than \$ 1 trillion.²⁻⁶

Mitochondria are primarily known as the "powerful stations" of the cell, which provide the cell's needs for macroergic compounds. However, along with the energy function, mitochondria participate in the regulation of calcium homeostasis, redox reactions, and apoptotic cascade.⁷ According to literature data, mitochondrial dysfunction contributes to increased A β formation. Studies conducted on neuronal cell culture have shown that mitochondrial dysfunction contributes to the A β production by increasing oxidative stress.⁸ Oxidative stress enhance the activity of γ -secretase by a mechanism

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involving covalent modification of the complex of the γ -secretase-nicastrin with the product of membrane lipid peroxidation - 4-hydroxynonenal. At the same time, it was found that the main source of 4-hydroxynonenal is arachidonic acid, which is widely represented in mitochondrial membranes. Thus, it is assumed that one of the first stages of the formation of A β plaques is the aberrant mitochondrial function.⁹ However, there is no denying that the amyloidogenic cascade itself significantly affects the change in mitochondrial function.

It was found that aggregation of A β leads to a decrease in the production of mitochondrial ATP, and the activity of mitochondrial enzymes, and an increase of the ROS level. Hou *et al.*,¹⁰ 2014 found that when exposed to A β_{1-42} fragments on the culture of primary cortical neurons during the day and after 72 hours, it leads to the opening of the mitochondrial permeability transition pore (mPTP), reducing cell proliferation and causes cell death. In isolated mitochondria, the toxic effect of A β is expressed in inhibiting the activity of the mitochondrial respiratory chain complexes, in particular complex IV, and reducing the activity of mitochondrial enzymes: α -Ketoglutarate dehydrogenase, cytochrome c-oxidase, and pyruvate dehydrogenase.¹¹ At the same time, neurons that overexpress APP and A β produce large amounts of mitochondrial ROS and are not able to synthesize ATP in physiological quantities.¹² The available literature suggests that mitochondrial dysfunction may be a factor preceding aggregation of A β and pTau, whereas aggregation of A β and pTau may exacerbate mitochondrial dysfunction, thus closing the "vicious cycle" in AD pathology.

Several experimental studies have shown the prospects of correcting mitochondrial dysfunction in AD.¹³ One of the most frequent directions of pharmacological correction of mitochondrial dysfunction is the administration of exogenous antioxidants.¹⁴ In previous studies, it was found that 4-hydroxy-3,5-ditretbutyl cinnamic acid has pronounced antioxidant properties, expressed in inhibiting the formation of superoxide and nitrosyl radicals, reducing the intensity of lipid peroxidation, and restoring the activity of antioxidant enzymes such as superoxydedismutase, catalase, and glutathione peroxidase.¹⁵ Also, this compound had a neuroprotective effect in experimental brain ischemia in rats mediated by increased ATP synthesis in the brain.¹⁶ In this regard, it was suggested that the use of 4-hydroxy-3,5-ditretbutyl cinnamic acid in AD will contribute to the reduction of the pathological process and restore the integrity of brain tissue, which was the aim of this study.

Materials and Methods

Experimental animals

One hundred male Wistar rats weighing 200-220 grams were used in this study as a biological model. The animals were obtained from the living system laboratory of the Pyatigorsk medical and pharmaceutical Institute. During the experiment, the rats were kept in T-3 macrolone boxes with free access to water and food. Litter material

-wood granulated fraction was changed 1 time in 3 days. Maintenance conditions: air temperature $22 \pm 2^\circ\text{C}$, relative humidity $60 \pm 5\%$, with a natural change of the daily cycle. The concept of a research was approved by the local Ethics Committee of Pyatigorsk medical and pharmaceutical Institute (Protocol No. 11 of 11.09.2019). The content and handling of animals were under generally accepted standards of experimental ethics (Directive 2010/63 / EC of the European Parliament and of the Council for the protection of animals used for scientific purposes, dated 22 September 2010).

Materials

In this study, 4-hydroxy-3,5-ditretbutyl cinnamic acid (laboratory code ATACL) in a suspension form was used as the test-object. The studied compound was obtained at the Department of organic chemistry of the Pyatigorsk medical and pharmaceutical Institute. IR, UV, and NMR spectroscopy confirmed the structure of the test-substance. Based on previous studies, the studied compound was administered at a dose of 100 mg/kg (*per os*).¹⁶ The reference drugs in this study were standardized *Ginkgo biloba* extract (EGB671) at a dose of 100 mg/kg¹⁷ and resveratrol at a dose of 20 mg/kg¹⁸ administered orally. The reference compounds were obtained from *Hunan Warrant Pharmaceuticals*, China. The choice of reference drugs was based on the presence of these compounds of neuroprotective action.^{17,18} Also, presumably, resveratrol and EGB 671 have a similar mechanism of action to the ATACL compound, which predetermined the choice of these substances as reference drugs.

Experimental model of Alzheimer's disease

AD in rats was modeled by intrahippocampal injection of a fragment of A β_{1-42} in an amount of 2 μl and a final concentration of 1 mmol. A β_{1-42} was obtained from Sigma-Aldrich (Darmstadt, Germany).¹⁹ Before injection, the A β_{1-42} fragment was solubilized in a cooled PBS (pH = 7.4) with continuous stirring for 36 h until fibrillar aggregates were formed. Next, the rats were anesthetized by chloral hydrate (intraperitoneal, 350 mg/kg), the parietal region was scalped, and A β_{1-42} was administered using a 5-ml micro dispenser connected to a G30 needle.²⁰ The injection was performed in the CA1 part of the hippocampus (anterior-posterior = -3.8 mm, medial-lateral = 2.0 mm, dorsal-ventral = 2.6 mm from the bregma, as determined by Paxinos.²¹ The needle remained at the injection site for 5 minutes, after which the needle was removed and the surgical wound was sutured and treated by a 10% povidone-iodine solution. Rats were left under a warming lamp until awakening. At the same time, a generalized brain lesion may be observed against the intrahippocampal injection of A β .²²

Study design

According to the developed design of the study, five equal groups of animals were formed during this work (n=20 each experimental group). The first group of

rats – sham-operated animals (SO), which were applied to all consecutive operating procedures except for the direct injection of the A β ₁₋₄₂ fragment. In the remaining experimental groups, AD was modeled according to the above experimental procedure, and the following groups were identified: a group of negative control animals (NC) that did not receive pharmacological support (bidistilled water was administered in an equal volume), a group of rats that received the studied compound ATACL, and a group of rats that were treated by reference drugs EGB671 and resveratrol, respectively. A SO group of animals received distilled water in an equivalent volume. The studied substance and reference drugs were administered as a fine aqueous suspension (these substances are completely insoluble in water) on the next day after injection of the A β ₁₋₄₂ fragment and then for 60 days (daily, once a day). On 10, 20, 30, 40, 50, and 60 days of the study, the memory trace of rats were evaluated using the “Morris water maze” test. Then, on the 61 day of the experiment, the animals were decapitated and the brain was extracted, which was used as the study biomaterial.

Morris water maze test

The Morris water maze device is a water arena with a diameter of 150 cm with a wall height of 60 cm and a movable platform with a diameter of 10 cm [Open science, Russia]. During the study, the installation was filled with water to the 50 cm level, after which the water was tinted by food dye. Before simulation AD, rats were trained in the testing procedure: within 2 minutes the animals were allowed to find a platform, provided no execution of the task, rats were moved to the platform for 10 sec. after that, the testing was repeated. The training lasted for 10 days. After reproducing the AD in similar conditions, the test was repeated. The latency period of reaching the platform in seconds and the distance that the animal spent on finding the platform in meters was recorded, for which the testing procedure was subjected to video recording followed by a frame-by-frame analysis of the image in the Adobe Photoshop CS5 software.

Biomaterial sampling. Mitochondrial isolation

The animal's brain was used in this work like test-material. Rats were decapitated, the cranium was opened and the brain was removed. Moreover, in 10 animals from the group, the brain was homogenized in PBS with a pH of 7.4 in a ratio of 1: 7 in a Potter mechanical homogenizer and centrifuged in mode 10000g for 5 min. The resulting supernatant was used in an ELISA study and for enzymes activity determination. In the remaining 10 rats, the brain was homogenized in an isolation medium (1 mmol EDTA + 215 mmol mannitol + 75 mmol sucrose + 0.1% BSA solution + 20 mmol HEPES, with a pH of 7.2). For mitochondrial isolation, the resulting homogenate was centrifuged at 1100g for 2 minutes. The resulting supernatant in the amount of 700 μ l was transferred into Eppendorf tubes and mixed with 75 μ l of 10% percol and centrifuged at 18000g

for 10 minutes. The precipitate was resuspended in 1 ml of the isolation medium and centrifuged for 5 minutes at 10,000g. All procedures were performed at 4°C.²³

Respirometric analysis

To assess the respirometric function of mitochondria, the method of respirometry was used, in which the AKPM 1-01L (Alfa Bassens, Russia) laboratory respirometer system was used. The analysis is based on the measurement of oxygen consumption rate (OCR) in the medium when mitochondrial respiration disconnectors are sequentially introduced into the analyzed medium with biomaterial. Mitochondrial respiration disconnectors are: oligomycin in concentration 1 μ g/ml; FCCP (4 - (trifluoromethoxy) phenyl) hydrazono) malonitrile) in concentration -1 μ M/ml; rotenone – in concentration 1 μ M/ml; sodium azide – in concentration 20 mmol/ml. The following oxidation substrates were also used at different stages of the research: glucose – 15 mmol/ml; pyruvate - 10 mmol/ml; malic acid - 1 mmol/ml; succinic acid - 10 mmol/ml; ascorbate - 2 mmol/ml; adenosinediphosphate - 1 mmol/ml; N, N, N', N' tetramethyl-1,4-phenylenediamine - 0.5 mmol/ml. Define parameters: ATP-generating ability; the maximum level of respiration and the respiratory capacity. The activity of glycolysis processes was evaluated when glucose was used as an oxidation substrate during the registration of oxygen consumption under the conditions of the sequential addition of glucose, oligomycin, and sodium azide to the medium. The intensity of anaerobic processes was also determined. Evaluated parameters: glycolytic capacity glycolytic reserve and intensity of glycolysis. Additionally, the activity of complexes I, II, IV, and V of the mitochondrial respiratory chain was evaluated. The activity of mitochondrial complexes was evaluated according to the principles outlined in Connolly *et al.*,²³ The complex I activity was investigated by the difference in oxygen consumption when malate/ pyruvate was used as oxidation substrates. The complex II activity was investigated by the difference in OCR when succinate was used as an oxidation substrate and adding of oligomycin to the medium. The activity of complex IV was defined by the difference in OCR after adding the mixture of rotenone / TMPD / ascorbate as an oxidation substrate. The activity of complex V was defined by the difference in OCR after the introduction of rotenone and ADP to the analyze medium. During the analysis, the biosample volume was 275 μ l, and 25 μ l of injected analyzers. OCR was determined in ppm.²⁴ The oxygen consumption in the sample was converted to the protein concentration in the sample, which was determined by the Bradford method, as described.²⁵

Evaluation of latent time of mitochondrial permeability transition pore (mPTP) opening

The latent time of mitochondrial pore transition permeability opening was evaluated by spectrophotometric method. The incubation medium contained: 0.5 ml of

the analyzed supernatant, 200 mM KCl, 0.5 ml of a 1 μ m solution of cyclosporin A. The resulting mixture was adjusted to 2 ml with HEPES buffer solution with a pH of 7.4. The optical density of the mixture was recorded at $\lambda = 540$ nm, then the resulting solution was incubated for 25 min at room temperature with constant stirring. At the same time, the latent time of opening of the mitochondrial pore in seconds was evaluated (by changing the optical density of the incubation medium).²⁶

Mitochondrial membrane potential evaluation

Mitochondrial membrane potential was evaluated by spectrophotometric method. The incubation medium contained: 0.5 ml of the analyzed supernatant, 0.5 ml of a 9 μ m solution of safranin O. The resulting mixture was adjusted to 2 ml with HEPES buffer solution with a pH of 7.4. The optical density of the mixture was recorded at $\lambda = 515$ nm and $\lambda = 525$ nm. The proton moving force (transmembrane electrochemical gradient, $\Delta\Psi$) was determined by the difference of the optical density: $\Delta\Psi = A_{515} - A_{525}$.²⁷

Evaluation of citrate synthase activity

The citrate synthase activity was evaluated according to the method proposed by *Shepherd & Garland* which is based on the spectrophotometric determination of colored decay products of 5,5'-di-thiobis- (2-nitrobenzoic acid) in the presence of acetyl-CoA and oxaloacetate. The reaction mixture contained: 5,5'-di-thiobis- (2-nitrobenzoic acid) 100 mM; Tris-HCl buffer with a pH of 7.8 to 100 mM; acetyl CoA 100mM; 0.1% Triton-X 100 μ l and 4 μ l of the investigated supernatant. The reaction was started by adding 100 μ l of oxaloacetate. The change of absorbance was recorded at a wavelength of 412 nm for 3 minutes at room temperature. Citrate synthase activity was expressed in U / mg protein. Protein concentration was estimated by the Bradford method.²⁷

Determination of aconitase activity

Aconitase activity was determined spectrophotometrically at 340 nm by detecting NADPH, which is formed in conjugated reactions catalyzed by aconitase and isocitrate dehydrogenase. The incubation medium contained: isocitrate dehydrogenase 0.03 U/l; NADP+ 0.32 mg / ml; PBS-55 μ l, the studied biomaterial-50 μ l (positive control-aconitase 0.03 U/l). The reaction was started by adding 0.1 mg/ml of sodium citrate. Changes in the optical density of the obtained solutions were registered at 340 nm at 37^o C for 2 minutes. Aconitase activity was calculated from changes in optical density using the extinction coefficient 0.0313 μ M⁻¹.²⁸

Evaluation of the calcium ions concentration

The content of ionized calcium in the analyzed samples was evaluated by the fluorescence method using Fura-2 / AM as a reporter. The incubation medium contained equimolar amounts (100 μ l) of the studied supernatant and

fluorescent reporter. The excitation wavelength of Fura-2 / AM is 340 nm. The emission wavelength of Fura-2 / AM is 380 nm. The concentration of calcium in the sample was calculated as the difference between the fluorescence signal at 340 nm and 380 nm, with a 510 nm filter installed (Hitachi MPF-4 spectrofluorimeter). The calcium content was converted to the protein concentration in the sample.²⁹ The concentration of calcium was determined in a supernatant obtained directly on the day of animal decapitation without freezing.

Reactive oxygen species (ROS) determination

The concentration of ROS, in particular mitochondrial hydrogen peroxide, was determined by fluorescence analysis using a standard set of Amplex Red (Thermo Fisher Scientific). The Amplex Red reagent is a colorless substrate that reacts with hydrogen peroxide with a stoichiometry of 1:1 to form a highly fluorescent resorufin (excitation/emission = 570/585 nm). The analysis process followed the manufacturer's instructions. The content of hydrogen peroxide was estimated in nmol/ml.

ELISA-study

Changes in the concentration of apoptosis-inducing factor (AIF), caspase-3, β -amyloid, and Tau protein (Ala34~Ser368) were evaluated using the ELISA method in the brain supernatant of animals. During the analysis, used species-specific reagents kits manufactured by *Cloud Clone* (USA). The analysis followed the manufacturer's recommendations.

Statistical analysis

The obtained results were statistically processed and expressed as $M \pm SEM$. A comparison of groups of means was carried out using the ANOVA method with post-processing Newman-Keuls test for multiple comparisons. Differences were considered statistically significant at $p < 0.05$. During the statistical analysis, the STATISTICA 6.0 application package (StatSoft, USA) for Windows was used.

Results

ATACL compound effect on the behavioral activity of rats in the "Morris water maze" test in experimental AD

During this block of experimental work, it was found that in SO animals, the time of finding the platform (Figure 1) and the swimming distance (Figure 2) did not significantly change throughout the experiment. At the same time, in the NC group of rats, the latent time of finding the platform exceeded the same indicator of SO animals by the 50th and 60th days of the experiment by 3.1 ($p < 0.05$) and 4.1 times ($p < 0.05$), respectively (Figure 1). The group of rats that received EGB671 to the endpoints of the experiment (50th and 60th days) showed a decrease in the time spent by the animal to find the platform, compared with the NC group of animals by 34.9% ($p < 0.05$) and 74.1% ($p < 0.05$), respectively. In the background, a resveratrol

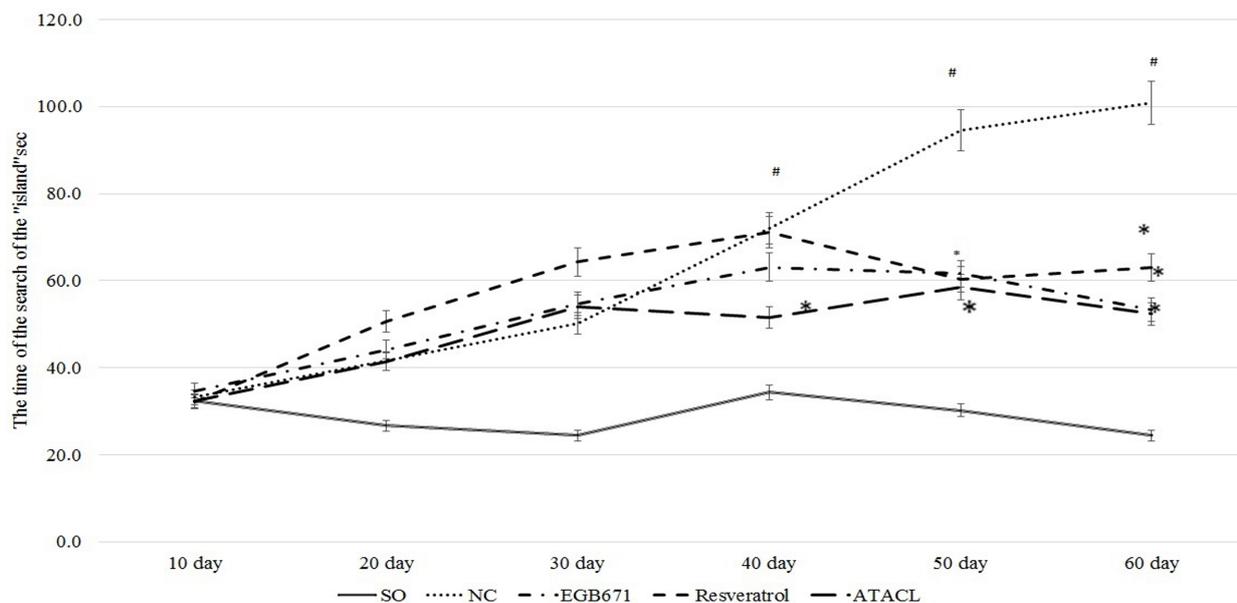


Figure 1. Change in the latent time of platform searching in the “Morris water maze” test in rats with experimental AD against the background of the administration of the test compound and reference drugs.

- statically significant relative to the SO animals group; * - statically significant relative to the NC animals group; SO-sham-operated rats; NC-negative control animals; EGB671 – group of rats treated by standardized Ginkgo biloba extract (EGB 671) in dose 100 mg/kg, per os; Resveratrol – group of rats treated by resveratrol in dose 20 mg/kg, per os; ATACL – animals treated by 4-hydroxy-3,5-ditertbutyl cinnamic acid in dose 100mg/kg, per os.

administration to rats, a decrease of the latent time of the platform searching on the 50th day of the experiment – 36.3% ($p < 0.05$) and 60th day of the study by 37.5% ($p < 0.05$) compared to the same indicators of the NC group of animals (Figure 1) was noted. When using the ATACL compound in rats, there was a decrease in the time of platform searching in comparison with the indicators NC of the group of animals by the 50th and 60th days of the study by 38.1% ($p < 0.05$) and 48.1% ($p < 0.05$), respectively (Figure 1).

It should be noted that the animals of the NC group on the 50th and 60th days of the experiment swam a much greater distance to the platform (Figure 2) than the rats of the SO group (by 3.7 times ($p < 0.05$) and 7.6 times ($p < 0.05$), respectively). Against the background of the administration of EGB671, there was a decrease in the distance covered to the platform relative to the NC group of animals by the 50th day of the study by 23.2% ($p < 0.05$) and by 29.2% ($p < 0.05$) – on the 60th day of the experiment. When using resveratrol, on the 50th and 60th days of the

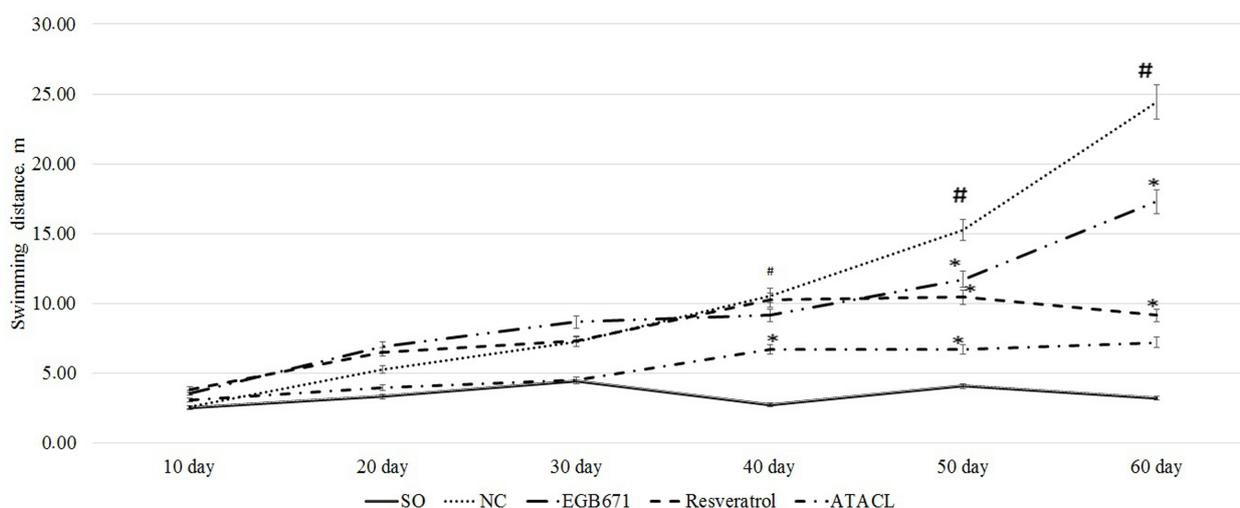


Figure 2. Changes of the swimming distance in the “Morris water maze” test in rats with experimental AD against the background of the administration of the test compound and reference drugs.

- statically significant relative to the SO animals group; * - statically significant relative to the NC animals group; SO-sham-operated rats; NC-negative control animals; EGB671 – group of rats treated by standardized Ginkgo biloba extract (EGB 671) in dose 100 mg/kg, per os; Resveratrol – group of rats treated by resveratrol in dose 20 mg/kg, per os; ATACL – animals treated by 4-hydroxy-3,5-ditertbutyl cinnamic acid in dose 100mg/kg, per os.

study, swimming distance was less than the same in the NC group of animals by 31.1% ($p < 0.05$) and 62.5% ($p < 0.05$), respectively. In rats treated by the ATACL compound, there was a decrease in the distance covered to the platform in comparison with the NC group of animals by the 50th day of the experiment-by 56.1% ($p < 0.05$) and on the 60th day of the study – by 70.5% ($p < 0.05$). It should be noted that the swimming distance of animals that received the ATACL compound on the 30th, 40th, 50th and 60th day of the experiment was less than rats treated by EGB671 by 49%; 35.6%; 43% and 58.3% (all values $p < 0.05$), respectively, as well as animals, which were treated by resveratrol 38.6%; 34,3%; 36% and 21.2% (all values $p < 0.05$).

ATACL compound effect on the concentration of A β and pTau in the brain supernatant in rats under experimental AD

The concentration of A β was higher by 6.8 times ($p < 0.05$), while the content of pTau increased by 19.5 times ($p < 0.05$) in the NC group of rats. Against an EGB671 administration to rats, there was a decrease in the content of A β and pTau in the brain supernatant by 18.9% ($p < 0.05$) and 27.6% ($p < 0.05$), respectively, relative to the NC group. At the same time, in rats treated by resveratrol, in comparison with animals deprived of pharmacological support, there was a decrease in the content of A β by 28.3% ($p < 0.05$) and pTau – by 30.8% ($p < 0.05$). At the same time, the concentration of A β and pTau was lower in animals treated by the ATACL compound by 38.9% ($p < 0.05$) and 42.3% ($p < 0.05$), respectively, relative to the NC group of rats (Figure 3).

When using the ATACL compound, the content of A β in the brain supernatant of animals was less by 24.2% ($p < 0.05$) and 14.2% ($p < 0.05$) than in rats receiving EGB671 and resveratrol, respectively, with a decrease of pTau content by

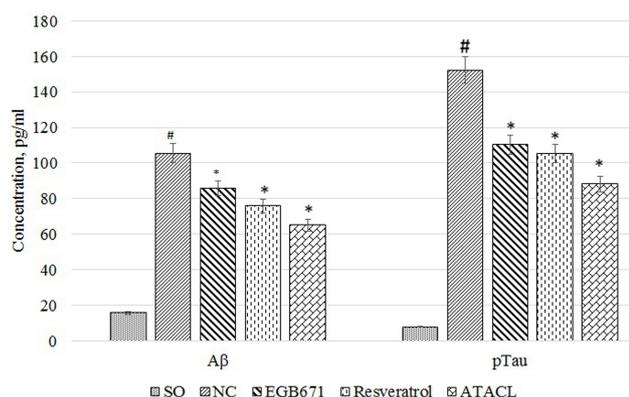


Figure 3. Changes of the A β and pTau concentration in the brain supernatant of rats with experimental AD on the background of the administration of the test compound and reference drugs.

- statically significant relative to the SO animals group; * - statically significant relative to the NC animals group; SO-sham-operated rats; NC-negative control animals; EGB671 – group of rats treated by standardized Ginkgo biloba extract (EGB 671) in dose 100 mg/kg, per os; Resveratrol –group of rats treated by resveratrol in dose 20 mg/kg, per os; ATACL – animals treated by 4-hydroxy-3,5-ditretbutil cinnamic acid in dose 100mg/kg, per os; A β - β -amyloid; pTau – tau-protein.

20.0% ($p < 0.05$) and 16.0% ($p < 0.05$), respectively.

ATACL compound effect on the respirometric function of brain mitochondria in rats under experimental AD

In the NC group of animals in comparison with SO rats a decrease in ATP-generating capacity, maximum respiratory rate, and respiratory capacity by 5.4 times ($p < 0.05$); 5.2 times ($p < 0.05$) and 6.8 times ($p < 0.05$), respectively (Figure 4) was observed. At the same time, in animals that were treated by EGB671 relative to the NC group of rats, an increase in ATP-generating capacity by 2.2 times ($p < 0.05$); the maximum level of respiration-1.8 times ($p < 0.05$) and respiratory capacity – 2.8 times ($p < 0.05$) was noted. Against the background of resveratrol administration to animals, an increase in ATP-generating capacity; the maximum level of respiration and respiratory capacity concerning the NC group of animals by 2.4 times ($p < 0.05$); 2 times ($p < 0.05$) and 2.73 times ($p < 0.05$), respectively was notified. In the group of rats that were treated by the ATACL compound ATP-generating capacity; the maximum level of respiration and respiratory capacity exceeded similar indicators of the NC group of rats by 3.2 times ($p < 0.05$); 3 times and 3.4 times ($p < 0.05$), respectively (Figure 4).

In rats NC group the intensity of glycolysis increased, and the glycolytic capacity and glycolytic reserve respectively decreased in comparison with the SO group of animals by 3.6 times ($p < 0.05$); 6.5 times ($p < 0.05$), and 7.7 times ($p < 0.05$), respectively. In rats that were treated by EGB671, a decrease in the intensity of glycolysis relative to the NC group of animals by 19.5% ($p < 0.05$), with an increase in the glycolytic capacity and glycolytic reserve by 2.9 times ($p < 0.05$) and 3.2 times ($p < 0.05$), respectively was noted. At the same time, in rats treated by resveratrol, the intensity of glycolysis was lower, and the glycolytic capacity and glycolytic reserve were respectively higher to the NC group of animals by 33.9% ($p < 0.05$); 3.4 times ($p < 0.05$) and 2.9 times ($p < 0.05$), respectively. While ATACL compound administered to the rats the decrease of the glycolysis intensity relative to the NC group of animals by 56.6% ($p < 0.05$), accompanied by an increase in glycolytic capacity by 4.0 times ($p < 0.05$) and glycolytic reserve 4.7 times ($p < 0.05$) was noted. (Figure 5)

The activity of mitochondrial complexes I, II, IV, and V in the NC group was lower than that in SO animals by 3.0 times ($p < 0.05$); 2.1 ($p < 0.05$); 2.0 ($p < 0.05$), and 2.3 times ($p < 0.05$), respectively (Figure 6). When using EGB671, there was an increase in the activity of mitochondrial respiratory chain complexes concerning the NC group of rats, while the activity of complex I increased by 62% ($p < 0.05$); complex II-by 44% ($p < 0.05$); complex IV - by 55% ($p < 0.05$) and complex V – by 37% ($p < 0.05$). In animals treated by resveratrol, compared with rats NC group, there was an increase in the activity of complexes I, II, IV, and V by 91% ($p < 0.05$); 57% ($p < 0.05$); 45% ($p < 0.05$) and 53% ($p < 0.05$), respectively. The activity of complex I in a group of rats treated by ATACL compound increased by 146% ($p < 0.05$); complex II-by 70% ($p < 0.05$); complex IV - by

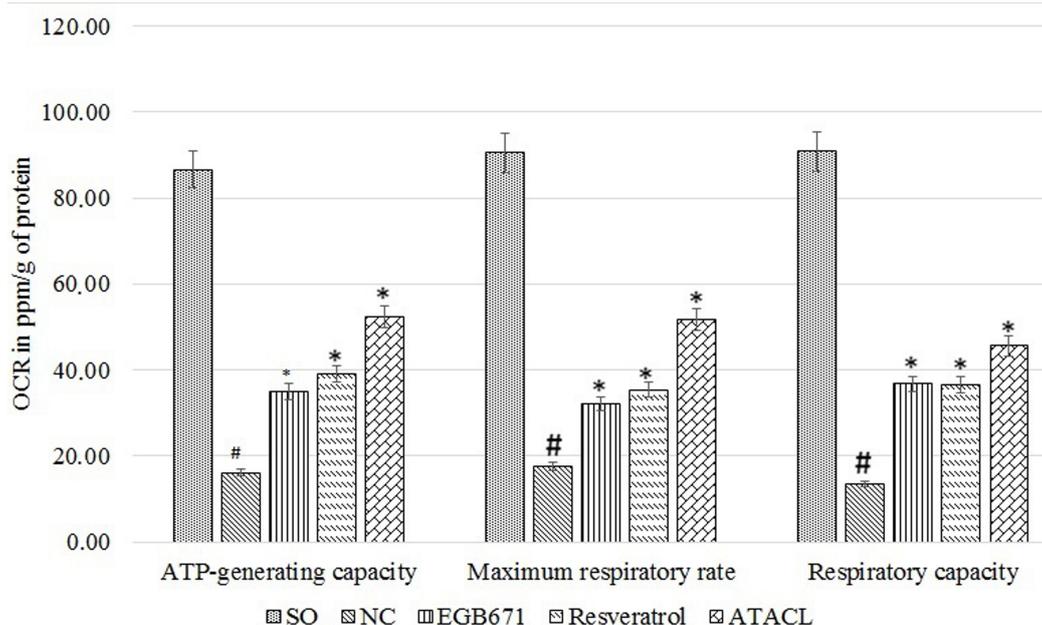


Figure 4. Changes in the overall respirometric function of brain mitochondria in rats with experimental AD on the background of the administration of the test compound and reference drugs.

- statically significant relative to the SO animals group; * - statically significant relative to the NC animals group; SO-sham-operated rats; NC-negative control animals; EGB671 – group of rats treated by standardized Ginkgo biloba extract (EGB 671) in dose 100 mg/kg, per os; Resveratrol – group of rats treated by resveratrol in dose 20 mg/kg, per os; ATACL – animals treated by 4-hydroxy-3,5-ditretbutil cinnamic acid in dose 100mg/kg, per os; OCR – oxygen consumption rate.

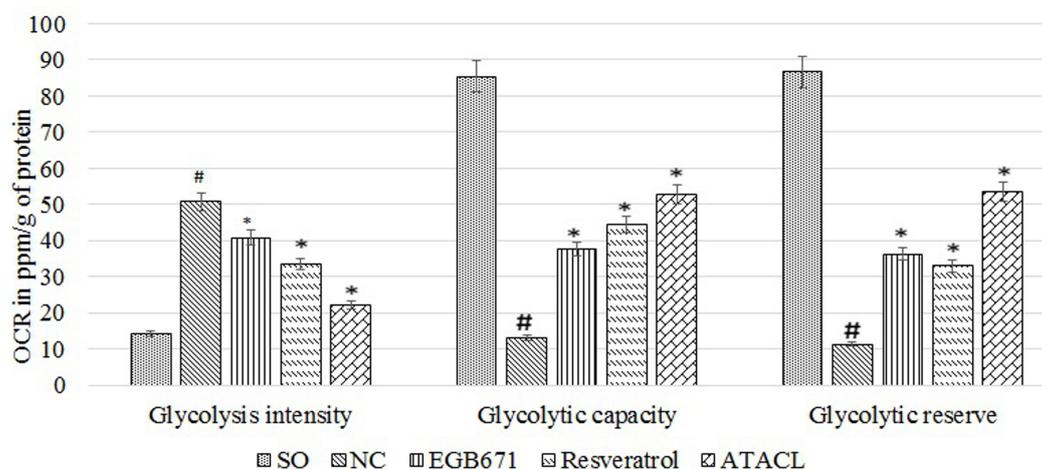


Figure 5. Changes in the activity of anaerobic processes in the brain supernatant in rats with experimental AD against the background of the administration of the test compound and reference drugs.

- statically significant relative to the SO animals group; * - statically significant relative to the NC animals group; SO-sham-operated rats; NC-negative control animals; EGB671 – group of rats treated by standardized Ginkgo biloba extract (EGB 671) in dose 100 mg/kg, per os; Resveratrol – group of rats treated by resveratrol in dose 20 mg/kg, per os; ATACL – animals treated by 4-hydroxy-3,5-ditretbutil cinnamic acid in dose 100mg/kg, per os; OCR – oxygen consumption rate.

71% ($p < 0.05$) and complex V – by 90% ($p < 0.05$) relative to the NC group of rats (Figure 6).

ATACL compound effect on the latent opening time of the mitochondrial permeability transition pore and the concentration of intracellular calcium in the brain supernatant in rats under experimental AD

During this stage of experimental work, it was found that in the NC group of rats, the latent opening time of mPTP (Figure 7) decreased by 6.7 times ($p < 0.05$), while

the content of intracellular calcium in this group of rats increased by 2.4 times ($p < 0.05$) (Figure 8).

The use of EGB671 increased the latent opening time of mPTP to the NC group of rats by 2.5 times ($p < 0.05$), while reducing the calcium content by 21.7% ($p < 0.05$). In rats that were treated with resveratrol, there was a decrease in the concentration of intracellular calcium and an increase in the latent opening time of mPTP in comparison with the NC group of animals by 26.8% ($p < 0.05$) and 2.8 times ($p < 0.05$), respectively. Against the administration of the

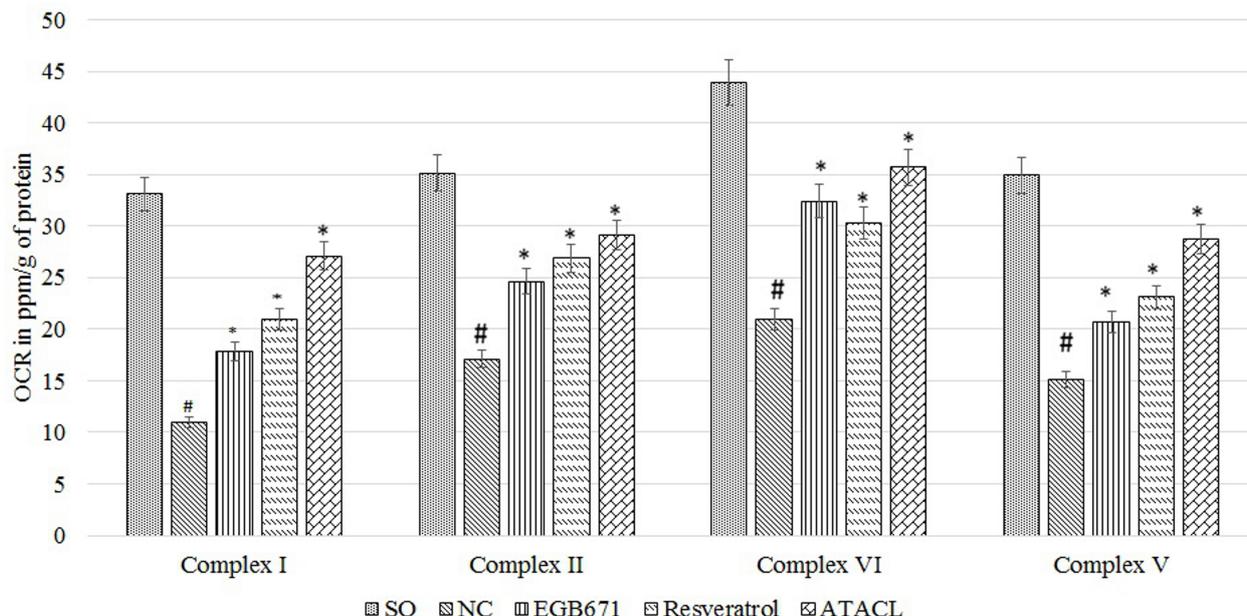


Figure 6. Changes in the activity of mitochondrial respiratory chain complexes of brain mitochondria in rats with experimental AD on the background of the administration of the test compound and reference drugs. # - statically significant relative to the SO animals group; * - statically significant relative to the NC animals group; SO-sham-operated rats; NC-negative control animals; EGB671 – group of rats treated by standardized Ginkgo biloba extract (EGB 671) in dose 100 mg/kg, per os; Resveratrol –group of rats treated by resveratrol in dose 20 mg/kg, per os; ATACL – animals treated by 4-hydroxy-3.5-ditretbutil cinnamic acid in dose 100mg/kg, per os; OCR – oxygen consumption rate.

ATACL compound increase of mPTP opening time by 3.8 times ($p < 0.05$) compared to the same period of NC group rats was noted, while the content of intracellular calcium in the ATACL-treated rats decreased by 39.7% ($p < 0.05$) in comparison with NC group. It is worth noting that the concentration of calcium in animals receiving ATACL was

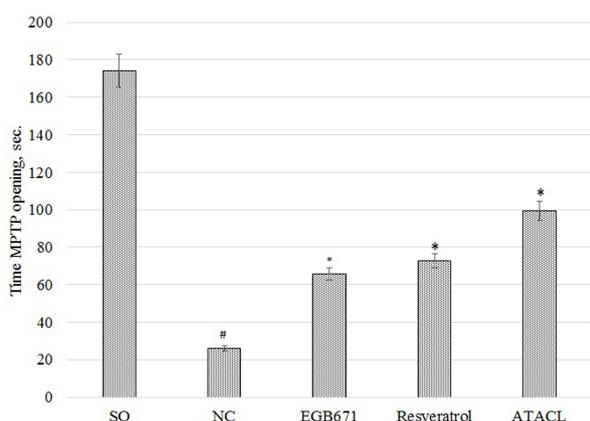


Figure 7. Change in the latent opening time of the mPTP in the brain supernatant in rats with experimental AD against the background of the administration of the test compound and reference drugs.

mPTP - mitochondrial permeability transition pore; # - statically significant relative to the SO animals group; * - statically significant relative to the NC animals group; SO-sham-operated rats; NC-negative control animals; EGB671 – group of rats treated by standardized Ginkgo biloba extract (EGB 671) in dose 100 mg/kg, per os; Resveratrol –group of rats treated by resveratrol in dose 20 mg/kg, per os; ATACL – animals treated by 4-hydroxy-3.5-ditretbutil cinnamic acid in dose 100mg/kg, per os.

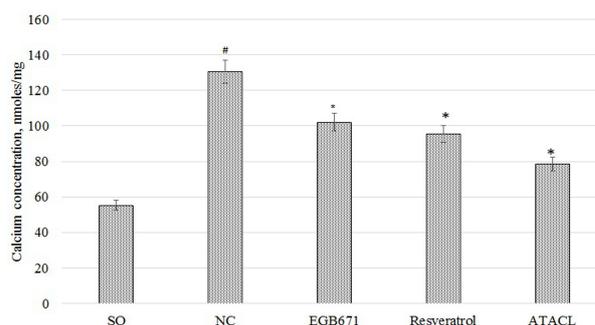


Figure 8. Changes in the concentration of calcium in the brain supernatant in rats with experimental AD against the background of the administration of the test compound and reference drugs.

- statically significant relative to the SO animals group; * - statically significant relative to the NC animals group; SO-sham-operated rats; NC-negative control animals; EGB671 – group of rats treated by standardized Ginkgo biloba extract (EGB 671) in dose 100 mg/kg, per os; Resveratrol –group of rats treated by resveratrol in dose 20 mg/kg, per os; ATACL – animals treated by 4-hydroxy-3.5-ditretbutil cinnamic acid in dose 100mg/kg, per os.

less than that in rats that were administered EGB671 and resveratrol by 23% ($p < 0.05$) and 17.6% ($p < 0.05$), respectively.

ATACL compound effect on mitochondrial membrane potential of brain mitochondria in rats under experimental AD

Evaluating the change in mitochondrial membrane potential (Figure 9), it was found that in the NC group of rats, this indicator decreased by 3.5 ($p < 0.05$) times in comparison with SO animals. The use of EGB671 and resveratrol helped to restore the mitochondrial membrane potential, which increased by 72% ($p < 0.05$) and 85% ($p < 0.05$)

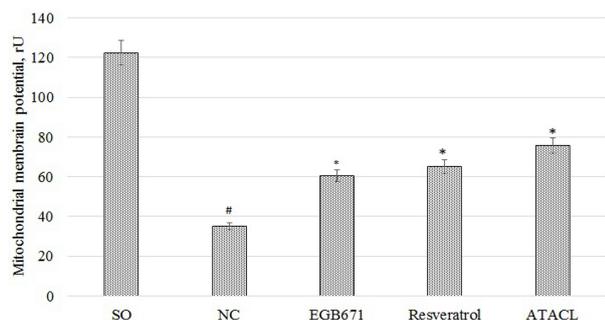


Figure 9. Changes in the membrane potential of brain mitochondria in rats with experimental AD against the background of the administration of the test compound and reference drugs.

- statically significant relative to the SO animals group; * - statically significant relative to the NC animals group; SO-sham-operated rats; NC-negative control animals; EGB671 – group of rats treated by standardized Ginkgo biloba extract (EGB 671) in dose 100 mg/kg, per os; Resveratrol –group of rats treated by resveratrol in dose 20 mg/kg, per os; ATACL – animals treated by 4-hydroxy-3.5-ditretbutil cinnamic acid in dose 100mg/kg, per os.

concerning the group of rats deprived of pharmacological support. Against the background of the administration of the ATACL compound, the mitochondrial membrane potential was higher than that of the NC group of animals by 115% ($p < 0.05$). At the same time, the mitochondrial membrane potential increased in rats treated by ATACL relative to animals that received EGB671 and resveratrol by 25.2% ($p < 0.05$) and 16% ($p < 0.05$) respectively.

ATACL compound effect on of the activity of citrate synthase and aconitase in the brain supernatant in rats under experimental AD

In the NC group of animals, the activity of citrate synthase and aconitase (Figure 10) in the brain's supernatant decreased by 3.6 times ($p < 0.05$) and 4.9 times ($p < 0.05$), respectively, compared to the SO group. At the same time, animals receiving EGB671 showed an increase of the activity of the citrate synthase by 54.9% ($p < 0.05$) and aconitase by 98% ($p < 0.05$). In the group of resveratrol-treated animals, the activity of citrate synthase exceeded a similar indicator of the NC group of rats by 109.8% ($p < 0.05$), with an increase in aconitase activity by 2.46 times ($p < 0.05$). In rats receiving the ATACL compound, there was an increase of the activity of citrate synthase and aconitase concerning the NC group by 2.6 times ($p < 0.05$) and 3.3 times ($p < 0.05$), respectively. At the same time, citrate synthase activity in rats treated by ATACL was higher than in animals treated by EGB671 and resveratrol by 69.6% ($p < 0.05$) and 29.2% ($p < 0.05$), respectively, accompanied by an increase in aconitase activity by 68% ($p < 0.05$) and 35.4% ($p < 0.05$), respectively.

ATACL compound effect on of the concentration of proapoptotic molecules in the brain supernatant in rats under experimental AD

During this research block, it was found that in the NC group of rats, the content of AIF and caspase -3 (Figure 11) exceeded that in SO animals by 15.5 ($p < 0.05$) and 5.9 times

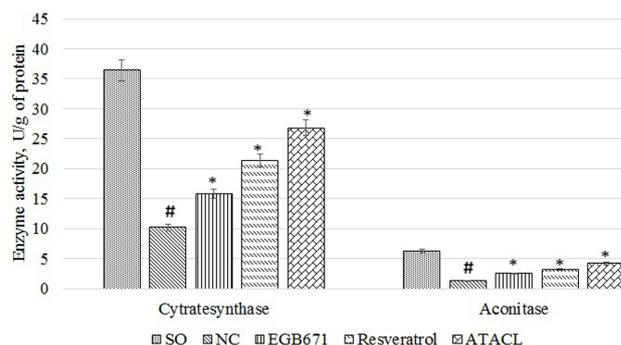


Figure 10. Changes in the activity of citrate synthase and aconitase in the brain supernatant in rats with experimental AD against the background of the administration of the test compound and reference drugs.

- statically significant relative to the SO animals group; * - statically significant relative to the NC animals group; SO-sham-operated rats; NC-negative control animals; EGB671 – group of rats treated by standardized Ginkgo biloba extract (EGB 671) in dose 100 mg/kg, per os; Resveratrol –group of rats treated by resveratrol in dose 20 mg/kg, per os; ATACL – animals treated by 4-hydroxy-3.5-ditretbutil. cinnamic acid in dose 100mg/kg, per os.

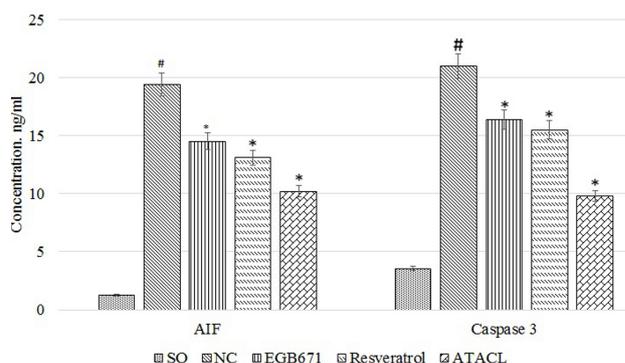


Figure 11. Changes in the concentration of proapoptotic molecules in the brain supernatant in rats with experimental AD against the background of the administration of the test compound and reference drugs.

- statically significant relative to the SO animals group; * - statically significant relative to the NC animals group; SO-sham-operated rats; NC-negative control animals; EGB671 – group of rats treated by standardized Ginkgo biloba extract (EGB 671) in dose 100 mg/kg, per os; Resveratrol –group of rats treated by resveratrol in dose 20 mg/kg, per os; ATACL – animals treated by 4-hydroxy-3.5-ditretbutil cinnamic acid in dose 100mg/kg, per os.

($p < 0.05$), respectively.

Against the background of EGB671 administration to rats, there was a decrease in the concentration of AIF – by 25.3% ($p < 0.05$) and caspase -3 – by 21.8% ($p < 0.05$) concerning the NC group of animals. At the same time, in rats treated by resveratrol, the content of AIF and caspase-3 in the brain supernatant was less than the similar indicators of the NC group of rats by 32.5% ($p < 0.05$) and 26.1% ($p < 0.05$), respectively. When using the ATACL compound, the concentration of AIF decreased (relative to the NC of the animal group) by 47.5% ($p < 0.05$), while the content of caspase-3 decreased by 53.3% ($p < 0.05$). In rats treated by ATACL, the AIF content decreased to animals

that were administered with EGB671 and resveratrol by 29.7% ($p < 0.05$) and 22.1% ($p < 0.05$), with a decrease in the concentration of caspase-3 by 40.2% ($p < 0.05$) and 36.8% ($p < 0.05$), respectively (Figure 11).

ATACL compound effect on ROS concentration in the brain supernatant in rats under experimental AD

It was found that the concentration of hydrogen peroxide in the NC group of rats in comparison with SO animals increased by 2.9 times ($p < 0.05$). At the same time, the use of resveratrol, EGB671, and ATACL compounds reduced the content of H_2O_2 in the brain supernatant in rats by 25.8% ($p < 0.05$); 21.1% ($p < 0.05$); and 41.6% ($p < 0.05$), respectively. Besides, the concentration of hydrogen peroxide in animals (Figure 12) that received the ATACL compound was less than that in rats that were administered EGB 671 by 26% ($p < 0.05$) and resveratrol by 21.2% ($p < 0.05$).

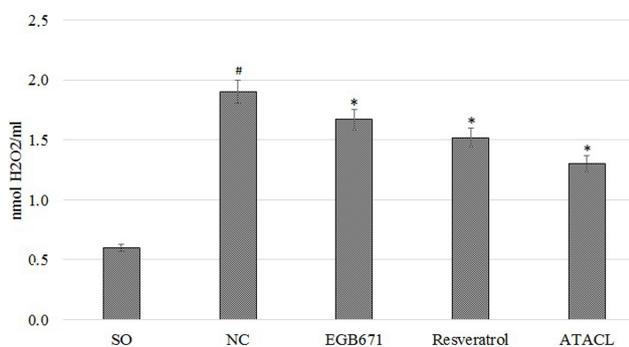


Figure 12. Changes in the concentration of H_2O_2 in the brain supernatant in rats with experimental AD against the background of the administration of the test compound and reference drugs.

- statically significant relative to the SO animals group; * - statically significant relative to the NC animals group; SO-sham-operated rats; NC-negative control animals; EGB671 – group of rats treated by standardized Ginkgo biloba extract (EGB 671) in dose 100 mg/kg, per os; Resveratrol –group of rats treated by resveratrol in dose 20 mg/kg, per os; ATACL – animals treated by 4-hydroxy-3,5-ditretbutyl cinnamic acid in dose 100mg/kg, per os.

Discussion

In this study, it was found that the administration of the ATACL compound helped restore mitochondrial function, reduced the concentration of $A\beta$ and Tau-protein in animal brain tissue, and helped to eliminate cognitive deficits in rats with experimentally reproduced Alzheimer's disease. The obtained results may be of some scientific and practical interest, because AD is one of the most common neurodegenerative pathologies, affecting more than 46 million people. To date, treatment of AD involves the use of symptomatic agents, such as acetylcholinesterase inhibitors and N-methyl-D-aspartate receptor antagonists.³⁰ However, the effect of these drugs is aimed only at dementia and does not act on the amyloidogenic cascade, which is the main pathogenetic chain of AD.³¹ Of course, targeted correction of neuronal damage caused by the accumulation of $A\beta$ and pTau pathology is one of the most promising directions of pathogenetic therapy of AD.

These strategies include the use of secretase inhibitors,³² or specific monoclonal antibodies to $A\beta$ and pTau.³³

γ -secretase inhibitors has high therapeutic efficacy in transgenic lines of $A\beta$ PP Tg mice, while modulating the activity of γ -secretase may be rather than its complete inhibition.³⁴ The use of specific antibodies has also demonstrated significant therapeutic potential. The monoclonal antibody based on IgG4 suppressed aggregation of $A\beta$ and induced disorganization of formed protofibrils, which prevented cytotoxicity of $A\beta$, both in cellular neuronal cultures and in animal models of AD.³⁵ At the same time, there are approaches to the AD treatment that are not directly related to the amyloidogenic cascade of brain damage. These include the use of drugs that normalize mitochondrial function, such as Mito Q or the SS protein (SS31). Under experimental conditions, the administration of these compounds restored mitochondrial ATP/GTPases activity, suppressed the formation of hydroperoxides, and reduced the concentration of water-soluble $A\beta$.³⁶ Thus, it seems promising to develop other mitochondrial-oriented compounds that are potentially effective in the treatment of AD.

This study showed that the use of 4-hydroxy-3,5-ditretbutyl cinnamic acid in experimental AD in rats contributed to the restoration of mitochondrial function, which was expressed in the normalization of aerobic-anaerobic metabolism, the activity of respiratory chain complexes. Recovery of aerobic metabolism in AD plays a crucial role in the progress of the amyloidogenic cascade. Vlassenko *et al.*,³⁶ was shown that a decrease in the intensity of aerobic reactions of glucose metabolism leads to the deposition of $A\beta$ and taupathology in the brain, with a decrease in neuroplasticity and deterioration of interneuron transmission. In this regard, the administration of 4-hydroxy-3,5 ditretbutyl cinnamic acid to animals must cause not only the restoration of mitochondrial respiratory function but also the reduction of $A\beta$ and pTau in the supernatant of the brain, which may indicate a decrease of the amyloidogenesis intensity. Also interesting is the comparable pharmacological activity of EGB671 and resveratrol. So in most cases (except for the intensity of glycolysis), the effects of these substances did not differ significantly from each other, which requires additional research. However, the pharmacological effect of the administration of referents was statistically significantly less than that of the ATACL compound, also in almost all experimental blocks. Comparable therapeutic potential of referents may be explained by the polyphenol composition of EGB671 similar to resveratrol.

Another important aspect of the pathogenesis of AD is the development of apoptotic events, in which one of the determining roles is assigned to neuronal mitochondria.³⁷ It is known that mitochondrial-mediated apoptosis is initiated when the critical value of the mitochondrial membrane potential is reached. As a result the activity of F_1F_0 -ATP synthase is inverted and the mPTP was formed.³⁸ It is worth noting that the mPTP opening is also an increased efflux of calcium into the mitochondrial

matrix via overexpressed VDAC channels.³⁹ The opening of mPTP induces mitochondria damage, with releasing in the cytosol, a large amount of pro-apoptotic molecules such as cytochrome C, apoptosis-inducing factor. These molecules can initiate DNA damage by ENDOG-dependent mechanism.⁴⁰

However, apoptotic reactions dependent on mitochondria can be implemented not only by the caspase-independent mechanism. It was found that after reaching a critically low level of ATP in the cell, there is a direct activation of caspase-3, which in turn leads to DNA sequencing and cell death. Due to the significant role of the apoptotic cascade in brain tissue damage in AD, this study evaluated the effect of 4-hydroxy-3,5-ditretbutyl cinnamic acid on changes in the concentration of the main pro-apoptotic molecules-caspase-3 and AIF, which implement programmed cell death by a caspase-dependent and caspase-independent mechanism, respectively. As a result, it was found that against the test-compound administration, the content of both proapoptotic markers significantly decreased. At the same time, some authors consider modulation of apoptosis, along with suppression of amyloidogenesis, to be one of the most promising methods of AD therapy. Thus, Chavoshinezhad *et al.*⁴¹, showed that intranasal administration of interferon β reduced the intensity of apoptosis reactions, improved the processes of neurogenesis and cognitive functions in rats with reproduced AD. Also, Jiang *et al.*,⁴² presented results indicating the antiapoptotic effect of hyperforin in neuronal cell culture, where apoptosis was induced by the injection of A β ₁₋₄₂ fragment. Rather *et al.*,⁴³ found that the administration of Asiatic acid had a pronounced neuroprotective effect, realized by reducing the expression of pro-apoptotic proteins in rats with Alzheimer's disease. In this regard, the anti-apoptotic effect of the ATACL compound may be an essential component of its neuroprotective action. However, excessive suppression of apoptosis can also have some negative effects, for example, in the form of hyperproliferation of cells, which can be fraught with the development of benign neoplasms.

Besides, inhibition of caspase-3 may be the basis for restoring the functional activity of the vascular endothelium.⁴⁴ It is known that caspase-3 in certain conditions can harm the vascular endothelium, which is manifested in the form of hypercoagulation, while the administration of the ATACL compound restored optimal blood rheology, which may also be associated with a decrease in endothelial apoptosis.⁴⁵ Of course, it cannot be denied that the mechanism of neuroprotective action of the ATACL compound is multifactorial and may include an impact on the processes of inflammation in the brain, which was confirmed in an earlier study. The administration of the ATACL compound at a dose of 100 mg/kg to rats with brain ischemia reduced the severity of the systemic inflammatory response, which was confirmed by lower levels of C-reactive protein and markers of inflammation (matrix metalloproteinase-1 activity).⁴⁶ It is also interesting to evaluate the effect of the ATACL

compound on the development of amyloidogenesis and the metabolic activity of neurons in different hemispheres of the brain, as well as in comparison with counter lateral in intact rats. This series of experiments will allow us to more fully establish the localization of action and the preferred mechanism of action of the ATACL compound and may be a precondition for further research.

Conclusion

Thus, based on obtained the data, it can be assumed that the neuroprotective effect of 4-hydroxy-3,5-ditretbutyl cinnamic acid in experimental AD is complex and is expressed in the form of restoration of mitochondrial function, suppression of apoptosis, and associated amyloidogenesis, which ultimately contributed to the restoration of the memory trace in rats. It seems promising to further study 4-hydroxy-3,5-ditretbutyl cinnamic acid as a treatment of AD.

Ethical Issues

The concept of a research was approved by the local Ethics Committee of Pyatigorsk medical and pharmaceutical Institute (Protocol No. 11 of 11.09.2019).

Author Contributions

PDI: Design of the work, conducting an experiment, interpretation of data, drafting and revising, and VAV: Design of the work, statistical analysis, interpretation of data for the work and drafting and revising. All authors have read and agreed to the published version of the manuscript.

Conflict of Interest

The author declare there is no conflict of interest in this study.

References

1. Takizawa C, Thompson PL, van Walssem A. Epidemiological and economic burden of Alzheimer's disease: a systematic literature review of data across Europe and the United States of America. *J Alzheimers Dis.* 2015;43(4):1271-84. doi:10.3233/JAD-141134
2. Prince MW, Anders G, Maëlenn A. World Alzheimer Report 2015. The Global Impact of Dementia. An Analysis of Prevalence, Incidence, Cost, and Trends London: Alzheimer's Disease International (ADI); 2015.
3. Crous-Bou M, Minguillón C, Gramunt N, et al. Alzheimer's disease prevention: from risk factors to early intervention. *Alzheimers Res Ther.* 2017;9(1):71. doi:10.1186/s13195-017-0297-z
4. Sperling RA, Aisen PS, Beckett LA. Toward defining the preclinical stages of Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimer's Dement.* 2011;7(3):280-92. doi:10.1016/j.

- jalz.2011.03.003
5. Olsson B, Lautner R, Andreasson U. CSF and blood biomarkers for the diagnosis of Alzheimer's disease: a systematic review and meta-analysis. *Lancet Neurol.* 2016;15(7):673-84. doi:10.1016/S1474-4422(16)00070-3
 6. Yeo JM, Waddell B, Khan Z. A systematic review and meta-analysis of 18F-labeled amyloid imaging in Alzheimer's disease. *Alzheimers Dement (Amst).* 2015;1(1):5-13. doi:10.1016/j.dadm.2014.11.004
 7. Albeni BC. Dysfunction of mitochondria: Implications for Alzheimer's disease. *Int Rev Neurobiol.* 2019;145:13-27. doi:10.1016/bs.irn.2019.03.001
 8. Esposito L. Reduction in mitochondrial superoxide dismutase modulates Alzheimer's disease-like pathology and accelerates the onset of behavioral changes in human amyloid precursor protein transgenic mice. *J Neurosci.* 2006;26(19):5167-79. doi:10.1523/JNEUROSCI.0482-06.2006
 9. Gwon AR. Oxidative lipid modification of nicastrin enhances amyloidogenic gamma-secretase activity in Alzheimer's disease. *Aging Cell.* 2012;11(4):559-68. doi:10.1111/j.1474-9726.2012.00817.x
 10. Hou Y. Permeability transition pore-mediated mitochondrial superoxide flashes mediate an early inhibitory effect of amyloid beta1-42 on neural progenitor cell proliferation. *Neurobiol Aging.* 2014;35(5):975-89. doi:10.1016/j.neurobiolaging.2013.11.002
 11. Casley CS. Beta-amyloid inhibits integrated mitochondrial respiration and key enzyme activities. *J Neurochem.* 2002;80(1):91-100. doi:10.1046/j.0022-3042.2001.00681.x
 12. Leuner K. Mitochondrion-derived reactive oxygen species lead to enhanced amyloid-beta formation. *Antioxid Redox Signal.* 2012;16(12):1421-33. doi:10.1089/ars.2011.4173
 13. Wilkins HM, Morris JK. New Therapeutics to Modulate Mitochondrial Function in Neurodegenerative Disorders. *Curr Pharm Des.* 2017;23(5):731-52. doi:10.2174/1381612822666161230144517
 14. Kook SY, Lee KM, Kim Y. High-dose of vitamin C supplementation reduces amyloid plaque burden and ameliorates pathological changes in the brain of 5XFAD mice. *Cell Death Dis.* 2014;5(2):e1083. doi:10.1038/cddis.2014.26
 15. Voronkov AV, Pozdnyakov DI. Endothelotropic activity of 4-hydroxy-3, 5-di-tert-butylcinnamic acid in the conditions of experimental cerebral ischemia. *Res Result Pharmacol.* 2018;4:1-10. doi:10.3897/rppharmacology.4.26519
 16. Voronkov AV, Abaev T, Oganessian ET, Pozdnyakov DI. Some aspects of the cerebroprotective activity of 4-hydroxy-3, 5-di-tert-butyl cinnamic acid in ischemic brain damage in an experiment. *Medical Bulletin of the North Caucasus.* 2018;13(1):90-3. Russian. doi:10.14300/mnnc.2018.13025
 17. Ba XH, Min LQ. Effects of Ginkgo biloba extract on the apoptosis of oxygen and glucose-deprived SH-SY5Y cells and its mechanism. *Indian J Pharmacol.* 2015;47(1):101-4. doi:10.4103/0253-7613.150372
 18. Arteaga O, Revuelta M, Urigüen L, Álvarez A, Montalvo H, Hilario E. Pretreatment with Resveratrol Prevents Neuronal Injury and Cognitive Deficits Induced by Perinatal Hypoxia-Ischemia in Rats. *PLoS One.* 2015;10(11):e0142424. doi:10.1371/journal.pone.0142424
 19. Paxinos G. *The Rat Nervous System.* Sydney, Australia: Academic Press; 1984.
 20. Rosales-Corral SA, Lopez-Armas G, Cruz-Ramos J. Alterations in Lipid Levels of Mitochondrial Membranes Induced by Amyloid- β : A Protective Role of Melatonin. *Int J Alzheimers Dis.* 2012;2012:459806. doi:10.1155/2012/459806
 21. Urrutia PJ, Hirsch EC, González-Billault C, Núñez MT. Hepcidin attenuates amyloid beta-induced inflammatory and pro-oxidant responses in astrocytes and microglia. *J Neurochem.* 2017;142(1):140-52. doi:10.1111/jnc.14005
 22. Wang X, Leverin AL, Han W. Isolation of brain mitochondria from neonatal mice. *J Neurochem.* 2011;119(6):1253-61. doi:10.1111/j.1471-4159.2011.07525.x
 23. Connolly NMC, Theurey P, Adam-Vizi V. Guidelines on experimental methods to assess mitochondrial dysfunction in cellular models of neurodegenerative diseases. *Cell Death Differ.* 2018;25(3):542-72. doi:10.1038/s41418-017-0020-4
 24. Voronkov AV, Pozdnyakov DI, Nigaryan SA, Khouri EI, Miroshnichenko KA, Sosnovskaya AV, et al. Evaluation of the mitochondria respirometric function in the conditions of pathologies of various geneses. *Pharm Pharmacol.* 2019;7(1):20-31. doi:10.19163/2307-9266-2019-7-1-20-31
 25. Carlsson N, Borde A, Wölfel S, Kerman B, Larsson A. Quantification of protein concentration by the Bradford method in the presence of pharmaceutical polymers. *Anal Biochem.* 2011;411(1):116-21. doi:10.1016/j.ab.2010.12.026
 26. Zhyliuk VI, Mamchur VV, Pavlov S. [Role of functional state of neuronal mitochondria of cerebral cortex in mechanisms of nootropic activity of neuroprotectors in rats with alloxan hyperglycemia]. *Eksp Klin Farmacol.* 2015;78(2):10-4. Russian
 27. Shepherd D, Garland PB. The kinetic properties of citrate synthase from rat liver mitochondria. *Bioch J.* 1969;114(3):597. doi:10.1042/bj1140597
 28. Ternette N, Yang M, Laroyia M. Inhibition of mitochondrial aconitase by succination in fumarate hydratase deficiency. *Cell Rep.* 2013;3(3):689-700. doi:10.1016/j.celrep.2013.02.013
 29. Field ML, Azzawi A, Styles P, Henderson C, Seymour AM, Radda GK. Intracellular Ca²⁺ transients in isolated perfused rat heart: measurement using the fluorescent

- indicator Fura-2/AM. *Cell Calcium*. 1994;16(2):87-100. doi:10.1016/0143-4160(94)90004-3
30. Kovács T. [Therapy of Alzheimer disease]. *Neuropsychopharmacol Hung*. 2009;11(1):27-33. Hu language.
31. Gao LB, Yu XF, Chen Q, Zhou D. Alzheimer's Disease therapeutics: current and future therapies. *Minerva Med*. 2016;107(2):108-13.
32. Mitani Y, Yarimizu J, Saita K. Differential effects between γ -secretase inhibitors and modulators on cognitive function in amyloid precursor protein-transgenic and nontransgenic mice. *J Neurosci*. 2012;32(6):2037-50. doi:10.1523/JNEUROSCI.4264-11.2012
33. Chai X, Wu S, Murray TK. Passive immunization with anti-Tau antibodies in two transgenic models: reduction of Tau pathology and delay of disease progression. *J Biol Chem*. 2011;286(39):34457-67. doi:10.1074/jbc.M111.229633
34. Garber K. Genentech's Alzheimer's antibody trial to study disease prevention. *Nat Biotechnol*. 2012;30:731-2. doi:10.1038/nbt0812-731
35. Reddy PH, Manczak M, Yin X, Reddy AP. Synergistic protective effects of mitochondrial division inhibitor 1 and mitochondria-targeted small peptide SS31 in Alzheimer's disease. *J Alzheimers Dis*. 2018;62(4):1549-65. doi:10.3233/JAD-170988
36. Vlassenko AG, Gordon BA, Goyal MS. Aerobic glycolysis and tau deposition in preclinical Alzheimer's disease. *Neurobiol Aging*. 2018;67:95-8. doi:10.1016/j.neurobiolaging.2018.03.014
37. Obulesu M, Lakshmi MJ. Apoptosis in Alzheimer's disease: an understanding of the physiology, pathology and therapeutic avenues. *Neurochem Res*. 2014;39(12):2301-12. doi:10.1007/s11064-014-1454-4
38. Ludtmann MHR, Angelova PR, Horrocks MH. α -synuclein oligomers interact with ATP synthase and open the permeability transition pore in Parkinson's disease. *Nat Commun*. 2018;9(1):2293. doi:10.1038/s41467-018-04422-2
39. Mazure NM. VDAC in cancer. *Biochim Biophys Acta Bioenerg*. 2017;1858(8):665-73. doi:10.1016/j.bbabi.2017.03.002
40. Parks RJ, Murphy E, Liu JC. Mitochondrial Permeability Transition Pore and Calcium Handling. *Methods Mol Biol*. 2018;1782:187-96. doi:10.1007/978-1-4939-7831-1_11
41. Chavoshinezhad S, Mohseni Kouchesfahani H, Ahmadiani A, Dargahi L. Interferon beta ameliorates cognitive dysfunction in a rat model of Alzheimer's disease: Modulation of hippocampal neurogenesis and apoptosis as underlying mechanism. *Prog Neuropsychopharmacol Biol Psychiatry*. 2019;94:109661. doi:10.1016/j.pnpbp.2019.109661
42. Jiang X, Kumar M, Zhu Y. Protective effect of hyperforin on β amyloid protein induced apoptosis in PC12 cells and colchicine induced Alzheimer's disease: An anti-oxidant and anti-inflammatory therapy. *J Oleo Sci*. 2018;67(11):1443-53. doi:10.5650/jos.ess18117
43. Rather MA, Thenmozhi AJ, Manivasagam T, Bharathi MD, Essa MM, Guillemin GJ. Neuroprotective role of Asiatic acid in aluminium chloride induced rat model of Alzheimer's disease. *Front Biosci (Schol Ed)*. 2018;10:262-75. doi:10.2741/s514
44. Suresh K, Carino K, Johnston L. A nonapoptotic endothelial barrier-protective role for caspase-3. *Am J Physiol Lung Cell Mol Physiol*. 2019;316(6):L1118-26. doi:10.1152/ajplung.00487.2018
45. Voronkov AV, Pozdnyakov DI. [Evaluation of the effect of 4-hydroxy-3,5-di-tert-butylcinnamic acid on changes in the antithrombotic potential of the endothelium in rabbits under conditions of brain ischemia. *Experimental and clinical pharmacology*]. 2018;81(8):3-7. Russian. doi:10.3897/rrpharmacology.4.26519
46. Voronkov AV, Pozdnyakov DI, Zolotykh D S. [Effect of the ATACL compound on the anti-inflammatory potential of the endothelium of rat brain vessels in experimental ischemia]. *Bull Pharm*. 2018; 1(79):65-8. Russian.