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Nootropic properties of a new combined cytoprotective agent

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Abstract. Medicinal products of nootropic action, which have a positive effect on neurometabolism and normalize memory and intellectual functions, are an important component of complex pharmacotherapy for various neurological and cerebrovascular diseases. The aim of the work was an experimental study of the nootropic activity of the new combined drug Melarginin, which includes 3-(2,2,2-trimethylhydrazinium) propionate (meldonium), L-arginine, and inosine in a fixed ratio.

In experiments on female Balb/c mice, it was established that Melarginin in doses of 250 and 500 mg/kg (per os for 14 days) statistically significantly increased the survival of animals in acute hypoxia, in a dose of 250 mg/kg - contributed to the preservation of cognitive function in experimental amnesia, improved spatial memory and recognition of the new location of the object, and in a dose of 500 mg/kg - increased muscle tone and endurance to physical and psychoemotional stress. The revealed effects indicate a nootropic effect due to the combined cyto- and cerebroprotective properties of the studied combined agent components. Based on the obtained data, the new pharmacological combination can be a promising drug for neurological recovery and improvement of physical and mental performance in chronic cerebrovascular pathology.

Keywords: combined drug Melarginin, hypoxia, amnesia, nootropic activity.

The constant growth of the frequency of brain vascular pathology determines the importance of finding new approaches, both to the prevention of these diseases and to the mitigation of their negative consequences, which limit the vital activities of patients and lead to the loss of work capacity.

It is known that in the pathogenesis of acute and chronic forms of cerebrovascular pathology, hypoxemia and the negative impact of oxidative stress play a decisive role [1–5]. Oxygen lack is especially dangerous for nerve cells, as it leads to a decrease in the mitochondrial synthesis of macroergic compounds, energy resources depletion, excitatory amino acids accumulation, excitotoxicity development, free radical oxidation activation, endogenous antioxidant reserves reduction, damage to neuronal and mitochondrial membranes, and brain cells death [6–8]. These processes are an important chain of strokes pathogenesis, chronic cerebral ischemia, encephalopathy, infectious and toxic lesions of the central nervous system, and also play a decisive role in the emergence of the so-called chronic fatigue syndrome (CFS) or asthenic syndrome - a psychopathological condition characterized by a loss of physical and mental energy [9–12].

The CFS pathophysiological mechanisms are complex and not fully understood. In general, CFS is considered a universal adaptive body reaction to any threat of energy resource depletion in extreme conditions. The prevailing hypothesis is that the main role in the CFS pathophysiology is played by the reticular activating system (RAS), which controls the body's energy resources [13]. As a result of neurohumoral, psychosocial, metabolic, or infectious-immune factors, RAS can experience overload, which leads to a decrease in physical activity, control weakening and emotional sphere exhaustion, and intellectual functions deterioration [9, 14]. According to numerous data, CFS is included in a complex of syndromes not only in cerebral strokes or

traumatic brain injuries [15–17] but also in other diseases that debilitate the body, such as diabetes [18], psychiatric pathology [19], COVID-19 infection [20–22]. Asthenic conditions occur at any age in various categories of the population, but they occur especially often in the elderly against the background of a general decrease in the functional system's activity of the ageing body and concomitant cerebrovascular and cardiovascular pathology. With long-term stress in the elderly, CFS occurs more quickly and as a result provokes depression, social isolation, the development of dementia, course deterioration of the course, and accompanying chronic disease prognosis [10]. Hypopexy-induced biochemical brain changes in asthenia are similar to those that develop in chronic stress and ageing [23, 24]. Today, the medical and social significance of CFS has increased significantly due to the spread of such risk factors as population ageing, chronic stress, military operations, viral infection, etc.

Therefore, to mitigate CFS complications, regardless of the conditions of its occurrence, it is advisable to use combined therapy with drugs of cerebroprotective action, which can influence several pathophysiological mechanisms at the same time: protect the brain from hypoxia, improve its energy supply, inhibit the development of oxidative and nitrosative stress, and also eliminate cognitive-behavioural and intellectual deficits, increase physical and mental activity [25]. Nootropics largely meet these requirements. Their use in asthenic conditions is quite encouraging [26–28].

Aim of the study: Nootropic activity experimental assessment of the new pharmacological combination Melarginin.

Materials and methods.

The object of study is the working name "melarginine" pharmacological combination, which includes 3-(2,2,2-trimethyl-hydrazinium)propionate (meldonium), L-arginine, and inosine in a fixed ratio. Experiments were performed on sexually mature female Balb/c mice, body weight 26–30 g, obtained from the "Biomodelservice" nursery (Ukraine). The animals were kept in conditions that meet the requirements of GLP (Good Laboratory Practice), with a modern ventilation system, stable temperature, and air humidity. Animal handling was carried out by the rules of the European Convention for the Protection of Vertebrate Animals Used in Experimental Research and Other Scientific Purposes (Strasbourg, 2005).

All animals were divided into 4 groups of 10 mice each. Group 1 was control; groups 2 and 3 were experimental mice injected with Melarginin at doses of 250 and 500 mg/kg, respectively, group 4 was mice that received the reference drug Piracetam at a dose of 300 mg/kg (an effective dose of a nootropic for laboratory rodents [29]). The drugs were administered to mice intragastrically, in the morning before regular feeding, for 14 days. After completing the drug administration course, experimental testing of animals was carried out.

The antihypoxic effect was evaluated by the mice's average lifespan under acute hypoxia with hypercapnia (hypoxic normobaric hypoxia), which was simulated in a hermetic chamber with a volume of 200 mm^3 [29].

The coordination, physical and psycho-emotional endurance of mice under the influence of the studied agent was evaluated in the "rotating shaft" test (Rotarod) [30]. The method also makes it possible to simulate "tension" endogenous physiological hypoxia, which occurs when the oxygen consumption rate by the tissues of the body increases relative to their intense physical activity [6]. Animals that could stay on the shaft for at least 3 minutes at a rotation speed of 4 revolutions per minute (rpm) were selected for the experiment. During testing, the shaft rotation speed was increased by 4 revolutions every 20 s. The maximum (critical) speed was set at which the animal refused to move and fell off the rotating shaft. The result was calculated as the mean of three runs for each animal.

The cognitive function effect was studied in the test of developing a conditioned passive avoidance response (PAR) in normal and scopolamine-induced amnesia. A camera with light and dark compartments was used to produce PAR. During "learning" after the transition from the light to the dark compartment of the chamber through the electrified floor, the animal received 10 electric shocks (0.45 mA) with an interval of 3 s. After 24 hours, the mice were again returned to the facility and the latent time of entry and the number of animals that did not enter the dangerous chamber, that is, keeping the memory trace, were determined. After that, the mice were injected with scopolamine (2 mg/kg, intraperitoneally) and a day later, the latency time and the proportion of mice that preserved the PAR with amnesia were re-determined [31].

To assess the impact on spatial memory, a recognition of the new location of the object test was used (OLT – object location task) [32]. The research was carried out in a rectangular installation - an arena (60 x 60 x 40 cm) in three 10-minute stages with an interval of 20 minutes between them. In the first stage, each animal was placed in the arena at the "start" location and allowed to get used to the setup for 10 minutes. In the second stage, familiarization with two identical objects (C1 and C2) of a cylindrical shape (height 10 cm and diameter 3 cm) took place, which was fixed with the help of tape in the left and right arena corners opposite the "start" place at a distance of 6 cm from its walls. During the third stage, object C2 was moved to the lower right arena corner, where it occupied a new location (C2new). The test uses the animal's innate tendency to prefer novelty, and if it remembers the position of the objects in the second stage, it will spend more time studying the displaced object during the third stage. During testing, before and after moving the objects, the approaches number to each of the objects and the duration of their active exploration (approaching at a distance of up to 2 cm, sniffing, touching) were recorded. The novelty preference index (PI) was calculated as the ratio of the time spent on research of the moving object, to the total research time of both objects, according to the formula PI= tC2new /
(tC2new + tC1) x 100%, where tC2new and tC1 are the research time of the moved and non-moved objects.

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Results and discussion.

It was established that Melarginin in both studied doses showed an antihypoxic effect and statistically significantly increased the mice survival in acute hypoxia with hypercapnia (Fig. 1).

The introduction of Melarginin improved coordination and increased the animal's endurance to physical exertion, which is simulated in the Rotarod test. (Fig. 2).

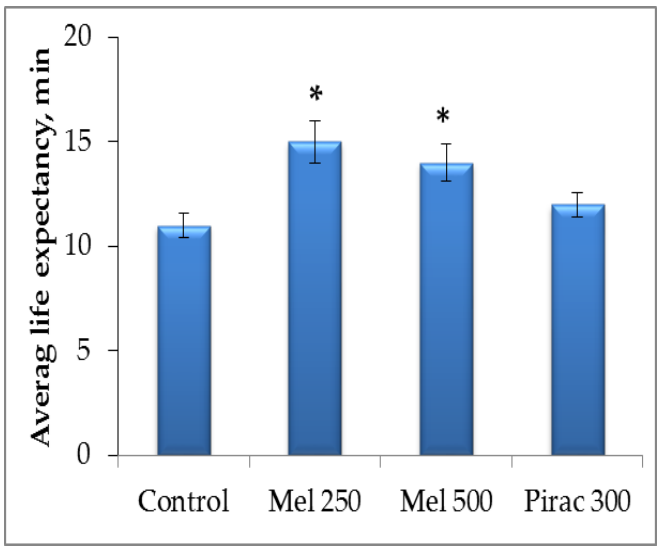


Figure 1. The average life mice expectancy in hypoxia under the Melarginin influence (* – P < 0.05 compared with the control group).

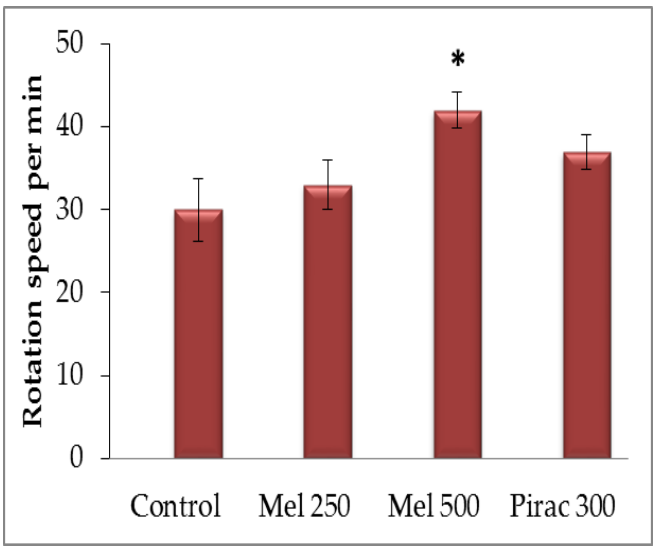


Figure 2. Maximum speed sustained by mice before falling in the Rotarod test under the Melarginin influence (* – P < 0.05 compared with the control group).

Thus, the mice of the experimental group, which received Melarginin at a dose of 500 mg/kg, could keep on the shaft at the maximum speed of rotation (41.5+2.2) rpm, while for the control animals this indicator was significantly lower, and amounted to (30.5+3.6) rpm ($P < 0.05$). After the introduction of Melarginin at a dose of 250 mg/kg, the indicator did not differ from the control and was (31.0+3.3) rpm. The reference drug Piracetam in this study was inferior in effectiveness to Melarginin at a dose of 500 mg/kg.

The PAR production test results showed that before the administration of scopolamine (before amnesia) 24 years after the training of animals, both experimental and control groups, demonstrated practically the same efficiency of producing a conditioned response, as evidenced by the latent time indicators of all and the percentage of mice that did not enter the dark compartment of the camera (Tab. 1). With amnesia induced by the administration of scopolamine, the latency time of entering the dark chamber in mice of all groups was reduced to a different degree, and the proportion of animals that preserved the PAR (did not enter the chamber) was significantly reduced, except for the mice group that was injected with melarginine at a dose of 250 mg/kg. Because amnesia "reverses" the produced PAR, a greater percentage of mice that did not enter the chamber showed preserved cognitive function, as observed after administration of Melarginine at a dose of 250 mg/kg, and to a lesser extent after administration of Piracetam. The proportion of animals that did not lose the produced URPU decreased in the control group by 50%; in the Piracetam group - by 20%; in the group of Melarginin at a dose of 500 mg/kg - by 37.5%, while in the group of mice injected with Melarginin at a dose of 250 mg/kg, the indicator did not change and was 57% both before and after amnesia. Melarginin at a 250 mg/kg dose exceeded the reference drug in anti-amnestic effect terms.

Table 1

Latency time and proportion of mice with a conditioned passive avoidance response (PAR) after scopolamine amnesia

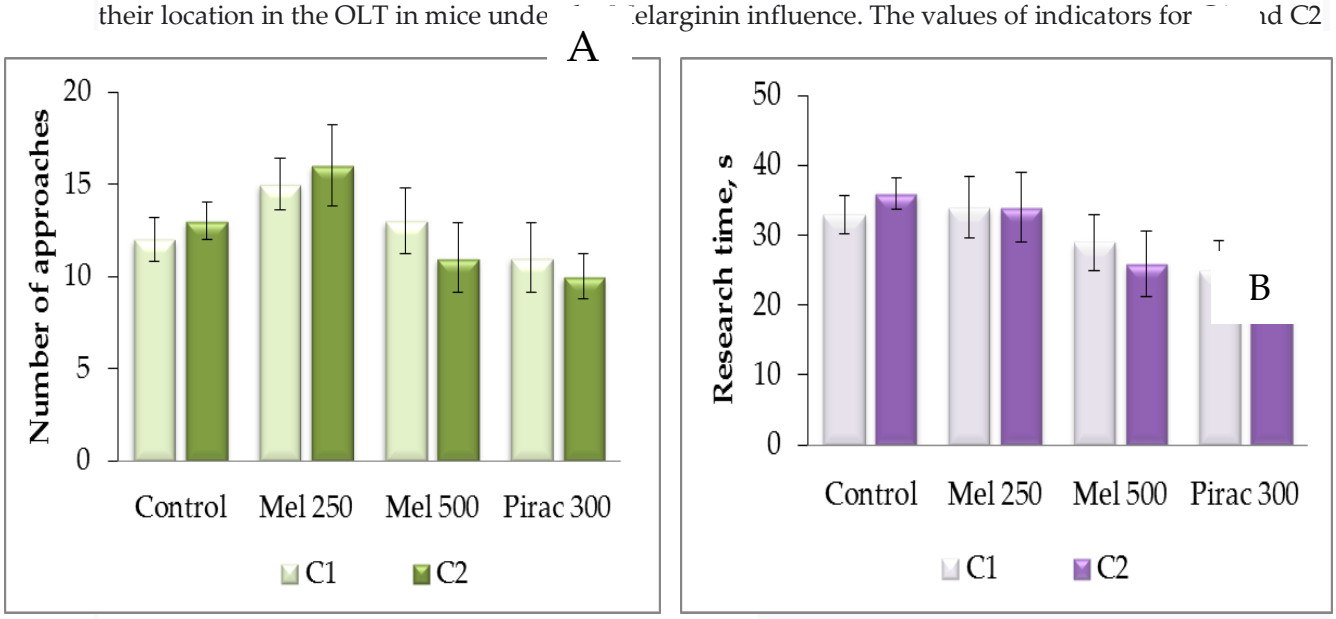
Group of mice, a dose of the drug	Latent time, s *			The proportion of mice that did not enter the camera (saved PAR), %	
	Before amnesia	After amnesia	Δ, s	Before amnesia	After amnesia
Control	161.0 ± 15.6	120.0 ± 21.4	–41.0	62.5	12.5
Melarginin, 250 mg/kg	164.2 ± 15.4	161.2 ± 18.2	–3.0	57.0	57.0
Melarginin, 500 mg/kg	165.7 ± 10.6	140.6 ± 17.6	–25.1	75.0	37.5
Piracetam, 300 mg/kg	164.3 ± 10.2	139.0 ± 20.0	–25.3	80.0	60.0

Notes. * – compared with the control group in all cases $P > 0.05$.

Memorization improvement and recognition of under-investigated agent influence are evidenced by the results of the OLT test. All animals, including the control ones, in the second stage of the experiment, did not prefer any of the objects and approached C1 and C2 objects with the same frequency (Fig. 3A). The study time of each of the objects also did not differ in all groups (Fig. 3B). Higher animal activity and a greater number of approaches to both objects were observed in the group of mice receiving Melarginin at a dose of 250 mg/kg.

At the third stage of the experiment, the number of approaches to the moving object C2new and the time of its study, compared to the unmoved object C1, increased in all groups. However, these indicators reached a statistically significant level only in the group of mice administered Melarginin at a dose of 250 mg/kg (Fig. 4C, 4D).

Figure 3. The number of approaches (A) and the study time of objects C1 and C2 (B) to the change in their location in the OLT in mice unde elarginin influence. The values of indicators for d C2 do not have statistically significant differences (P > 0.05)



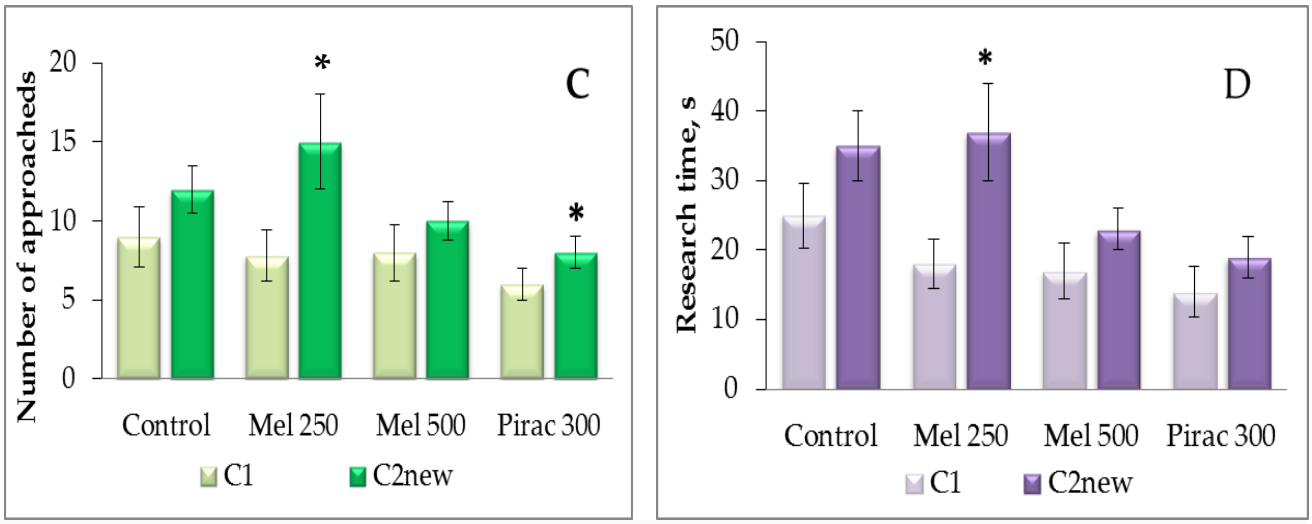


Figure 4. The number of approaches (C) and the study time of objects C1 and C2new (D) after changing their location in the OLT in mice under the Melarginin influence
(* – P<0.05 when comparing C1 and C2new)

Under the Melarginin influence at a dose of 250 mg/kg, the number of approaches of mice to the moved C2new object (15.0+3.0) and the time of its examination (36.6+7.7) exceeded these indicators for the non-moved object by 2 times object C1 (respectively (7.8+1.6) and (17.9+3.5) at P<0.05. The preference index for a new location (location index) in these animals was (66.4+3.0)%, which statistically significantly exceeded the index of control mice - (59.4+3.0)%, which was not observed in other experimental groups (Fig. 5). Values of the indicator greater than 50% indicate a preference for a new location and a significant improvement of spatial memory under the Melarginine influence at a dose of 250 mg/kg in animals with intact cognitive function (Fig. 5).

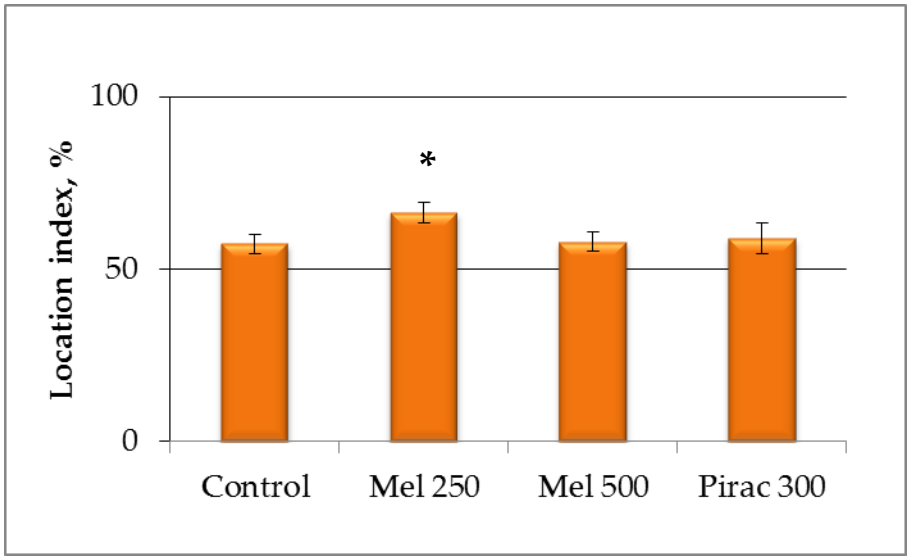


Figure 5. An indicator of the novelty advantage in the OLT in mice under the Melarginin influence (* – P <0.05 compared with the control group).

The study established that Melarginin exhibited an antihypoxic effect, improved coordination and increased endurance to physical and psychoemotional stress, contributed to the preservation of cognitive function in experimental amnesia and had a positive effect on the process of spatial learning and memorization. The established effects correspond to the nature of the nootropic agent's action and are due to the properties of the components 3-(2,2,2-trimethylhydrazinium) propionate, L-arginine, and inosine combination. According to the literature, 3-(2,2,2-trimethylhydrazinium)propionate corrects energy metabolism at the level of mitochondria, blocking β-oxidation of fatty acids and stimulating the glycolytic pathway of energy production; has a positive effect on cerebral blood flow and brain oxygenation [33]; reduces oxidative stress; reduces manifestations of neurological deficit [34–36]. This active substance can produce and accumulate gamma-butyrobetaine - a choline-like metabolite of carnitine, which can affect the central departments of the neuro-endocrine system, increasing the body's resistance to psycho-emotional and physical stress [37, 38]. As a precursor of nitric oxide synthesis, the amino acid L-arginine improves endothelial function, exhibits a vasotropic effect, and reduces the nitrosative level and oxidative stress [39–42]. Inosine stimulates metabolic processes, activates Krebs cycle enzymes, increases the contractility of muscle fibres, improves blood supply to the brain in hypoxia conditions, and increases the adaptive capabilities of the hypothalamic-pituitary system in stress conditions [43–46]. The neuroprotective potential of inosine and its ability to stimulate compensatory growth of axons have been shown [47]. The active substances of metabolic action combination in Melarginin, which complement each other, opens up new possibilities in the correction of the pathogenesis of many chronic neurological diseases and their most common complications, such as asthenic syndrome or CFS.

Conclusion.

The combined drug Melarginin has nootropic properties and can be an effective tool for neurological recovery and increasing physical and mental performance.

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Conflicts of Interest: The authors declare no conflict of interest.

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