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# The Study of the Effectiveness of Ethylmethylhydroxypyridine Succinate in Acute Alcohol Intoxication

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The effectiveness of ethylmethylhydroxypyridine succinate (EMHPS) in acute alcohol intoxication was tested in a study on SPF male outbred ICR mice. Ethanol (concentration 40%) was administered to animals once intraperitoneally at a dose of 4 g/kg. Control animals were injected with saline in an equivalent volume. In 15 min after the administration of alcohol, the animals were injected intravenously or intramuscularly with EMHPS at a dose of 50 or 100 mg/kg or with saline via the same route in an equivalent volume. Animal behavior was tested 3 and 24 h later after administration of the substances. After 3 and 24 h, mice in the pathological control groups developed semiptosis, the gait and the turning over reflex were impaired, the strength of the hind limbs decreased and the distance between the hind limbs increased when landing; in the open-field test, the latency of the first movement increased, and the number of rearing postures decreased. Intravenous and intramuscular administration of EMHPS in doses of 50 and 100 mg/kg had a pronounced antitoxic and neuroprotective effect in acute alcohol intoxication: all studied parameters did not differ significantly from the control.

**Key Words:** *ethylmethylhydroxypyridine succinate; Mexidol; acute alcohol intoxication; SPF outbred mice ICR*

Ethanol is the most consumed psychoactive substance; the euphoric and empathic effects of ethanol were experienced by the vast majority of people [1]. At the same time, 5.3% of all deaths in the world are associated with alcohol consumption, and in the age group of 20-39 years this figure increases to 13.5% [2].

The brain is one of the main targets of the toxic effects of ethanol. It has been shown that acute exposure to ethanol has a heterogeneous effect on neuronal activity in the striatum, hippocampus, cerebellum, amygdala, substantia nigra, and ventral tegmental area. Ethanol causes diverse changes in both GABAergic and glutamatergic signaling in neurons, which affects long-term potentiation and CNS activity [3]. Gene expression analysis has shown that alcohol abuse altered neurovascular blood flow, astrocyte reactivity, neuronal glutamatergic signaling, and ion transport [4]. Acute ethanol exposure increased glutamate uptake by astrocytes through excitatory amino acid transporters [5]. Therefore, the development of approaches to protect CNS from the toxic effects of ethanol is a pressing medical and social problem.

In view of the important role of disturbances in energy and metabolic processes, as well as the activation of free radical oxidation processes in the development of alcohol-induced neuronal damage [6], the use of antioxidants in the complex therapy of this pathology is pathogenetically justified. These drugs reduce the rate of oxidative processes, block the negative effects of free radicals, increasing the excitation threshold in the brain. Ethylmethylhydroxypyridine succinate (EMHPS), original drug Mexidol, is a highly active antioxidant with antihypoxic activity.

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Our aim was to study the effectiveness intravenous and intramuscular administration of EMHPS to ICR male mice with acute alcohol intoxication.

## MATERIALS AND METHODS

Male mice of outbred stock ICR (SPF status; age 9-10 weeks, body weight 30.5±2.8 g) were used in the study. The experiment was approved by the Bioethical Committee of the Ryazan State Medical University (Protocol No. 777/21 of February 12, 2021) and was performed in accordance with the Directive 2010/63/EU of the European Parliament and of the Council (September 22, 2010; On the Protection of Animals Used for Scientific Purposes).

Acute alcohol intoxication was modeled by single intraperitoneal injection of 40% ethanol (4 g/kg) [7]. Control animals were injected with saline in an equivalent volume. In 15 min after alcohol administration, the animals received intravenous or intramuscular injection of EMHPS (Pharmasoft) in doses of 50 or 100 mg/kg [8]. The administration route was chosen in accordance with the recommendations for the administration of medicinal substances in acute intoxications with psychoactive substances in clinical practice [9].

The animals were divided into 7 groups (8 animals in each): controls (administration of saline; group 1); alcohol intoxication+administration of saline intravenously (10 ml/kg) or intramuscularly (2 ml/kg) (groups 2 and 3, respectively); alcohol intoxication+intravenous administration of EMHPS at doses of 50 and 100 mg/kg (groups 4 and 5, respectively); alcohol intoxication+intramuscular administration of EMHPS at doses of 50 and 100 mg/kg (groups 6 and 7, respectively).

In 3 and 24 h after administration of EMHPS, the condition of the animals was evaluated using the following tests [10]: development of ptosis and semiptosis (the number of mice with this symptom); open field behavior (latency of the first step (sec)); number of rearing postures and number of animals with gait disturbances (the body was dragged and swayed, the stomach is in contact with the floor) for 2 min; impairment of the righting reflex, *i.e.* the ability to roll over from the back onto 4 legs (number of animals with impaired reflex) [11]; hind limb placement when landing (characterizes the development of ataxia): all 4 paw were marked with ink, and then, the animal was lowered from a height of 30 cm onto a sheet of absorbent paper and the distance between the prints of each hind limb was measured (in mm) [12]; Grip Strength (hind limb strength, in kg) (Columbus Instruments) [13]. The tests were performed in the specified sequence.

Statistical analysis was performed using Statistica 7.1 software (StatSoft, Inc.). The obtained quantitative data are presented as the Me (min; max), frequency indicators are presented as the percentage without deviations in relation to the total number of animals. The significance of differences was assessed using the Kruskal–Wallis test; for subsequent pairwise comparisons, the Mann–Whitney test was used. To analyze frequency indicators, the $\chi^2$ test was applied. The differences were considered statistically significant at $p<0.05$.

## RESULTS

In the pathological control groups (intravenous and intramuscular injection of saline after ethanol administration), pronounced disturbances of mouse behavior were observed both after 3 and 24 h. After 3 h, semiptosis developed in 62.5% mice ($p<0.05$) receiving intravenous injection of saline against the background of alcoholization (Fig. 1), gait disturbances were observed in 37.5% animals ($p<0.05$) (the body dragged and swayed, the stomach was in contact with the floor surface) (Fig. 2), the righting reflex was impaired in 50% ($p<0.05$) (Fig. 3).

In the open-field test, the latency of first movement increased by 130.6% ($p<0.05$) and the number of rearing postures decreased by 92.8% ($p<0.05$) compared to the control (Table 1). There was also a decrease in the strength of the hind limbs by 42.9% ($p<0.05$) and an increase in the distance between the hind limbs when landing from a height by 42.7% ($p<0.05$) (Table 2). After intramuscular injection of saline against the background of alcohol treatment, 75% ($p<0.05$) of mice developed semiptosis (Fig. 1), 62.5% ($p<0.05$) developed gait disturbance (Fig. 2), 62.5% ($p<0.05$) had righting reflex impairment (Fig. 3), the latency of the first movement increased by 127.8% ($p<0.05$), the number of rearing postures in the open-field test decreased by 85.7% ($p<0.05$) (Table 1), the strength of the hind limbs decreased by 42.9% ($p<0.05$), and the distance between the hind limbs when landing from a height increased by 50.1% ($p<0.05$) (Table 2).

Similar changes (by the direction and severity) were observed 24 h after intravenous and intramuscular injection of saline, except the time of the first step in the open-field test that did not differ significantly from that in the control.

The changes in the animal behavior observed in the experiment were a consequence of the toxic effect of ethanol on CNS and indicated successful modeling of acute alcohol poisoning. When the concentration of ethanol in the blood increases to 12 mM, euphoria developed in animals and humans and an anxiolytic effect is observed; at a concentration of 18 mM, the reaction time increases, coordination of movements is impaired, and cognitive impairment develops; at a

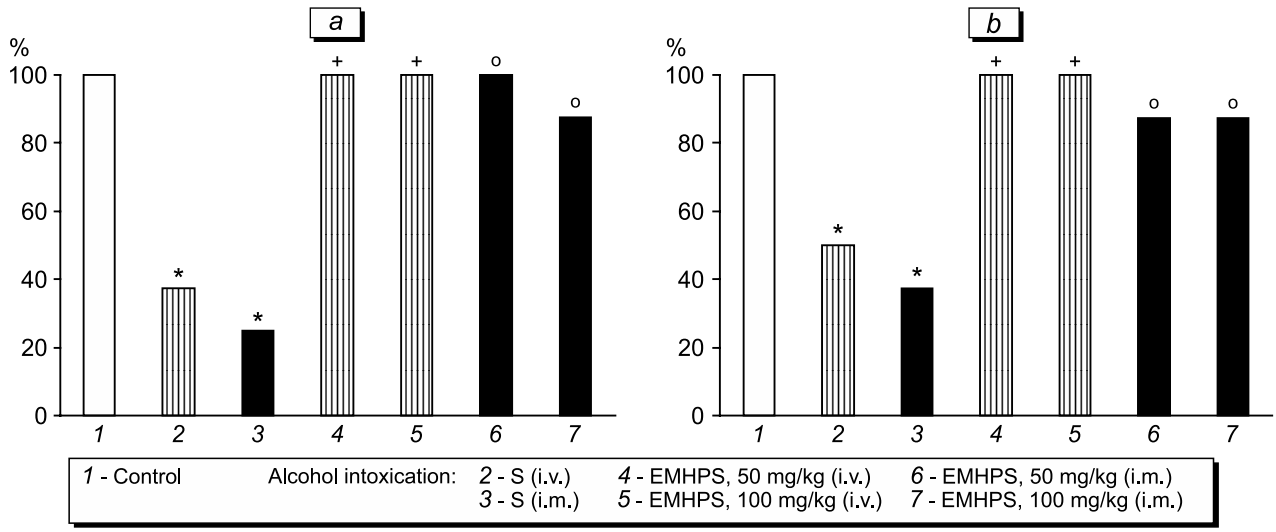


**Fig. 1.** Effect of EMHPS on semiptosis in male mice in a model of acute alcohol intoxication 3 h (*a*) and 24 h (*b*) after ethanol administration. Proportion of animals without deviations. $p<0.05$ in comparison with *control, +saline intravenously (S (i.v.)), °saline intramuscularly (S (i.m.)).

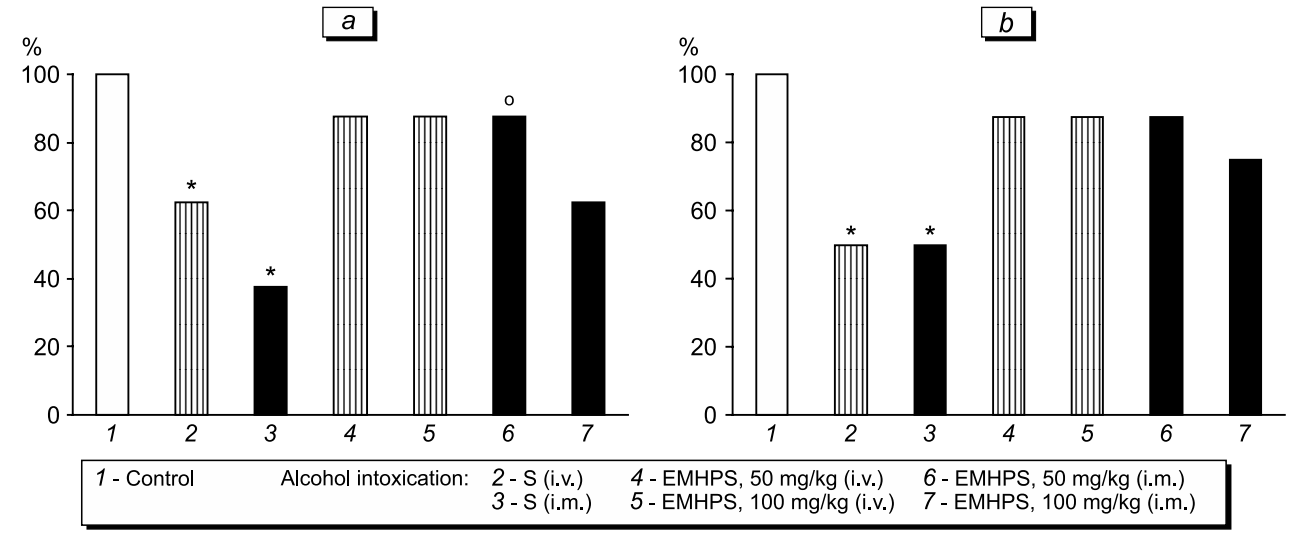


**Fig. 2.** The effect of EMHPS on the gait of male mice (the body drags and sways, the stomach can contact the floor surface) in a model of acute alcohol intoxication 3 h (*a*) and 24 h (*b*) after ethanol administration. Proportion of animals without deviations. $p<0.05$ in comparison with *control, +saline intravenously (S (i.v.)), °saline intramuscularly (S (i.m.)).

concentration of up to 50 mM, the motor and cognitive impairments increase and a sedative effect appears. At higher concentrations, severe sedation and respiratory depression can lead to coma and death [3].

Intravenous administration of EMHPS in doses of 50 and 100 mg/kg (groups 4 and 5) had a pronounced pharmacological effect: all studied indicators (development of semiptosis, open field behavior of animals, righting reflex, strength of the hind legs, and position of the hind limbs) did not differ significantly from the control values. At the same time, the proportion of animals without ptosis and with normal righting reflex, as well as the number of rearing postures in the open-field test, significantly exceeded the corresponding indicators in the pathological control ($p<0.05$). There were no significant differences between the effect of EMHPS at doses of 50 and 100 mg/kg (Figs. 1-3; Tables 1-2).

Intramuscular administration of EMHPS at doses of 50 and 100 mg/kg (groups 6 and 7) also had a pronounced antitoxic and neuroprotective effect. The studied indicators did not differ significantly from the control. At the same time, the proportion of animals without ptosis and with a normal righting reflex, as well as the number of rearing postures in the open-field test, as with intravenous administration, significantly exceeded the indicators of the corresponding pathological control ($p<0.05$). There were also no

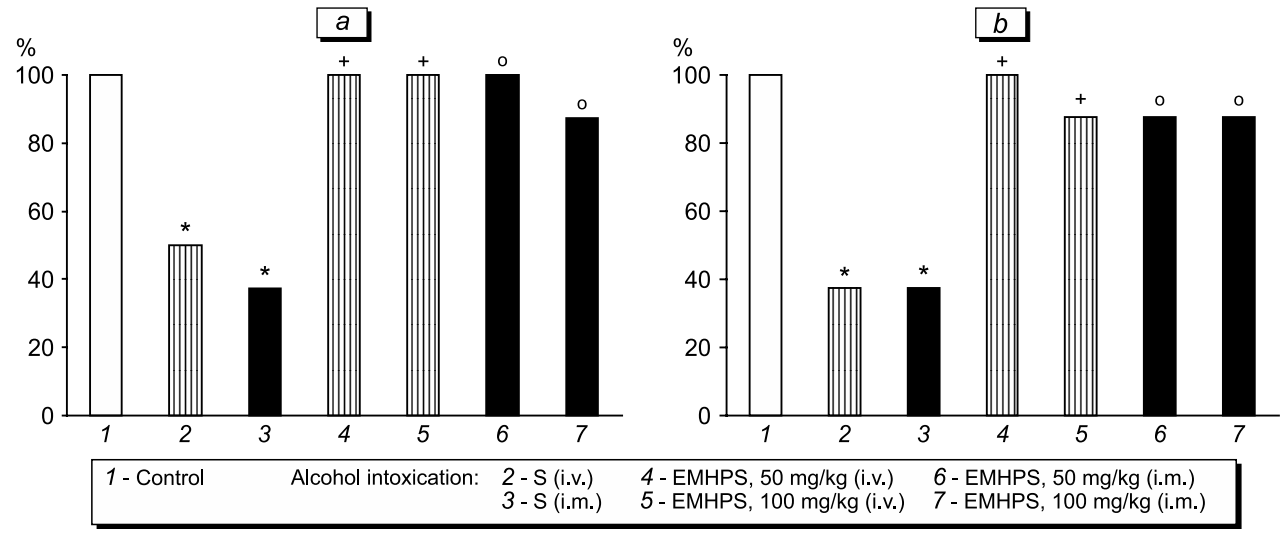


**Fig. 3.** Effect of EMHPS on the righting reflex of male mice in a model of acute alcohol intoxication 3 h (*a*) and 24 h (*b*) after ethanol administration. Proportion of animals without deviations. $p<0.05$ in comparison with *control, $^{+}$saline intravenously (S (i.v.)), $^{o}$saline intramuscularly (S (i.m.)).

significant differences between the effect of EMHPS at doses of 50 and 100 mg/kg (Figs. 1-3; Tables 1-2).

Activation of free radical oxidation processes plays an important role in the pathogenesis of neuronal damage under the influence of ethanol. Thus, it has been shown that ethanol metabolism of primary human neurons by alcohol dehydrogenase or cytochrome P450-2E1 is accompanied by generation of ROS and NO through the induction of NADPH/xanthine oxidase [14]. On the other hand, mitochondrial tissue respiration is impaired after acute and chronic exposure to ethanol, ATP production is suppressed, and ROS generation is enhanced, which increases the susceptibility of cells to apoptosis and/or their death from oxidative stress [15].

Numerous studies have shown that EMHPS inactivates free radicals (superoxide anion radical, hydroxyl radical), increases activity of antioxidant enzymes (glutathione peroxidase, superoxide dismutase), suppresses the development of glutamate excitotoxicity, and enhances the expression of transcriptional factors Nrf2 and HIF-1α [16]. That is, EMHPS can prevent damage to biomacromolecules caused by activation of LPO during acute alcohol intoxication due to its direct and indirect antioxidant activity.

Succinate, a component of EMHPS, supports the work of complex II of the respiratory chain [16,17] and can improve energy production in neurons during alcohol intoxication. In addition, it has been shown that succinate activates specific succinate receptors, which also contributes to the neuroprotective activity of EMHPS [18]. EMHPS has a membrane-stabilizing effect, modulates membranes receptor complexes in the brain, in particular GABA-benzodiazepine [19], which also determines its therapeutic effect in acute alcohol intoxication.

Thus, EMHPS (Mexidol) after single intravenous and intramuscular administration in doses of 50 and 100 mg/kg to male ICR mice has a pronounced antitoxic and neuroprotective effect, which can significantly reduce the severity of symptoms of acute alcohol intoxication.

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**TABLE 1.** Effect of EMHPS on the Behavior of Male Mice in the Open-Field Test in the Model of Acute Alcohol Intoxication (Me (min; max))

| Parameter | Control | Alcohol intoxication | | | | | |
|---|---|---|---|---|---|---|---|
| | | saline (i.v.) | saline (i.m.) | EMHPS, 50 mg/kg (i.v.) | EMHPS, 100 mg/kg (i.v.) | EMHPS, 50 mg/kg (i.m.) | EMHPS, 100 mg/kg (i.m.) |
| **3 h after ethanol administration** | | | | | | | |
| First step latency, sec | 0.36 (0.21; 0.75) | 0.83* (0.49; 1.78) | 0.82* (0.23; 1.32) | 0.63 (0.32; 0.95) | 0.47 (0.26; 0.82) | 0.53 (0.23; 0.72) | 0.39 (0.23; 0.58) |
| Number of rearings | 7 (5; 10) | 0.5* (0.0; 3.0) | 1* (0; 3) | 5.5$^{+}$ (3; 8) | 5$^{+}$ (3; 9) | 6$^{o}$ (4; 9) | 7$^{o}$ (4; 11) |
| **24 h after ethanol administration** | | | | | | | |
| First step latency, sec | 0.40 (0.21; 0.72) | 0.55 (0.32; 1.34) | 0.50 (0.23; 1.28) | 0.42 (0.29; 0.73) | 0.40 (0.20; 0.72) | 0.58 (0.27; 0.85) | 0.39 (0.29; 0.76) |
| Number of rearings | 9 (5; 12) | 1.5* (0.0; 3.0) | 1.5* (0.0; 3.0) | 5$^{+}$ (2; 7) | 4$^{+}$ (3; 7) | 5$^{o}$ (2; 8) | 4.5$^{o}$ (3.0; 8.0) |

**Note.** i.v.: intravenously; i.m.: intramuscularly. $p<0.05$ in comparison with *control, $^{+}$with saline (i.v.), $^{o}$with saline (i.m.).

**TABLE 2.** Effect of EMHPS on the Development of Ataxia and Strength of the Hind Limbs in Male Mice in a Model of Acute Alcohol Intoxication (Me (min; max))

| Parameter | Control | Alcohol intoxication | | | | | |
|---|---|---|---|---|---|---|---|
| | | saline (i.v.) | saline (i.m.) | EMHPS, 50 mg/kg (i.v.) | EMHPS, 100 mg/kg (i.v.) | EMHPS, 50 mg/kg (i.m.) | EMHPS, 100 mg/kg (i.m.) |
| **3 h after ethanol administration** | | | | | | | |
| Hind limb placement, mm | 33.5 (25.5; 43.5) | 47.8* (44.5; 53.5) | 50.3* (45.5; 56.5) | 40 (29; 48) | 41.5 (38.0; 54.0) | 41.3 (28.0; 63.0) | 41.5 (36.5; 46.5) |
| Hind limb strength, kg | 0.14 (0.1; 0.16) | 0.08* (0.04; 0.12) | 0.08* (0.05; 0.1) | 0.11 (0.09; 0.13) | 0.10 (0.07; 0.12) | 0.09 (0.06; 0.13) | 0.10 (0.08; 0.14) |
| **24 h after ethanol administration** | | | | | | | |
| Hind limb placement, mm | 33.0 (27.0; 35.5) | 48.5* (41.5; 51.0) | 45.8* (43.0; 51.0) | 34.3$^{+}$ (27.0; 41.0) | 31.5$^{+}$ (29.0; 45.0) | 35.5 (26.5; 54.0) | 32.0$^{o}$ (30.5; 40.5) |
| Hind limb strength, kg | 0.13 (0.1; 0.17) | 0.10* (0.07; 0.11) | 0.11* (0.08; 0.13) | 0.14 (0.05; 0.17) | 0.15 (0.0; 0.2) | 0.13 (0.08; 0.16) | 0.110 (0.095; 0.180) |

**Note.** i.v.: intravenously; i.m.: intramuscularly. $p<0.05$ in comparison with *control, $^{+}$with saline (i.v.), $^{o}$with saline (i.m.).

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