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CORRECTION OF PSYCHONEUROLOGICAL SIGNS OF ACUTE ALCOHOL INTOXICATION IN RATS WITH A NEW ACETYLCYSTEINE-BASED COMPOSITION

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The aim of the study is an experimental confirmation of the use of a new combination of biologically active substances with tonic and antioxidant effects. This combination contains acetylcysteine in its composition to reduce the severity of psychoneurological consequences of alcohol intoxication.

Materials and methods. The study was conducted on male Wistar rats. The post-intoxication state was simulated by a single injection of ethanol (3 g/kg, intraperitoneally). Half an hour after awakening, the rats were divided into groups, which were injected with saline, acetylcysteine (1 g/kg), taurine (20 mg/kg), caffeine (20 mg/kg), succinic acid (100 mg/kg), lipoic acid (100 mg/kg), pyridoxine (400 mg/kg), or a combination of acetylcysteine with all these substances taken in a twice lower dose (except taurine). Before the treatment and 3 hours after it, the degree of neurological disorders was fixed according to the Combs and D'Alecy scale, in the Open Field test and the Adhesion test. Then the animals were euthanized to assess the level of glutathione, triglycerides and malondialdehyde (MDA) in liver homogenates, to determine the activity of enzymatic antioxidant systems and serum aminotransferases.

Results. In the animals injected with alcohol, there were evident signs of neuropsychiatric disorders, manifested in a low motor activity and a decrease in fine motor skills. This state did not change after an oral administration of saline. After the administration of acetylcysteine, taurine, caffeine, succinic and lipoic acids, pyridoxine and, to a greater extent, their compositions, the compensation of neuropsychiatric disorders and improvement of fine motor skills were notified. In the liver of these animals, the levels of glutathione, MDA, triglycerides, and the activity of antioxidant defense enzymes corresponded to the physiological norm.

Conclusion. The introduction of a combination of acetylcysteine with taurine, caffeine, pyridoxine, lipoic and succinic acids after an acute alcohol intoxication, to a greater extent than each of the substances separately, contributes to the function retention of the antioxidant system of hepatocytes. Besides, it reduces the level of their dystrophic changes and leads to a decrease in the severity of psychoneurological disturbances in the experimental animals.

Keywords: ethanol; acetylcysteine; taurine; caffeine; pyridoxine; lipoic acid; succinic acid; composition; preclinical studies

Abbreviations: AST – aspartate aminotransferase; ALT – alanine transaminase; MDA – malonic dialdehyde; SOD – superoxide dismutase; TNF-α – tumor necrosis factor-alpha; GPR91 – G protein-coupled receptor; GSH – glutathione.

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ОРИГИНАЛЬНАЯ СТАТЬЯ

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КОРРЕКЦИЯ ПСИХОНЕВРОЛОГИЧЕСКИХ ПОСЛЕДСТВИЙ ОСТРОЙ ИНТОКСИКАЦИИ АЛКОГОЛЕМ У КРЫС НОВОЙ КОМПОЗИЦИЕЙ НА ОСНОВЕ АЦЕТИЛЦИСТЕИНА

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Цель. Экспериментальное обоснование применения новой комбинации биологически активных веществ с тонизирующим и антиоксидантным действием, содержащей в составе ацетилцистеин для снижения выраженности психоневрологических последствий интоксикации алкоголем.

Материалы и методы. Исследование проведено на крысах-самцах линии Вистар. Постинтоксикационное состояние моделировали однократным введением этанола (3 г/кг, внутрибрюшинно). Через полчаса после пробуждения крыс разделяли на группы, которым вводили: физиологический раствор, ацетилцистеин (1 г/кг), таурин (20 мг/кг), кофеин (20 мг/кг), янтарную (100 мг/кг), липоевую кислоту (100 мг/кг), пиридоксин (400 мг/кг), или комбинацию ацетилцистеина со всеми данными веществами, взятыми в меньшей (в 2 раза) дозе (кроме таурина). До лечения и спустя 3 часа фиксировали степень неврологических нарушений по шкале «Combs и D'Alecy», в тесте «Открытое поле» и «Адгезивный тест». Далее животных подвергали эвтаназии для оценки уровня глутатиона, триглицеридов и малонового диальдегида в гомогенатах печени, определения активности ферментативных антиоксидантных систем и сывороточных аминотрансфераз.

Результаты. У животных, которым вводили алкоголь, отмечались выраженные признаки психоневрологических нарушений, проявляющихся в низкой двигательной активности и снижением мелкой моторики. Данное состояние не изменялось после перорального введения физиологического раствора. После введения ацетилцистеина, таурина, кофеина, янтарной кислоты, липоевой кислоты, пиридоксина и, в большей степени, их композиции, отмечали компенсацию психоневрологических нарушений, улучшение мелкой моторики. Уровни глутатиона, малонового диальдегида, триглицеридов и активность ферментов антиоксидантной защиты в печени этих животных соответствовали физиологической норме.

Заключение. Введение комбинации ацетилцистеина с таурином, кофеином, пиридоксином, липоевой и янтарной кислотами после острой алкогольной интоксикации, в большей степени способствует сохранению функций антиоксидантной системы гепатоцитов, снижает уровень дистрофических изменений в них и приводит к снижению тяжести психоневрологических нарушений у экспериментальных животных.

Ключевые слова: этанол; ацетилцистеин; таурин; кофеин; пиридоксин; липоевая кислота; янтарная кислота; композиция; доклинические исследования

Список сокращений: АСТ – аспартатаминотрансфераза; АЛТ – аланинаминотрансфераза; МДА – малоновый диальдегид; СОД – супероксиддисмутаза; TNF-α – tumor necrosis factor-alpha/факто некроза опухоли-альфа; GPR91 – G protein-coupled receptor/рецептор, связанный с G-белком; GSH – glutathione/глутатион.

INTRODUCTION

Alcohol consumption is one of the leading risk factors for death and disability. Social and economic consequences of large quantities consumption of alcohol-containing beverages negatively affect many social institutions (industry, trade, education, health care). Globally, alcohol use was the seventh leading risk factor for premature death and disability in 2016, accounting for 2.8 million deaths [1]. A post-intoxication state is a primary medical problem for people who drink alcohol in small and moderate amounts (up to 100 g of pure alcohol per day), and a common cause of professional and home injuries due to impaired motor skills and attention [2].

The most commonly used medical products or food supplements do not significantly reduce the overall severity of the post-intoxication state. Although some of them relieve certain symptoms (vomiting and headache), they do not affect tremors or drowsiness The most promising drugs in terms of the effective reduction of the alcohol consumption consequences are sorbents (effective in pre- or co-intake with alcohol), which inhibit the synthesis of prostaglandins and accelerate the metabolism of ethanol [3].

It should be notified that such drugs have a significant drawback. Alcohol metabolism and, what is more important in terms of the relief of post-intoxication consequences, acetaldehyde biotransformation mainly depends on the hepatocyte antioxidant system activity. The use of antioxidants, such as acetylcysteine, in order to correct the complex of negative consequences of a high dose alcohol consumption, is justified and confirmed by the results of preclinical studies [4]. The development of fixed combinations of known medications is a well-known and promising strategy for working out and implementing new drugs in practice, which makes it possible not only to increase the effectiveness of treatment, but also to make the therapy more accessible and/or more convenient for the patient.

THE AIM. To justify experimentally the use of a new combination of biologically active substances containing acetylcysteine, with tonic and antioxidant effects, to reduce the severity of the psychoneurological consequences of the acute alcohol intoxication.

MATERIALS AND METHODS

Laboratory animals

The study was conducted in accordance with the legislation of the Russian Federation and the technical standards of the Eurasian Economic Union on good laboratory practice (GOST R 53434-2009, GOST R 51000.4-2011). The protocol was approved by the Regional Independent Ethics Committee at Volgograd State Medical University (IRB 00005839 IORG 0004900 (OHRP), protocol No. 132 dated 20.05.2019).

In the study, male Wistar rats (300–350 g, Rappolovo animal house) were used. The animals were kept in a 12/12 hour alternating light-dark cycle, the temperature of 20 ± 2°C and the humidity of 40–60%.

Design

48 hours before the alcohol intoxication, all the rats were trained in the conditioned passive avoidance reaction (CPAR) test, and the training success was confirmed during 24 hours.

The doses of the study drugs were selected according to the literature data [4- 8], and the following 9 groups (n = 10) were formed:

1. Intact group – normal saline (15 mL/kg i.p. and 5 mL/kg p.o.);
2. Ethanol (3 g/kg) + normal saline (5 mL/kg; placebo);
3. Ethanol (3 g/kg) + acetylcysteine (1 g/kg);
4. Ethanol (3 g/kg) + taurine (20 mg/kg);
5. Ethanol (3 g/kg) + caffeine (20 mg/kg);
6. Ethanol (3 g/kg) + succinic acid (100 mg/kg);
7. Ethanol (3 g/kg) + lipoic acid (100 mg/kg);
8. Ethanol (3 g/kg) + pyridoxine (400 mg/kg);
9. Ethanol (3 g/kg) + combination of substances.

The composition of the substances combination was as follows: acetylcysteine (500 mg/kg), taurine (20 mg/kg), caffeine (10 mg/kg), succinic acid (50 mg/kg), lipoic acid (50 mg/kg), pyridoxine (200 mg/kg).

The animals from the intact group were administrated with normal saline (i.p. and p.o.). The rats of the other groups were administrated with a 20% aqueous solution of ethyl alcohol (3 g/kg) (i. p.) and after awakening and assessing the level of neurological deficits, they were administrated with normal saline, the substances or their combination given as prescribed single doses. The volume of the injected fluid was 15 mL/kg for the intraperitoneal administration and 5 mL/kg for the administration p. o.

The average sleep duration in the rats was comparable between the groups (8 h ± 30 min) [4]. After waking up, the level of neurological deficits was determined using the Combs & D'Alecy scale, and a few tests. They were: the Adhesive test (the time of detection and removal of the 5x5 mm patch on the palmar surface of the forepaws for 180 seconds), the Open field test (the motor activity was recorded as the number of the sectors crossed for 3 minutes), and the exploratory activity – as a sum of the number of examined holes and the number of standing on the hind paws) [9]. After that, the animals were administrated with normal saline, one of the substances tested, or their combination in a twice lower dose (with an exception of taurine, which was administered as a part of the combination in a similar dose to maintain its potential cardioprotective effect notified in a number of studies) [10]. A two-fold dose reduction in the composition of the combination was performed to assess the drug-drug potentiation effects of the individual components in the combination.

The behavioral tests were repeated 3 hours later. Euthanasia (cardiac puncture) was performed under anesthesia with zoletil 20 mg/kg (Zoletil®100, Valdepharm, France) + xylazine 8 mg/kg (Xyla, Interchemie, Netherlands). After euthanasia, the liver tissue samples were taken for the subsequent biochemical analysis. The concentration of reduced glutathione was measured in the reduction reaction of 5.5-dithiobis-(2-nitrobenzoic acid).

The analysis was carried out in triplicates. The activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in plasma was determined using appropriate reagents (manufactured by Diacon-DS, Russia). The content of triglycerides in homogenates of the liver tissue was determined after the extraction with heptane and isopropanol followed by photometrical fractionation with sodium alcoholate (after the incubation with 2,4-pentanedione at the wavelength of 410 nm). The concentration of malonic dialdehyde (MDA) in homogenates was determined by the reaction with thiobarbituric acid; the concentration of reduced glutathione was determined in the reduction reaction of 5,5-dithiobis-(2-nitrobenzoic acid). A superoxide dismutase (SOD) activity was determined by a photometric method based on assessing the degree of inhibition of the epinephrine oxidation reaction.

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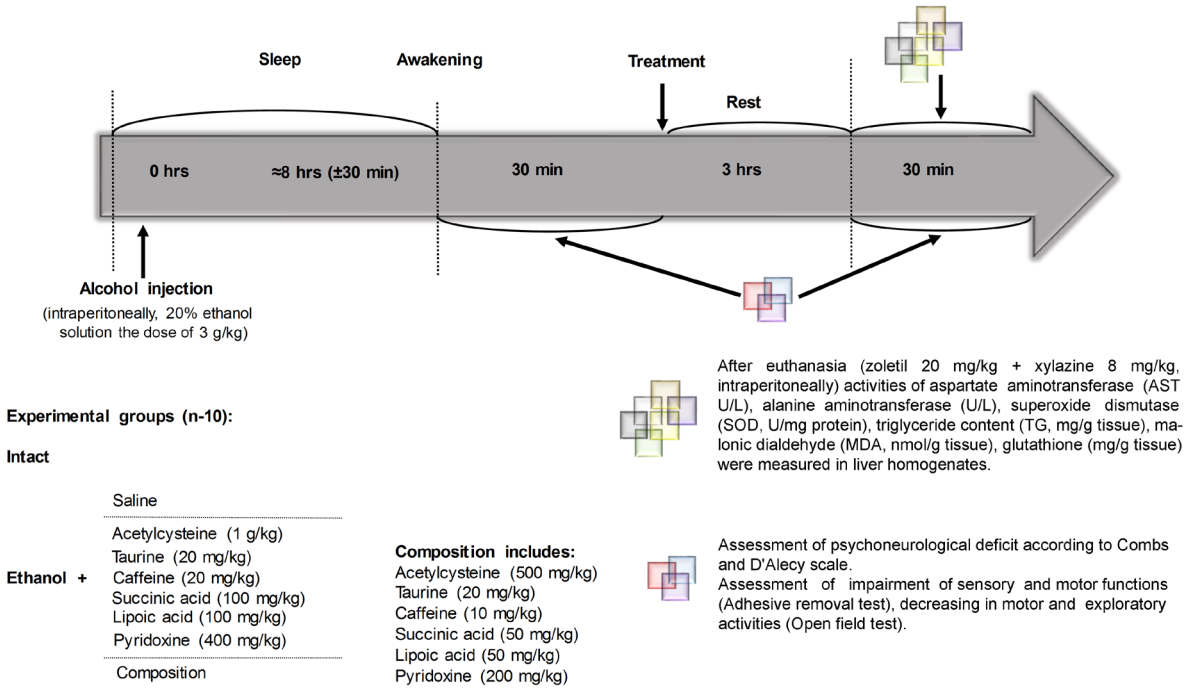

Figure 1 – Study design

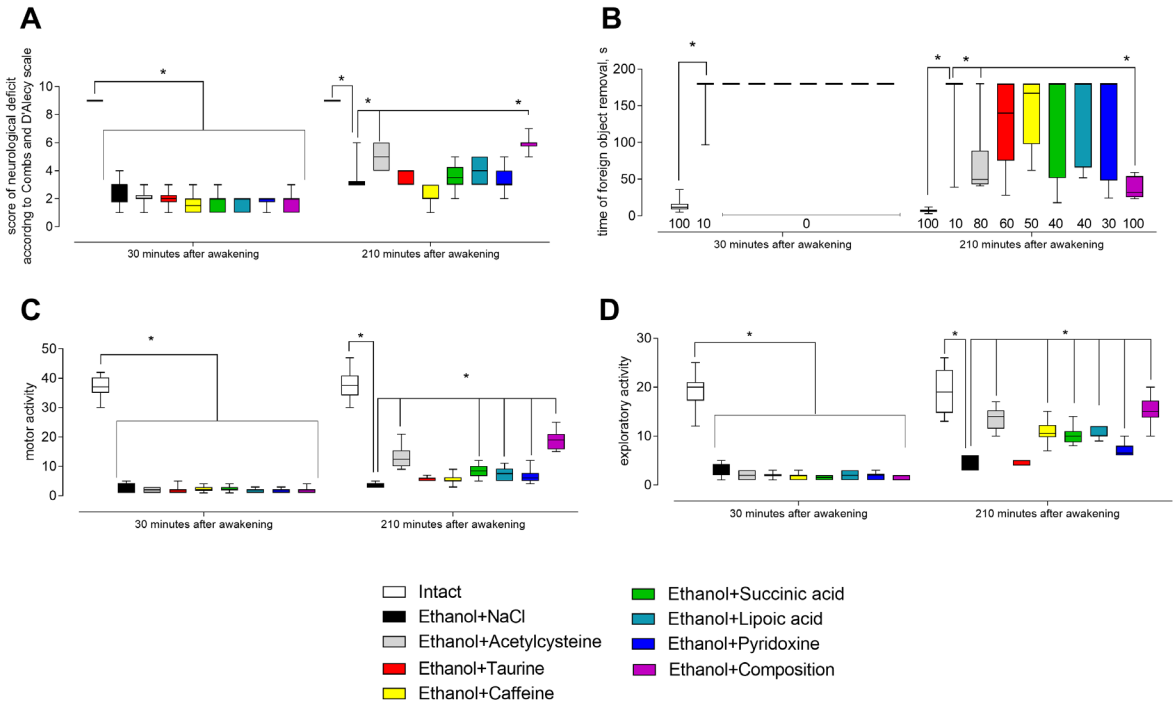

Figure 2 – The levels of neurological deficits assessed by the Combs & D'Alecy scale (A) and the time of removing a foreign object from the volar surface of the rats' fore paws (B), indicators of locomotor (C) and exploratory (D) activities in the open field test in the rats after acute alcohol intoxication

Note: * – $p < 0.05$, one-way variance analysis with Newman-Keuls post-hoc test; the compared samplings are indicated by lines; exploratory activities are the sum of the number of pointing acts and the number of holes examined; locomotor activity is the number of crossed sectors of the installation; numbers represent the numbers of the animals that found and got rid of foreign objects fixed on the volar surface of their forepaws (%)

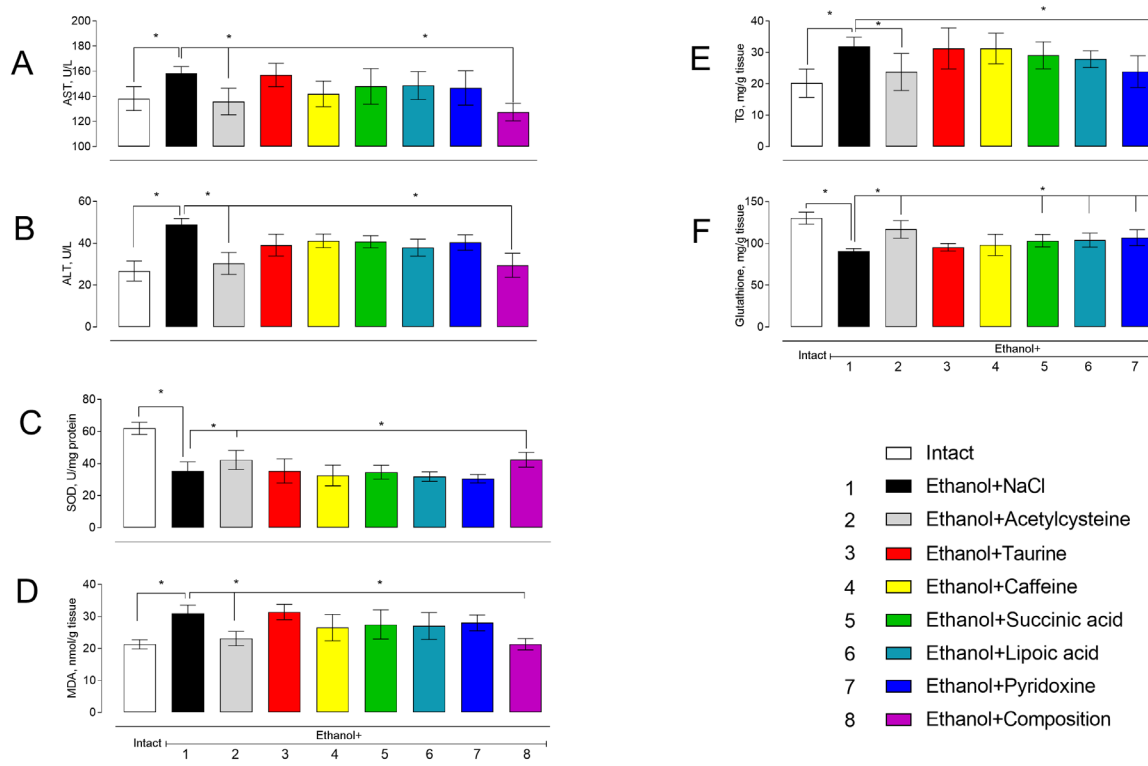

Figure 3 – Activity of superoxide dismutase (SOD), aspartate aminotransferase (AST) and alanine aminotransferase (ALT); the content of triglycerides, malonic dialdehyde (MDA), glutathione in rat liver homogenates, obtained after acute alcohol intoxication

Note: AST – aspartate aminotransferase (U/L, A), ALT – alanine aminotransferase (U/L, B), SOD – superoxide dismutase (U/mg protein, C), TG – triglyceride content (mg/g tissue, D), MDA – malonic dialdehyde (nmol/g tissue, E), glutathione (mg/g tissue, F); the data are presented as averaged individual values (n = 3 at each of the points), the arithmetic mean and standard error of the arithmetic mean; * – $p < 0.05$ (one-way variance analysis with Newman-Keuls post-hoc test); the compared groups are indicated by horizontal lines.

The results were statistically processed using descriptive and analytical statistics. The distribution of quantitative variables was assessed using the Shapiro-Wilk test. Intergroup differences were assessed using a one-way variance analysis with the Newman-Keuls post-hoc test, and the numerical values were presented as the arithmetic mean and standard error of the mean. The chi-square test was used to assess the differences in categorical data.

RESULTS

Ethyl alcohol injection (3 g/kg, i. p.) caused a progressive sedative effect. The animals fell asleep within 2–3 minutes and were asleep for 8 ± 0.5 hours. The animals that fell asleep after a longer period of time or slept longer than the designated period, were excluded from the experiment.

After awakening, the animals did not demonstrate active actions, all the observed behavioral acts were at the minimum level: the animals tended not to move, staying in one place (if they moved, they did it very slowly), they practically did not react to irritating stimuli (touching vibrissae, lateral pushes). After awakening, the rats, compared to the intact rats in the open field test, showed low motor and exploratory activities.

Three hours after the treatment in the control group (Ethanol + 0.9% sodium chloride solution) in the Open field test, the assessed variables were significantly lower than in the intact group. In the groups administrated with acetylcysteine, succinic acid, lipoic acid, pyridoxine, or a composition of the listed substances, motor and exploratory activities were significantly higher (Fig. 2, C), which indicates an accelerated recovery after the ethanol intoxication.

After awakening, the rats showed signs of pronounced neurological deficits (by the Combs & D'Alecy scale), which slightly decreased in the control group 180 minutes later (Ethanol + 0.9% sodium chloride solution) and significantly decreased after the administration of acetylcysteine, succinic and lipoic acids, pyridoxine, or their composition (Fig. 2, A).

During the Adhesive test, after awakening, the animals did not react to foreign objects fixed on the volar surface of their paws, i.e. their sensory and motor functions were significantly reduced, and this was not corrected by the oral administration of normal saline and having a rest for 3 hours. The oral administration of

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acetylcysteine, succinic and lipoic acids, pyridoxine, or their composition (more pronounced) led to a significant improvement in the sensory and motor functions of the animals (Fig. 2, B). Thus, 80% and 100% of the animals, which had been orally administered with acetylcysteine or the test composition, respectively, when tested in the Adhesive test, removed foreign objects from the palmar surface of the forepaws ($p < 0.05$, chi-square test).

After a biochemical analysis of liver homogenates obtained from the intact animals or rats, the animals were first injected with ethanol, and then with one of the above-mentioned substances or their composition. It was found out that the administration of acetylcysteine and, which was more pronounced, its composition with taurine, caffeine, succinic acid, lipoic acid, pyridoxine, reduced the expression of hepatic metabolism disorders to a minimum. In the control group (Ethanol + 0.9% NaCl solution), the activities of AST and ALT in liver homogenates reached 158.3 ± 5.4 U/L and 48.8 ± 3 U/L, respectively (versus 138.2 ± 9.6 U/L and 26.7 ± 4.8 U/L, respectively, in intact animals; $p < 0.05$), the triglyceride content was higher (31.7 ± 2.9 mg/g tissue versus 20.2 ± 4.5 mg/g tissue; $p < 0.05$), and glutathione was lower (90.5 ± 3.4 mg/g tissue versus 130.5 ± 7.2 mg/g tissue; $p < 0.05$). In the placebo group animals, the disturbances in the hepatocyte antioxidant system functioning were also manifested to a greater extent, as evidenced by the low activity of SOD (35.4 ± 5.6 U/mg versus 62 ± 3.8 U/mg in the intact group, $p < 0.05$) and a high content of MDA (30.9 ± 2.6 nmol/g tissue versus 21.3 ± 1.4 nmol/g tissue in the intact group, $p <0.05$).

In liver homogenates of the animals that had been orally administered with acetylcysteine or its combination with the above-mentioned substances after the alcohol injection, the activities of AST and ALT were 135.9 ± 106 U/L, 30.3 ± 5.2 U/L and 127.5 ± 7.0 U/L, 29.4 ± 5.8 U/L, respectively ($p < 0.05$, relative to the animals in the placebo group), triglyceride content 23.8 ± 5.9 mg/g tissue and 22.5 ± 4.5 mg/g tissue ($p < 0.05$, relative to the animals in the placebo group), glutathione 117 ± 10.6 mg/g tissue and 133.3 ± 7.6 mg/g tissue, respectively ($p < 0.05$). Functioning of the antioxidant system of these animals' hepatocytes was close to normal. The SOD activity in the animals' liver of these groups was 42.3 ± 5.9 U/mg and 42.4 ± 4.6 U/mg, respectively ($p < 0.05$), and the MDA content was 23.1 ± 2.2 and 21.3 ± 1.8 nmol/g tissue, respectively ($p < 0.05$). The indicated biochemical parameters in the animals of the other groups were of the intermediate values, and the differences between the intact group, placebo group, acetylcysteine or combination group did not reach statistical significance (with the exception of the positive effect of pyridoxine, succinic and lipoic acids, on the level of glutathione, which is obviously insufficient to affect other variables).

A single administration of acetylcysteine and, to a greater extent, its combination with taurine, caffeine, pyridoxine, succinic and lipoic acids obviously restored or increased the glutathione content in the liver, prevented the development of the oxidative stress and dystrophic changes, which helped to reduce the severity of the alcohol intoxication and, accordingly, all its neuropsychiatric manifestations.

DISCUSSION

The increased stressogenic conditions, combined with the growing variety and availability of alcoholic beverages, contribute to an increase in their consumption. As mentioned above, the main medical problem of the alcohol consumption is post-intoxication neuropsychiatric and somatic symptoms (a post-intoxication state), and not the consequences of chronic ethanol effects on the body, such as cardiomyopathy, cirrhosis, or mental illnesses. It is the pathogenetic symptom complex, interpreted as "an alcohol withdrawal syndrome" that causes significant economic and medical consequences for a modern society. Available and effective treatments (infusion therapy, despite the effectiveness and safety, is available to a minimum number of people) for the relief of this condition are currently limited and require significant expansion. The key factor in the pathogenetic action of ethanol is a violation of metabolism of its main derivative, acetaldehyde, which occurs as a result of depletion of substrates involved in oxidoreductions occurring in hepatocytes and largely dependent on functioning of their antioxidant system. The main antioxidant of hepatocytes is glutathione, which does not only protects the cell from free radicals, but also determines the redox characteristics of the intracellular environment, and the intake of acetylcysteine leads to the replenishment of glutathione reserves. In previous studies, the authors have established that both preliminary and therapeutic administration of acetylcysteine prevents or promotes the recovery of the experimental animals after acute alcohol poisoning. In these studies, acetylcysteine was administered at the dose of 1 g/kg, which limits its clinical use. The authors tried to reduce the effective dose of acetylcysteine by potentiating its action with other substances that can affect alcohol metabolism or add additional positive effects (psychostimulating).

Caffeine, having an activating and attention-maintaining effect, is found (sometimes in combination with related substances) in energy drinks and is often consumed with alcohol. The animal studies have shown the ability of methylxanthines, including caffeine, to modulate the psychopharmacological effects of certain psychoactive substances, such as amphetamine [11], nicotine [12], cocaine [13] and ethanol [6].

There is a widespread belief that caffeine can counteract the intoxicating effects of alcohol. Caffeine indirectly modulates the activity of many neurotransmitters and neuromodulators, including dopamine, acetylcho-

line, or glutamate, in various areas of the brain. The main effect of caffeine is associated with an antagonistic activity against adenosine receptors (A_1 and A_{2A}) in the central nervous system [6, 14]. Ethanol increases the extracellular levels of adenosine by increasing its synthesis (which is facilitated by acetate formed during metabolism of ethanol), secretion, and a decrease in absorption, as a result of dysfunction of the nucleoside transporter [15]. Thus, caffeine can have a stimulating effect in post-alcoholic intoxication conditions.

In the experimental studies, ethanol and caffeine affected a locomotor activity with a bell-curve dependence of the observed effect from the following doses: low doses are stimulating, while high doses are depressive [16]. Caffeine can affect a locomotor activity in a two-phase manner [17]. At low doses, an acute administration of caffeine can enhance the stimulatory effect of ethanol. However, when the dose of caffeine or ethanol is higher, there is a pronounced suppressive effect of the both substances. Low doses of caffeine reduce the discoordination effects of ethanol, while high doses enhance them.

Adenosine mediates the intoxicating effects of ethanol such as ataxia and sedation [15]. Adenosine agonists prolong the duration of sleep induced by high doses of ethanol, while its antagonists shorten it.

A combined administration of ethanol and caffeine has a neuroprotective effect on various models of brain damage [18, 19]. A single oral dose of caffeinol (a combination of 10 mg/kg caffeine and 0.65 g/kg alcohol) 15 minutes after a traumatic brain injury improved the performance of animals in the Morris water maze. These data are important because it is known that the risk of cardiovascular complications increases with the intake of large doses of alcohol and in a post-intoxication state. Caffeine prevents memory loss caused by high doses of ethanol. Given the potentially beneficial effects of caffeine, it was included in the study composition.

A taurine pre-administration slows down the ethanol-induced increase in acetaldehyde in rats and humans without affecting blood ethanol levels. In a clinical study, taurine was used at the dose of 20 mg/kg (1 hour before and 1 hour after ethanol), which led to a decrease in the level of acetaldehyde in the blood by one third [20].

In alcohol intoxication, taurine has a hepatoprotective effect: it reduces oxidative stress, TNF-α levels, and steatosis. Taurine had a positive effect on the adipocytes damaged by ethanol, it is anti-inflammatory and maintains the normal secretion of adiponectin [21]. Taurine supplementation (1% in drinking water) prevents the induction of hypertension in rats chronically administrated with ethanol (15% in drinking water) [22].

Taurine decreases the concentration of acetaldehyde in blood after the oral administration of ethanol [23]. In some models, taurine has anti-atherosclerotic and antihypertensive effects; limited clinical data indicate that it also exhibits antihypertensive activity [23], which is important given the damaging effect of high doses of ethanol on the cardiovascular system. Taurine has a stabilizing effect on platelets, which is expected to reduce the risk of myocardial infarction or stroke. Taurine has a positive inotropic effect in congestive heart failure [10]. In the central nervous system, taurine prevents the development of excitotoxicity, and a long-term intake of taurine reduces age-related memory decline in mice [24]. Thus, taurine was chosen as the third component of the combination.

Lipoic acid supports the optimal activity of aldehyde dehydrogenase 2 (ALDH-2), which has a protective effect against an oxidative stress, negatively affecting the efficiency of hepatic ALDH-2 and, accordingly, the metabolism of alcohol and acetaldehyde. Several studies in rats have shown that the administration of lipoic acid protects the stomach lining, liver and developing brain from the side effects of alcohol [8].

ALDG-2 provides the metabolism of acetaldehyde and physiologically acts as an antioxidant, it also participates in metabolism and, therefore, detoxifies certain toxic aldehydes such as 4-hydroxynonenal (4-HNE), decomposition products of oxidized membrane lipids. It is important for protecting mitochondria from the oxidative stress [25, 26]. Due to its antioxidant effect, a prophylactic course of the oral administration of lipoic acid at the dose of 100 mg/kg effectively prevents ethanol-induced damage to the gastric mucosa [8].

Lipoic acid and taurine have protective effect in ischemia-reperfusion models [8, 27]. Lipoic acid increases the activity of ALDH-2 in the heart, which has a protective effect in postischemic reperfusion [27] and may be a promising agent for the prevention of alcoholic cardiomyopathy [26].

As a metabolic intermediate, succinic acid is a dicarboxylic acid that plays several biological roles: it is involved in the production of ATP and, as a signaling molecule, reflects the state of cellular metabolism [28]. Succinate is produced in mitochondria through the tricarboxylic acid (TCA) cycle and functions in the cytoplasm as well as in the extracellular space, altering gene expression patterns, modulating epigenetic metabolism or hormone-like signaling [28], linking cellular metabolism, especially in terms of ATP production.

Succinate signaling often occurs in response to a state of hypoxia. In the liver, succinate serves as a paracrine signal secreted by anoxic hepatocytes, and acts on stellate cells via GPR91 [29]. This leads to the activation of stellate cells and fibrogenesis. Succinate plays a significant role in liver homeostasis [29] and alcohol metabolism. Thus, lipoic and succinic acids were included in the composition as the substances similar to acetylcysteine, they have a pronounced antioxidant action and some unique effects.

Pyridoxine and some of its derivatives (pyrithioxine and metadoxine) showed hepatoprotective and neuroprotective properties under intoxication conditions. Intramuscular administration of pyridoxine to rats

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(187 mg/kg) significantly reduced the lethality from ethanol and increased the LD_{50} of ethanol from 4.46 to 5.19 g/kg ($p < 0.005$) [30]. Metadoxine (pyridoxine pyrrolidone carboxylate) has a hepatoprotective effect at the doses of 200 and 400 mg/kg reducing oxidative stress and preventing depletion of reduced glutathione levels, when rats are intoxicated with alcohol, CCl_4 and paracetamol. [31]. Pyritinol (pyrithioxine) is a combination of two molecules of vitamin B_6 (pyridoxine) with a disulfide bond. Its pharmacokinetic profile affects the profile of the parent compound, which easily penetrates the blood-brain barrier and regulates the signaling pathways of various neurotransmitters, including acetylcholine, γ-aminobutyric acid, NMDA. It also acts as an antioxidant and anti-inflammatory agent and reduces plasma viscosity. For pyrithioxine (pyritinol), its ability to reduce the severity of post-intoxication state symptoms in people aged 21–40 years, was noted after taking alcohol [32]. In experimental studies, pyridoxine was assessed in a wide range of doses: while high doses can be dangerous with a chronic administration, a dose of 400 mg/kg was chosen for monotherapy, and 200 mg/kg in the combination was given when taking into consideration the acute nature of the reproducible pathology.

Thus, a combined administration of the substances described above, taken in twice lower doses than administered separately, effectively improved the restoration of psychoneurological functions, impaired by the acute administration of alcohol. It should be notified that the isolated intragastric administration of each of the substances, with the exception of acetylcysteine, did not contribute to a significant reduction in the symptoms of psychoneurological deficit after acute ethanol intoxication. This is obviously due to their insufficient dose, but when combining these substances, a significant potentiation of the protective effect was observed.

The main active ingredient of the composition, in the authors' opinion, is acetylcysteine, the antioxidant effects of which are potentiated by pyridoxine, lipoic and succinic acids, taurine, and the introduction of caffeine has a psychoactivating and stimulating effect. The developed combination can be used not only for the prevention and/or correction of post-intoxication neuropsychiatric disorders, but also in the conditions accompanied by antioxidant system disorders.

CONCLUSION

Under the conditions of acute alcohol intoxication, an oral administration of a combination of acetylcysteine with taurine, caffeine, pyridoxine, lipoic and succinic acids, improves the functional state of the antioxidant system, reduces the severity of destructive changes in the liver, and helps to reduce the severity of neurological deficits in the laboratory animals in a more pronounced way than the substances, administered separately. The obtained results indicate that the use of the studied combination is promising in order to eliminate the severity of the post-intoxication state (hangover syndrome).

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHOR'S CONTRIBUTION

D.V. Kurkin – research idea and planning, article writing; E.I. Morkovin – participation in the development of the research design, correction of the article, translation; N.A. Osadchenko – carrying out biochemical research; D.A. Bakulin – modeling of pathology, assessment of neurological deficit, preparation of the final version of the article; E.E. Abrosimova – article translation, conduct of behavioral tests; M.A. Dubrovina – conduct of behavioral tests; N.S. Kovalev – introduction of compounds, conduct of behavioral tests; Yu.V. Gorbunova – conduct of behavioral tests; I.N. Tyurenkov – general project management.

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