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THE ALLEVIATING EFFECT OF ZINC SULFATE ON THE LONG-TERM POSTNATAL ALCOHOL-INDUCED MORPHOLOGICAL, BEHAVIORAL AND METABOLIC IMPAIRMENTS IN THE OFFSPRING OF ALCOHOLIZED FEMALE ALBINO RATS

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ეთანოლის პრენატალური ინტოქსიკაციით გამოწვეული მორფოლოგიური, მეტაბოლური და ქცევითი პარამეტრების ცვლილებების გამოვლენა გვიან პოსტნატალურ პერიოდში და მათი კორექცია თუთიის სულფატის მეშვეობით

[1]ნეიროტოქსიკოლოგიის ლაბორატორია, ივანე ბერიტაშვილის ექსპერიმენტული ბიომედიცინის ცენტრი, თბილისი, საქართველო; [2]ფიზიკის, ბიოფიზიკის, ბიომექანიკის და ინფორმაციული ტექნოლოგიების დეპარტამენტი, თბილისის სახელმწიფო სამედიცინო უნივერსიტეტი, საქართველო

რეზიუმე

ჩვენი კვლევის მიზანს წარმოადგენდა ეთანოლით პრენატალური ინტოქსიკაციისას მორფოლოგიური, მეტაბოლური და ქცევითი პარამეტრების ცვლილებების გამოვლენა გვიან პოსტნატალურ პერიოდში და მათი კორექცია თუთიის სულფატის მეშვეობით. ლიმბური სისტემის ქერქულ და ქერქქვეშა სტრუქტურებში განისაზღვრა ნეირონებისა და გლიის უჯრედების საშუალო რაოდენობა, შეფასდა სივრცითი მეხსიერების ცვლილება და ოქსიდაციური სტრესის ინდექსი.

მიღებული შედეგების საფუძველზე შეიძლება დავასკვნათ, რომ ცხოველთა ჯგუფში, რომელთა დედა იღებდა ალკოჰოლს, პოსტნატალური პერიოდის მოგვიანებით ვადაზე შენარჩუნებულია ალკოჰოლის მოქმედებით გამოხატული ცვლილებები, რაც ვლინდება ლიმბური სისტემის ქერქულ და ქერქქვეშა სტრუქტურებში ნეირონებისა და გლიის უჯრედების რაოდენობის შემცირებით, შესაბამისად დასწავლისა და მეხსიერების პროცესის ცვლილებით, ასევე ოქსიდაციური სტრესის ინდექსის მომატებით. თუთიის სულფატის ანტიოქსიდანტური ეფექტი ვლინდება ოქსიდაციური სტრესის დონის შემცირებით, დასწავლის პროცესის გაუმჯობესებით და უჯრედების რაოდენობის შემცირებით, მაგრამ აღსანიშნავია, რომ იგი სრულად ვერ ახდენს ალკოჰოლით გამოწვეული ცვლილებების პრევენციას.

Introduction. One of the most severe consequences of prenatal ethanol exposure is damage to the developing central nervous system, which is manifested by long-term cognitive and behavioral deficits in the offspring [1,2]. Ethanol can affect the neurochemical and cellular components of the developing brain and interfere with all stages of brain development. The extent of damage depends on the dose of ethanol consumed and the duration of exposure [3,4,5]. Ethanol's toxic effects are not uniform, and some brain regions are more vulnerable to its exposure. At the cellular level, ethanol disrupts the development process by generating reactive oxygen species (ROS), leading to an imbalance in the intracellular redox state and an increase in oxidative stress. This interference can affect cell division, proliferation, differentiation, and migration in structures of the limbic system [6,7,8,9]. The mechanisms underlying ethanol-induced changes in hippocampal functions are highly complex, and ethanol-induced hippocampus-dependent decline in spatial navigation and memory formation process is associated with a combination of changes in neuronal activity in the intrahippocampal circuit and extrahippocampal regions [10,11]. During the early postnatal period, ethanol-related CNS damage may be linked to oxidative stress processes or the insufficiency of antioxidants. Therefore, antioxidant treatment could potentially serve as a therapy for preventing or alleviating alcohol-induced brain damage [12,13]. Our previous studies showed that ethanol

intoxication, leading to the formation of FAS, causes inhibition of stem cell proliferation, violates the process of neuro- and glioblasts' migration, and axonal growth, and causes the development of oxidative stress in young rats. Zinc sulfate weakens the violations of neurogenesis, behavioral acts, NO signals, and the formation of radicals [14].

Our study **aimed** to assess morphological, behavioral, and metabolic changes in the offspring of female white rats given 15% ethanol solution during pregnancy and to address the possibility of correcting ethanol-induced impairments at long-term developmental consequences using the antioxidant Zinc sulfate.

Materials and Methods. The research involved three groups of white mongrel rats: Group 1 was the 6-month-old intact rats (7 animals). Group 2 - was 6-month-old offsprings of females, who consumed a 15% ethanol solution during pregnancy, and group 3 was 6-month-old offsprings of alcoholized females given Zinc sulfate (5 mg/kg) in their morning feed.

Histology. The total numbers of nerve cells were counted in the cortical and subcortical structures of the limbic system (Cingulate Gyrus, Entorhinal cortex, medial and lateral nuclei of Septum). Serial paraffin sections of thickness 10 μm were stained with Cresyl Violet. Cell count was carried out with the eyepiece micrometer grid at a magnification of 10x40 by the light microscope (Amplival Zeiss, Germany).

Behavioral tests. In a study by Mitagvaria [15], spatial learning and memory formation were assessed using an elevated multiway labyrinth. The labyrinth consisted of 10 platforms (40×10 cm) fixed at a height of 25 cm. Animals were motivated to reach a box nest located at the end, serving as an escape from ethologically negative conditions. We used numerical data analysis to help us estimate the learning process's dynamics and outcomes. Successfully navigating the maze in 50-60 seconds without errors and achieving automatic behavior were the criteria for completing the learning process.

Assessment of Oxidative Damage. Oxidative stress was assessed using the FRAS5 photometric system. The levels of d-ROMs (1U. Carr=0.08 mg H2O2/dl), PAT (1 U.Cor =1.4 mcmol/L ascorbic acid), OBRI, and OSI were determined.

Statistical analysis. Statistical analysis was performed using analyses of variants (ANOVA).

Results. In our study, two groups of animals were observed to understand their spatial learning process in an elevated maze. The control group (group I) consisted of 6-month-old rats. On the first day of testing, they required minimal help from the experimenter and independently explored the maze. Their errors decreased, and their average passage time on the 7th day was reduced to 54 seconds. In contrast, animals in group 2 showed errors, and the time to reach the target increased throughout the experiment, with their average passage time on the 7th day increased to 75 seconds. Group 3 showed decreased errors and improved crossing times compared to group 2 (Fig.1).

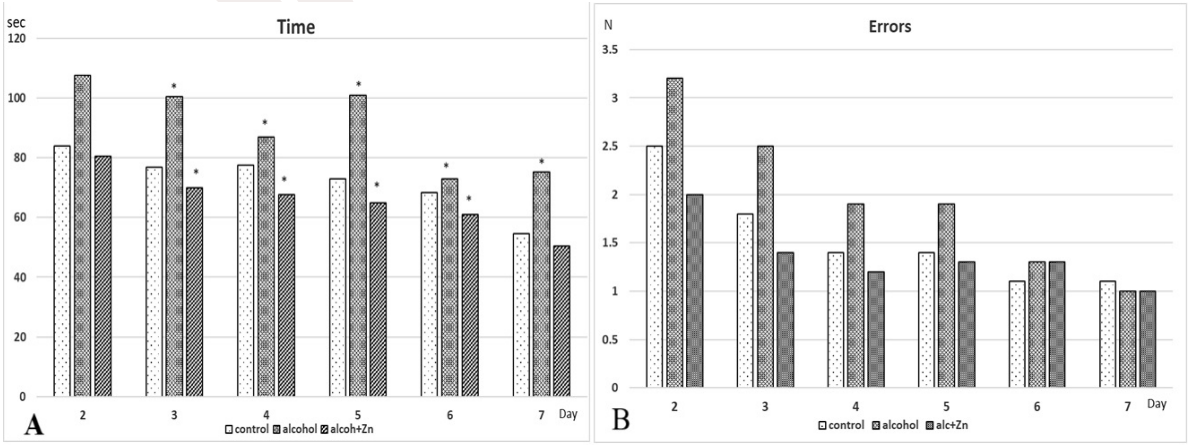

Fig.1 A - Elevated multi-way maze (Mean number of times for crossing the maze) * P < 0.05
B - Elevated multi-way maze (Mean number of errors in the maze)

Based on the assessment of oxidative stress parameters, it was found that the number of free radicals in the I and II Groups is within normal levels. However, in group 2 the number of antioxidants (PAT) has decreased, while the value of OBRI has remained unchanged. Additionally, the oxidative stress index (OSI) has increased in Group 2. In the third experimental group, the level of antioxidants (PAT) is within the normal range, but the oxidative stress index (OSI) is borderline (Table 3).

Discussion. The developing central nervous system (CNS) is affected by ethanol, leading to morphological changes in the cerebral cortex. These changes can disrupt the normal development of both cortical and subcortical brain structures by impairing proliferation and migration processes [16,17]. Alcohol consumption leads to oxidative stress, and excessive levels of reactive oxygen species (ROS) can damage cells and induce cell death [18]. In our study, six-month-old offspring of females who consumed a 15% ethanol solution during pregnancy exhibited increased oxidative stress levels compared to the control group (Table 3). This could explain the decreased number of neurons and glial cells in the cortical and subcortical structures of the limbic system.

Normal brain development and function require not only neurons but also glial cells, which support neuronal growth and development. Newly formed neurons must migrate to their final locations in the developing brain, a process that relies on the presence of radial glia [19]. However, prenatal alcohol exposure can cause radial glia to differentiate prematurely into astrocytes, resulting in a loss of radial glia that assists neuronal migration. This cell death or misdevelopment in the limbic system can lead to behavioral changes (Table 1,2).

Table 1. Number of neurons. Group 1 - the 6-month-old intact rats. Group 2 - 6-month-old offsprings of females who consumed a 15% ethanol solution during pregnancy, and group 3 - 6-month-old offsprings of alcoholized females given Zinc sulfate (5 mg/kg). *P < 0.05, ** P < 0.01

Structures	Group 1	Group 2	Group 3
Cingulate gyrus	103.8 ± 3.5	94.9 ± 2.2*	100.6 ± 1.6
Entorhinal cortex	153 ± 2.9	140.1 ± 2.9**	140.2 ± 3.3*
Lateral septum	108.8 ± 2.3	93.1 ± 2.4**	99.1 ± 1.5**
Medial septum	94.0 ± 1.4	78.1 ± 1.6**	83.8 ± 1.3**
CA3	42.3 ± 0.8	39.2 ± 0.8*	40.9 ± 0.9
CA1	83.9 ± 1.0	82.9 ± 1.5	77.7 ± 0.9**
Dentata Gyrus	123.4 ± 1.8	118.9 ± 1.1*	113.9 ± 1.8**

Table 2. Number of glial cells. Group 1 - the 6-month-old intact rats. Group 2 - 6-month-old offsprings of females who consumed a 15% ethanol solution during pregnancy, and group 3 - 6-month-old offsprings of alcoholized females given Zinc sulfate. *P < 0.05, ** P < 0.01

Structures	Group 1	Group 2	Group 3
Cingulate gyrus	49.7 ± 0.9	48.2 ± 1.1	46.2 ± 0.9**
Entorhinal cortex	56.7 ± 2.1	53.7 ± 1.7	56.4 ± 1.1
Lateral septum	90.4 ± 1.4	50.1 ± 1.3**	70.4 ± 2.7**
Medial septum	81.2 ± 1.1	51.2 ± 1.2**	62.4 ± 1.7**
CA3	34.5 ± 0.8	29.9 ± 1.0**	35.5 ± 0.8
CA1	39.1 ± 1.0	29.5 ± 0.6**	40.9 ± 1.0

Results from the spatial learning and memory formation tests in an elevated multi-way maze revealed a relationship between morphological changes and impairments in the spatial learning process [20]. Zinc sulfate as an antioxidant showed improvements in the learning process, reduced cell death, and a lower oxidative stress index. Antioxidant treatment could potentially serve as a therapy for preventing or mitigating alcohol-induced brain damage, though it may not exhibit significant antioxidant activity in the case of long-term developmental consequences.

Table 3. Oxidative stress parameters. Group 1 - the 6-month-old intact rats. Group 2 - 6-month-old offsprings of females who consumed a 15% ethanol solution during pregnancy, and group 3 - 6-month-old offsprings of alcoholized females given Zinc sulfate.

	Group 1	Group 2	Group 3
d-ROMs	291	216	210
PAT	2658	2067	2305
OBRI	0.9	0.9	0.8
OSI	22	55	47

Conclusion: Based on the obtained results, it can be concluded that alcohol consumption induces long lasting changes during the postnatal period (up to 6 months). These changes are manifested through a reduction in the number of neurons and glial cells in the cortical and subcortical structures of the limbic system. This results in changes to learning and memory processes and the development of oxidative stress. Zinc sulfate acts as an antioxidant and reduces oxidative stress levels, leading to an improvement in the learning process and a decreased level of cell death. However, it is important to note that though Zinc Sulfate has been found to have some positive effects, it cannot completely reverse the changes caused by alcohol consumption.

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SUMMARY

Background: The present work aimed to assess morphological, behavioral, and metabolic changes in the offspring of female white rats given 15% ethanol solution during pregnancy and to address the possibility of correcting ethanol-induced impairments at long-term developmental consequences using the antioxidant Zinc Sulfate.

Methods: The research involved three groups of white mongrel rats: 1- 6-month-old intact rats (7 animals). Group 2 - was the 6-month-old offsprings of females, who consumed a 15% ethanol solution during pregnancy, and group 3 was 6-month-old offsprings of alcoholized females given Zinc sulfate (5 mg/kg) in their morning feed.

The total number of neurons in the limbic system's cortical and subcortical structures was counted. Spatial learning and memory formation were estimated in the elevated-type multiway labyrinth. Oxidative stress was measured using the FRAS5 photometric system.

Results: Alcohol consumption during pregnancy induces long-lasting changes during the postnatal period (up to 6 months). These changes are manifested through a reduction in the number of neurons and

glial cells in the cortical and subcortical structures of the limbic system. Zinc sulfate acts as an antioxidant and reduces oxidative stress levels, leading to an improvement in the learning process and a decreased level of cell death.

Conclusion: Zinc sulfate acts as an antioxidant and reduces oxidative stress levels, leading to an improvement in the learning process and a decreased level of cell death. However, it cannot completely reverse the changes caused by alcohol consumption.

Keywords: Prenatal alcohol exposure, limbic system, oxidative stress, zinc sulfate