ORIGINAL ARTICLE

INDICATORS OF OXIDATIVE STRESS IN THE ORGANS OF THE REPRODUCTIVE SYSTEM OF RATS UNDER CONDITIONS OF LONG-TERM ETHANOL EXPOSURE

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ABSTRACT

Aim: To investigate changes in oxidative stress indicators in rats under conditions of long-term ethanol exposure.

Materials and Methods: We studied the effect of prolonged exposure to ethanol on the activity of free radical processes in the gonads of rats of both sexes. Experimental animals were divided into 2 groups: Group I (control group) rats, which were injected with distilled water orally for 28 days; II group – which for 28 days were injected intragastrically with a 30% ethanol solution at the rate of 2ml/100g of body weight once a day.

Results: The conducted experimental studies showed that the level of free radicals increases in animals that were injected with ethanol for 28 days, as indicated by an increase in the ROS index by 1.6 times in males and 1.7 times in females. Lipid peroxidation processes are also activated, as evidenced by an increase in the level of initial products of the lipoperoxidation chain – HPL by 54.6%, intermediate products – TBP by 57.9% and final products – SHO – by 80.3% in males and by 68.2%, respectively , 80.9% and 84.6% in females. Along with this, the activity of oxidative modification of proteins in the organs of the reproductive system of the experimental group of animals also increased. In particular, the level of OMP370 in the testes of male rats increased by 89.1%, and OMP430 increased by 56.4% from the level of animals that were not given ethanol. In the ovaries of female rats, the level of OMP370 increased by 112.%, and OMP430 increased by 60.7% from the level of animals without simulating ethanol intoxication. Therefore, we were able to establish significant changes and disturbances in the processes of free radical oxidation in the organs of the reproductive system of animals under the toxic effect of ethanol, which can negatively affect the reproductive function and quality of offspring in animals that were simulated ethanol intoxication.

Conclusions: In animals of both sexes, under the conditions of chronic ethanol exposure, there is an increase in free radical oxidation indicators, namely ROS, lipid peroxidation, oxidative modification of proteins, and the growth indicators in females are more significant than in males. This indicates a negative effect of chronic ethanol intoxication on the state of the membrane structures of germ cells, with DNA damage, which can lead to a violation of the function of the genital organs.

KEY WORDS: ethanol, free radical oxidation, lipid peroxidation, oxidative modification of proteins, oxidative stress, reproductive system

Pol Merkur Lek,2024;52(5):500-504. doi: 10.36740/Merkur202405104

INTRODUCTION

Alcoholism is a significant medical and social problem worldwide. In recent years, there has been an intensification of scientific research characterized by a comprehensive approach to addressing the issue of alcohol-related damage to organs and systems, their diagnosis, and the selection of treatment methods [1].

Due to the toxic effects of ethanol, many systems and organs are affected as it influences a large number of vital processes in the body, thereby causing disturbances in metabolic processes [2].

A particular issue is the impact of excessive ethanol consumption on the reproductive system. Several studies by foreign researchers emphasize that alcohol is one of the main factors of male and female infertility [3]. The literature indicates that excessive ethanol consumption leads to disruptions in the functioning of key metabolic pathways in the human body [3, 4]. Ethanol metabolism occurs through two main mechanisms: oxidative (primarily in the liver) and non-oxidative (in other tissues) [4]. The primary metabolic pathway of ethanol is its oxidation involving cytoplasmic alcohol dehydrogenase (ADH). This reaction results in the formation of an intermediate product—acetaldehyde, which is toxic and highly reactive. The reaction also results in the reduction of NAD+ to NADH. Consequently, a high concentration of reducing equivalents accumulates in the cytoplasm [5]. However, the major role in ethanol oxidation to acetaldehyde is played by the cytochrome P-450 system (CYP), including its isoenzymes CYP2E1, 1A2, and 3A4, and catalase [6]. Moreover, in conditions of chronic ethanol consumption, cytochrome P450 isoenzymes play a major role in its metabolism. The conversion of ethanol by CYP2E1 is accompanied by the formation of reactive oxygen species (ROS), such as hydroxyethyl, superoxide dismutase, and

hydroxyl radicals. These ROS are characterized by short lifespans, instability, and active interactions with cellular molecules [7].

As noted by most researchers, it is difficult to identify a single mechanism that explains all the effects of alcohol on the body or specific organs [2, 3, 4]. Many different mechanisms work in concert and reflect the spectrum of the body's response to numerous direct and indirect effects of ethanol. One factor that is thought to play a central role in the development of alcohol-induced cellular damage is excessive production of free radicals and oxidative stress. This is believed to be the result of a combination of impaired antioxidant defense and excessive ROS production by the mitochondrial electron transport chain, induced by ethanol CYP2E1 and activated phagocytes [6, 8, 9].

Given that the membranes of reproductive organs are rich in unsaturated fatty acids, which are susceptible to oxidative damage, ROS may be involved in the development of gonadal dysfunction due to chronic alcohol consumption [10, 11, 12, 13].

AIM

To investigate changes in oxidative stress indicators in rats under conditions of long-term ethanol exposure.

MATERIALS AND METHODS

To study the effects of prolonged ethanol exposure on oxidative stress indicators, an experimental study was conducted on 48 sexually mature rats-24 males and 24 females, weighing 220±39 g, bred in the vivarium of I. Horbachevsky Ternopil National Medical University. The experiments were conducted in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1985, amendments made in 1998), General Ethical Principles of Animal Experiments approved by the First National Congress on Bioethics (Kyiv, 2001), and the Helsinki Declaration of the World Medical Association (2000) [14]. The Bioethics Committee of I. Horbachevsky Ternopil National Medical University, Ministry of Health of Ukraine, confirmed that there were no bioethical violations during this research. Animals for modeling experimental chronic alcohol intoxication were selected by free choice. In the cages where the animals were kept, alongside the water dispensers, dispensers with a 20% ethanol solution were placed, and the behavior of the animals was observed for 10 days. During the observation, animals that preferred the 20% alcohol were identified and marked, forming the experimental and control groups. Experimental animals that did not consume alcohol were discarded and not used in further research [15]. The experimental animals were divided into two groups: Group I, which was given distilled water intragastrically at a dose of 2 ml per 100 g of body weight for 28 days as a control, and Group II, which was given a 30% ethanol solution intragastrically at a dose of 2 ml per 100 g of body weight once daily for 28 days. The animals were kept under standard vivarium conditions with free access to food and water. Euthanasia of the animals was performed by exsanguination under thiopental anesthesia.

The testes in males and ovaries in females were decapsulated, placed in a 0.9% NaCl solution on ice, cut into small pieces, and homogenized, followed by centrifugation at 1000 g for 10 minutes. The supernatant was collected and used for analysis.

In the tissues of the testes (in males) and ovaries (in females), as well as in the blood serum of the animals, the concentrations of lipid hydroperoxides (LHP), thiobarbituric acid-reactive products (TBARS) [16], and Schiff bases [17] were determined. The content of aldehyde and ketone derivatives of neutral (OMB370) and basic (OMB430) nature in the reproductive organs of the animals was determined according to the methodology proposed by I.F. Meshchysyn [18]. The production of reactive oxygen species (ROS) in mononuclear leukocytes (MNL) was determined by flow cytometry using the Accuri C6 device with dichlorofluorescein diacetate (DCFH-DA) dye (Sigma Aldrich). The values of the studied parameter were expressed in arbitrary units (intensity of fluorescence per cell).

RESULTS

Analysis of the indicators of free radical oxidation in animals of different sexes revealed a higher level of ROS in the MNL blood as well as indicators characterizing the activity of lipid peroxidation and oxidative modification of proteins in females compared to males.

Modeling of ethanol intoxication was accompanied by a significant increase in indicators of free radical oxidation. In particular, in MNL blood of males (Table 1), the level of ROS was 59.9% higher than in animals without pathology. The indicator of initial lipid peroxidation products-lipid hydroperoxides-was 64.6% higher than in animals not subjected to alcohol intoxication, TBARS was 57.9% higher, and the final products-Schiff bases were 80.3% higher. Modeling of ethanol intoxication in female rats was accompanied by a more pronounced increase in free radical processes compared to males. Specifically, the level of ROS was 69.5% higher than in animals without pathology, exceeding the corresponding value in males by 1.3 times. The concentration of LHP in this sex group also increased to 168.2% of the level in animals not given ethanol and also exceeded the value in males by 7.4%. The increase in TBARS was similarly directed: the value was 180.9% of the level in animals without pathology and was 20.7% higher than in males. The same applies to Schiff bases—the level in animals with ethanol intoxication was 184.6% of the level in animals without pathology and 110.9% compared to males with ethanol intoxication.

Studies conducted in the reproductive organs of rats of both sexes (Table 2) showed similar directional but quantitatively more pronounced results. In particular, the concentration of LHP in the testes of rats with ethanol intoxication increased 1.9 times compared to animals without pathology. The concentration of intermediate products of lipid peroxidation – TBARS-active products increased by 66.5%, and final products (Schiff bases) by

Table 1. ROS Indicators in MNL Blood and Lipid Peroxidation in the Blood Serum of Rats with Ethanol Intoxication (M±m)

Animal Groups / Indicator		ROS, arb. units	LHP, arb. units/ml	TBARS, μmol/l	Schiff Bases, arb. units/ml
No Pathology	Males, n=12	0,334±0,011	4,24±0,12	3,52±0,11	0,076±0,010
	Females, n=12	0,361±0,12	4,47±0,09	3,71±0,12	0,082±5,8
Ethanol Intoxication	Males, n=12	0,543±0,015*	6,98±0,12*	5,56±0,14*	0,137±0,011*
	Females, n=12	0,612±0,014*#	7,52±0,15*	6,71±0,016*#	0,152±0,012*#

**Notes: Here and in the following tables: 1 – * indicates significant changes relative to the control group; 2 – # indicates significant changes in females relative to males.*

Table 2. Lipid Peroxidation Indicators in Testes and Ovaries of Rats with Ethanol Intoxication (M±m)

Animal Groups / Indicator		LHP, nmol/mg	TBARS, nmol/mg	Schiff Bases, nmol/mg
No Pathology	Males, n=12	4,24±0,12	3,52±0,11	0,076±0,010
	Females, n=12	4,52±0,13#	3,76±0,12	0,091±0,008#
Ethanol Intoxication	Males, n=12	7,97±0,15*	5,86±0,12*	0,127±0,011*
	Females, n=12	8,72±0,12*#	6,57±0,14*#	0,162±0,009*#

*Notes:1 – * indicates significant changes compared to animals without pathology; 2 – *# indicates significant changes in females compared to males.*

67.1%. The activity of lipid peroxidation processes in the ovaries of females increased even more intensively than in males. Specifically, the concentration of LHP in the ovarian homogenate was 192.9% of the level in animals not administered ethanol, which is 1.8 times higher than in males with ethanol intoxication. The concentration of TBARS increased by 2.1 times compared to animals without pathology and 1.5 times higher than in males with ethanol intoxication. The concentration of Schiff bases was 2.2 times higher compared to animals without pathology and 1.8 times higher than in males with ethanol intoxication.

In studying oxidative modification of proteins, it is evident that in animals not subjected to ethanol intoxication, the baseline level of oxidative modification of proteins (OMP) in female rats is higher than in males. Research on the activity of oxidative modification of proteins has shown that ethanol intoxication affects not only lipids but also leads to protein peroxidation. Specifically, the concentration of OMP_{370} in male rats was 89.1% higher than in animals not subjected to intoxication, while OMP_{430} was 56.4% higher. In female rats, the level of OMP_{370} increased 2.1 times, which is also 61.4% higher than in males. The level of OMP_{430} increased by 60.7%, which is 13.9% higher than in males (Table 3).

Thus, our results and data from several researchers indicate that ethanol administration can seriously disrupt the metabolism, structure, and functions of lipids, proteins, and nucleic acids both directly and through its metabolites. Reactive oxygen species (ROS) formed during ethanol oxidation are a major source of DNA damage, causing strand breaks, nucleotide removal, and various modifications of the organic bases of nucleotides [7, 19]. Ethanol-induced oxidative stress can also play a significant role in the damage of germ cell DNA, uncontrolled apoptosis, and disruption of the interactions between Sertoli cells and epithelial cells during spermatogenesis [12].

DISCUSSION

Given the prevalence of alcohol abuse and the contribution of oxidative stress to the development of pathological

Table 3. Indicators of aldehyde and ketone derivatives of neutral (OMP_{370}) and basic (OMP_{430}) nature in the reproductive organs of rats with ethanol intoxication, (M±m)

Animal Groups / Indicator		OMP_{370}, (μmol/g protein)	OMP_{430}, (μmol/g protein)
No Pathology	Males, n=12	0,83±0,11	0,55±0,08
	Females, n=12	0,94±0,13	0,61±0,11
Ethanol Intoxication	Males, n=12	1,57±0,13*	0,86±0,10*
	Females, n=12	1,92±0,11*#	0,98±0,10*#

*Notes:1 – * indicates significant changes compared to animals without pathology; 2 – *# indicates significant changes in females compared to males.*

conditions, studying oxidative stress indicators in the reproductive organs of rats under conditions of prolonged ethanol exposure is a current issue.

An imbalance between the formation of reactive oxygen species (ROS) and the cell's ability to neutralize them is characterized as oxidative stress and occurs in almost any pathological condition. The reactions converting ethanol to acetic acid are accompanied by increased ROS production.

Mechanisms by which oxidative stress causes ethanol-induced gonadotoxicity have been intensely studied for quite some time [8]. It should be noted that in the testes, ROS generation can be beneficial or even necessary in the complex process of proliferation and maturation of male germ cells through meiosis from diploid spermatogonia to mature haploid spermatozoa. Conversely, high doses and/or inadequate removal of ROS, caused by many mechanisms involved in ethanol metabolism, can be very dangerous, altering sensitive testicular molecules such as DNA, lipids, and proteins [8, 9, 10].

All types of stress are accompanied by a viola-tion of pro-antioxidant status, which is an important factor in the pathogenesis of stress-induced disorders, including reproductive disorders [20, 21]. On the other hand, oxidative stress may be one of the long-term negative consequences of chronic stress. Unfortunately, the few retrospective studies of this problem in humans leave the exact age at which they were exposed to early life stress unknown [22, 23, 24]. For the first time, it was found that chronic pubertal stress causes a state of oxidative stress in the testes in adult rats, which is confirmed by a 50% increase in the content of malonic dialde-hyde and diene conjugates in them. There are reports on delay of testicular maturation and decreased spermatogenic and androgenic testicular functions in adult rats submitted to immobilization-induced stress from prepuberty [25, 26, 27]. It is likely that the damaging effect of oxidative stress may cause disturbances in the generative and steroid-synthesizing function of the testicles.

CONCLUSIONS

Prolonged exposure to ethanol induces oxidative stress in rats of both sexes. However, females experience more pronounced changes in the parameters of lipid peroxidation, which is evident in a higher level of ROS in MNL blood, LHP, TBARS, and Schiff bases in the blood serum and tissues of reproductive organs compared to males.

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The work is a fragment of scientific research work of the Clinical Anatomy and Operative Surgery Department of the I. Horbachevsky Ternopil National Medical University "Structural and functional regularities of the course of adaptive and compensatory processes in organs and systems during surgical interventions on abdominal organs and chest cavity under the influence of toxic endogenous and exogenous factors" (registration number 0122 U 000031).

CONFLICT OF INTEREST

The Authors declare no conflict of interest

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RECEIVED: 15.06.2024
ACCEPTED: 08.09.2024