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GENDER DIFFERENCES IN THE EFFECT OF ANTIULCER DRUGS AND PLACENTA CRYOEXTRACT ON THE INTENSITY OF LIPID PEROXIDATION AND THE ACTIVITY OF THE ANTIOXIDANT SYSTEM IN EXPERIMENTAL HEPATITIS WITH ETHANOL-INDUCED CIRRHOSIS

Illia V. Koshurba[1,2], Fedir V. Hladkykh[1,3], Mykola O. Chyzh[1], Mykhailo M. Marchenko[4], Yurii V. Koshurba[2], Volodymyr B. Hrishyn[5]

1 – Institute for Problems of Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine, Kharkiv, Ukraine
2 – Communal non-profit enterprise «Chernivtsi Regional Perinatal Center», Chernivtsi, Ukraine
3 – State Organization «Grigoriev Institute for Medical Radiology and Oncology of the National Academy of Medical Sciences of Ukraine», Kharkiv, Ukraine
4 – Yuriy Fedkovych Chernivtsi National University of the Ministry of Education and Science, Chernivtsi of Ukraine, Chernivtsi, Ukraine
5 – State Institution of Science «Research and Practical Center of Preventive and Clinical Medicine» State Administrative Department, Kyiv, Ukraine

Summary

Introduction. It is well recognized that drug metabolism products in the liver can induce oxidative stress and mitochondrial dysfunction, leading to the development of hepatocellular injury. As a potential agent capable of counteracting the hepatotoxic effects of drugs, we focused our attention on a domestic biotechnological preparation – cryopreserved placental extract (CPE).
The aim. To characterize gender differences in the effect of esomeprazole, clarithromycin, metronidazole (E/C/M), and CPE on the intensity of lipid peroxidation and the activity of the antioxidant system in tetrachloromethane (CCl_4) hepatitis with a background of ethanol-induced cirrhosis (ETCM).
Materials and methods. The study was conducted with varying levels of sex hormones on 112 male and female rats. Chronic ETCM was induced by administering a 50.0% oil solution of CCl4 at a dose of 8 ml/kg body weight of the animals twice a week, in combination with a 5.0% ethanol solution for drinking over a period of 45 days. The content of TBA-RP in liver homogenates was determined spectrophotometrically by the method described by Asakawa T. et al. Catalase activity in liver homogenates was determined spectrophotometrically according to the method of Korolyuk M. A. and co-authors.
Results. The most pronounced increase in lipid peroxidation processes was observed in females with chronic ETCM-induced liver damage and administration of antiulcer drugs following ovariectomy, resulting in a TBA-RS content of 36.1±2.79 μmol/kg of tissue. Administration of E/C/M in animals with chronic liver damage led to a suppression of the antioxidant system, as evidenced by a decrease in catalase activity in liver tissues.
Conclusion. The combined use of anti-ulcer drugs and CPE on the background of chronic ETCM mitigated the activation of lipid peroxidation processes, which was indicated by a statistically significant ($p < 0.001$) 2.7-fold lower content of TBA-RP in liver homogenates. Additionally, it was established that the administration of CPE was accompanied by a statistically significant increase in catalase activity in females, more prominently than in males. In females without changes in hormonal status, the introduction of CPE resulted in a growth ($p < 0.001$) of catalase activity by 75.0%, with the most significant increase observed in females after ovariectomy – catalase activity statistically significantly ($p < 0.001$) increased by 2.6 times compared to the indicators of females not administered with CPE. The administration of CPE in female rats without altering hormonal status was accompanied by a twofold ($p < 0.01$) increase in the antioxidant-prooxidant index compared to male rats, indicating more pronounced antioxidant properties of CPE in female rats.

Key words: **cryopreserved placenta extract, peptic ulcer disease, hepatitis, gender determinism, lipid peroxidation**

INTRODUCTION

The comorbidity of digestive system diseases represents a complex medical and social issue, significantly complicating the course of both primary and associated illnesses. The prevalence of peptic ulcer disease (PUD) in patients with liver cirrhosis is approximately 5.0-20.0%, compared to 2.0-4.0% in the general population [1]. It has been shown that patients with liver cirrhosis have a significantly higher risk of bleeding compared to the general population. According to Leontiadis G. I. et al. [2], the mortality rate of patients with ulcer bleeding in the context of liver diseases was 26.9%, compared to a mortality rate of 6.3% among those without liver diseases. Given the aforementioned information, adequate treatment of PUD in patients with liver diseases becomes an important prognostic factor for the course of both conditions.

The standard first-line therapy for peptic ulcer disease (PUD) is triple therapy, consisting of a proton pump inhibitor (PPI) and two antibiotics such as clarithromycin, amoxicillin, or metronidazole, administered for 7-14 days [3, 4, 5]. However, a challenge for effective ulcer treatment is the hepatotoxicity of antibacterial and acid-suppressive agents. Reactive chemical metabolites formed during drug metabolism in the liver can lead to hepatocellular damage due to oxidative stress and mitochondrial dysfunction, causing drug-induced liver injury [6].

According to the literature [7], rabeprazole and pantoprazole were found to more frequently induce fulminant hepatitis than other proton pump inhibitors (PPIs). Mechanisms involved in the hepatotoxicity of omeprazole PPI include the disruption of actin filaments, which may lead to cell lysis through changes in membrane transport pumps, as well as apoptosis triggered by the activation of tumor necrosis factor α [8].

Individual variations in drug metabolism and pharmacokinetics pose a significant challenge in modern clinical pharmacology. Considering the pivotal role of hepatic enzymes in regulating the pharmacological and biological activity of drugs, it's crucial to understand the regulatory peculiarities that lead to individual differences in their expression. Gender differences in drug metabolism are a major contributor to sex-dependent pharmacokinetics and reflect fundamental gender-related variations in the expression of hepatic enzymes involved in the metabolism of drugs, steroids, fatty acids, and chemicals, including cytochrome P450 enzymes, sulfotransferases, glutathione transferases, and glucuronosyltransferases [9].

As a potential agent capable of mitigating the hepatotoxic effects of drugs while exhibiting its own anti-ulcer activity, our attention was drawn to cryopreserved placental extract (CPE). Previous research has shown that the administration of CPE leads to the attenuation of lipid peroxidation (LPO) activation in experimental hepatitis and ulcerogenesis [10, 11]. The cryopreserved human placental tissue preparation was initially developed by scientists from the Institute for Problems of Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine (hereinafter referred to as IPC&C NAS of Ukraine). They designed and implemented a methodology for its prolonged storage in a low-temperature environment [11].

The placenta functions as a natural «depot» and a producer of a wide range of biologically active substances (Table 1), which support the growth and development of the fetus during intrauterine development. It facilitates trophic processes and protein synthesis, gas exchange, hormone secretion, blood pressure regulation, blood clotting, detoxification, metabolite elimination, deposition of biologically active substances, immune regulation, regulation of lipid peroxidation, and more [12].

Technology of Obtaining CPE. Placenta donors are healthy parturients who must undergo similar screening as blood donors. The placenta is harvested after cesarean section. The preparations are mandatory tested for prenatal infections, syphilis, human immunodeficiency virus, hepatitis A, B, C, cytomegalovirus infection. Before cryopreservation, the placenta is washed of blood, fragmented, the amniotic membrane is separated and placed in 0.2 liters of 0.9% saline solution, 250 mg of kanamycin, and 4 ml of dimexide. Fragments of placental tissue are placed in a flask with 0.5 liters of 0.9% NaCl solution. The flask is shaken for 1-2 minutes, the supernatant is drained, and fresh physiological solution is added. This procedure is repeated 5-6 times. To the dispersed tissue, add 0.9% NaCl solution (1:2), incubate for a day at 4 °C and centrifuge for 15-20 minutes at 4000 rpm. The obtained supernatant is filtered through Millipore filters (diameter 0.22 μm), packaged in transparent glass ampoules of 1.8 ml and stored in liquid nitrogen [10, 11].

The aim of the study is to characterize gender differences in the impact of esomeprazole, clarithromycin, metronidazole, and placental extract on the intensity of lipid peroxidation and the activity of the antioxidant system in carbon tetrachloride (CCl_4)-induced hepatitis with concomitant ethanol-induced cirrhosis.

MATERIALS AND METHODS

The study was conducted at the Department of Experimental Cryomedicine at the Institute for Problems of Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine, and the Educational and Scientific Institute of Biology, Chemistry, and Bioresources at Yuriy Fedkovych Chernivtsi National University under the Ministry of Education and Science of Ukraine. The hepatotropic effects of placental extract (CPE) and the three-component anti-ulcer therapy were investigated on 112 male and female rats weighing 200-220 g, divided into **4 groups** of 28 animals each: **Group I (males)** and **Group III (females)** – rats with

CCl_4-induced hepatitis and background ethanol-induced liver cirrhosis, receiving daily intragastric administration of esomeprazole (50 mg/kg), clarithromycin (91 mg/kg), and metronidazole (91 mg/kg) for 7 days [14, 15, 16].

Table 1

Biologically active substances contained in cryopreserved placental extract [10, 11]

Name of the biologically active substance	Characteristic	Content
α-fetoprotein	Activator (or inhibitor) of growth of embryonic, transformed, and activated immune-competent cells	429 ± 75 mMol/ ml
Chorionic gonadotropin	Immune system activator, stimulates the production of steroid hormones (testosterone and estradiol)	26.8 ± 8 mMol/ ml
Estradiol	Reproductive function, cardioprotective effect	755 ± 48 pMol/ ml
Progesterone	Reproductive function, cardioprotective effect	226 ± 110 nMol/ml
Prolactin	Influence on the development of secondary sexual characteristics, erythropoietic action, regulation of lipid metabolism	705 ± 129 mMol/ ml
α-fertility microglobulin	Preparation for pregnancy, conception process, normal development of fetoplacental unit	1470 ± 173 ng/ml
Lactoferrin	Stimulation of lactation	1270 ± 223 ng/ml
Somatotropic hormone	Growth hormone, anabolic effect	5.64 ng/ml
Luteinizing hormone	Pituitary hormone, secretion of estrogens, progesterone, testosterone	7.8 ± 1.9 IU/L
Follicle-stimulating hormone	Pituitary hormone, promotes maturation of follicles in the ovaries and spermatogenesis	7.1 ± 2.3 mIU/ ml
Testosterone	Differentiation and functioning of the reproductive system, anabolic effect	3.68 ± 1.06 nmol/ml
Thyrotropic hormone	Stimulation of thyroid gland function, immunomodulatory effect	291 ± 13 mIU/L
Triiodothyronine	Stimulation of metabolism, growth, and tissue differentiation, processes of reproduction, hematopoiesis	2.1 ± 0.6 pMol/ ml
Thyroxine	Stimulation of metabolism, growth, and tissue differentiation, processes of reproduction, hematopoiesis	5.6 ± 0.99 pMol/ ml
Cortisol	Metabolism of proteins, carbohydrates, fats, and nucleic acids	1392 ± 515 nMol/ml
Colony-stimulating factor	Proliferation of bone marrow cells	9.87 ng/ml
TNF-α	Inhibition of proliferation of cancer cells	84,5 pg/ml
IL-1β	Regulation of differentiation of pluripotent stem cells, immune-endocrine system	201,7 pg/ml
IL-4	Regulation of differentiation of pluripotent stem cells, immune-endocrine system	21,7 pg/ml
IL-6	Regulation of differentiation of pluripotent stem cells, immune-endocrine system	114.9 pg/ml
Total protein	Plastic function	76.5 ± 14
Proteins with a molecular weight of 20-100 kDa	Plastic function	70-80%
Proteins with a molecular weight below 20 kDa	Plastic function	20-30%

Group II (males) and **Group IV (females)** – rats with CCl_4-induced hepatitis and background ethanol-induced liver cirrhosis, receiving the same treatment as Group I and III rats for 7 days and then, from day 3 to day 7 of anti-ulcer treatment, intramuscular injections of CPE (0.16 mg/kg) were administered.

Each group had **four subgroups** with different hormonal status, consisting of 7 animals each:

– **Subgroup a)** – pseudooperated rats of both sexes undergoing replacement hormone therapy (excessive).

– **Subgroup b)** – pseudooperated rats of both sexes without changes in hormonal status (comparison group).

– **Subgroup c)** – rats of both sexes subjected to orchidectomy or ovariectomy.

– **Subgroup d)** – rats of both sexes that underwent gonadectomy and subsequently received replacement hormone therapy.

Animals were withdrawn from the experiment 24 hours after the last administration of CPE through cervical dislocation under inhalation anesthesia.

Modeling of Experimental Pathology. Chronic CCl4-induced hepatitis with background ethanol-induced liver cirrhosis *(hereinafter referred to as ETHM)* was induced by intragastric administration of a 50.0% oil solution of CCl_4 at a dose of 8 ml/kg body weight of animals twice a week, combined with a 5.0% ethanol solution for drinking, for a period of 45 days [17].

Modulation of the levels of sex hormones was achieved through surgical ovariectomy or testectomy

in female and male rats, respectively, following well-established methods [18, 19, 20]. The investigations were carried out 21 days after gonadectomy. For the non-castrated animals in the control groups, an incision was made on the anterior abdominal wall and the wound was sutured (sham-operated animals). Replacement and excessive hormone therapy were conducted for 14 days in males by subcutaneous injection of testosterone propionate («Pharmak», Ukraine) at a dose of 1 mg/kg once daily, and in females by intragastric administration of estradiol hemihydrate (Abbott Biologicals B. V., Netherlands) at a dose of 150 mg/kg [21, 22, 23]. The placental cryoextract (CPE) was obtained from the Interdepartmental Scientific Center for Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine, National Academy of Medical Sciences, and Ministry of Health of Ukraine, in the form of an ampouled preparation named «Cryocell – placental cryoextract».

Biochemical Research Methods. To obtain liver homogenate, the liver was perfused with an isotonic solution and homogenized at 3000 rpm (using a Teflon-glass homogenizer) in a buffer solution at a ratio of 1:10 (weight/volume: 250 mg tissue + 2.25 ml of a 1.15% solution), resulting in a 10.0% homogenate.

The content of thiobarbituric acid reactive products (TBA-RP) in liver homogenates was determined spectrophotometrically using the method described by Asakawa et al. [24]. The optical density was measured at a wavelength of $\lambda = 535$ nm, considering the molar extinction coefficient of the red-colored complex, which is 1.56 105 mol-1/cm-1. The results were expressed in micromoles per kilogram of tissue.

The activity of catalase in liver homogenates was determined spectrophotometrically using the method described by Korolyuk et al. [25]. The measurements were taken at a wavelength of $\lambda = 410$ nm. The assay involves comparing the experimental sample (0.1 ml of serum + 2 ml of 0.03% H_2O_2) with the control sample (0.1 ml of serum + 2 ml of H_2O).

The method is based on catalase's ability to decompose H_2O_2 and form a stable yellow-colored complex with ammonium molybdate (4.0% – 1.0 ml), which is added to stop the reaction between H_2O_2 and catalase. The catalase activity (CA) was calculated using the formula: CA = (Ek - Ed) V t k, where Ek is the absorbance of the control sample (2.0 ml H_2O); Ed is the absorbance of the experimental sample (2 ml of 0.03% H_2O_2), in absorbance units; V is the sample volume (0.1 ml); t is the incubation time; k is the molar extinction coefficient of H_2O_2 (22.2 10^3 $M^{-1}cm^{-1}$). The catalase activity was expressed in micromoles per kilogram of tissue.

The antioxidant-prooxidant index (API) was calculated using the formula: API = (Catalase Activity 100) / Thiobarbituric Acid Reactive Substances (TBA-RP) content [26].

The bioethical aspects of the research were conducted in accordance with the requirements of Good Laboratory Practice, as outlined in the guideline «Medicinal Products. Proper Laboratory Practice» approved by the Order of the Ministry of Health of Ukraine No. 95 dated February 16, 2009, and in compliance with the fundamental principles of the Council of Europe Convention on the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes of March 18, 1986, Directive 2010/63/EU of the European Parliament and of the Council of September 22, 2010, on the protection of animals used for scientific purposes, the Order of the Ministry of Health of Ukraine No. 944 dated December 14, 2009, «On Approval of the Procedure for Conducting Preclinical Studies of Medicinal Products and Expertise of Materials of Preclinical Studies of Medicinal Products», the Law of Ukraine No. 3447-IV dated February 21, 2006, «On the Protection of Animals from Cruel Treatment». The comprehensive research program was reviewed and approved by the Bioethics Committee at the Institute for Problems of Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine (extract from Protocol No. 2 dated January 3, 2022; extract from Protocol No. 5 dated November 22, 2022).

Statistical data analysis. The nature of distribution of variables in each group of the sample was assessed using the Shapiro-Wilk test. Homogeneity of variances was determined using Levene's test. For normally distributed independent variables, differences between groups were assessed pairwise using the Student's t-test and through analysis of variance (ANOVA) using the parametric F-test. In the case of non-normally distributed variables in at least one of the groups, differences between them were determined pairwise using the non-parametric Mann-Whitney rank-sum test and through rank-based analysis of variance using Kruskal-Wallis. Numeric data for normally distributed variables are presented as «M ± m» (M ± SE), where M is the mean, m (SE) is the standard error of the mean, and the 95% confidence interval (95% CI): 5% – 95%. For non-normally distributed data, values are presented as Me [LQ; UQ], where Me is the median, [LQ; UQ] represents the upper limit of the lower quartile and the lower limit of the upper quartile [27].

The work was carried out as a part of the departmental research project of the Institute for Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine titled «Features of Destructive-Inflammatory and Reparative Processes under the Influence of Low Temperatures and Cryoextracts of Mammalian Organs» (duration: 2022-2026, project leader – Acting Head of the Department of Experimental Cryomedicine of the Institute, Ph.D. in Medicine, Senior Researcher Chizh M. O.).

RESULTS AND DISCUSSION

The pathophysiological mechanisms of CCl_4-induced hepatotoxicity are primarily associated with the metabolic conversion into active oxygen forms, which induce oxidative stress and damage cellular macromolecules [28, 29]. Oxidative stress, characterized by an imbalance between endogenous reactive oxygen species (ROS) generation and antioxidant defense mechanisms, has recently been recognized as a key factor in the pathophysiological changes observed in a wide range of liver diseases, including subclinical hepatitis without jaundice, inflammatory necrotic hepatitis, liver cirrhosis, and hepatocellular carcinoma [30, 31]. A better understanding of the role of oxidative stress in these liver diseases could lead to the proper use of antioxidants as a therapeutic approach for liver disorders.

According to the results of the research on the influence of sex hormone levels on the hepatotropic effects of E/K/M and placental extract (CPE), it was determined that the highest activation of lipid peroxidation (LPO) processes in sham-operated rats without alteration of hormonal status was observed in females. This was indicated by a 44.8% higher ($p = 0.01$) level of thiobarbituric acid reactive products (TBA-RP) compared to the levels in males (Table 2). This suggests a greater activation of LPO processes in the liver of sham-operated females without hormonal status changes in the context of CCl_4-induced hepatotoxicity and the administration of E/K/M. These findings align with literature data indicating that females are more susceptible to drug-induced liver damage [32, 33].

The combined application of E/K/M and placental extract (CPE) in the context of CCl_4-induced hepatotoxicity (EHTM) led to a statistically significant reduction in the content of TBA-RP in liver homogenates in both males and females ($p < 0.001$). This reduction was approximately 2.2 times in males and 2.7 times in females compared to rats receiving only E/K/M under EHTM conditions (see Table 2). These findings indicate that CPE has the ability to mitigate the hepatotoxic effects of E/K/M under CCl_4-induced hepatotoxicity in rats, regardless of their hormonal levels, whether they are males or females.

The lowest content of TBA-RP in liver homogenates of female rats under CCl_4-induced hepatotoxicity (EHTM) was observed ($p < 0.01$ compared to females without hormonal status change) in the context of excessive hormone therapy with estradiol hemihydrate (20.6±1.17 μmol/kg tissue). This suggests a protective role of female sex hormones in the development of oxidative stress in liver tissues under EHTM conditions and the administration of E/K/M. This finding is consistent with the effectiveness of the investigated placental extract, as it contains a range of biologically active substances with hormone-like effects.

The highest activation of lipid peroxidation processes was observed in females to whom CCl_4 and E/K/M were administered after ovariectomy, with a TBA-RP content of 36.1±2.79 μmol/kg tissue. The combined application of E/K/M and placental extract in the context of EHTM reversed the activation of lipid peroxidation processes, as indicated by a significantly lower TBA-RP content in liver homogenates, reduced by 2.7 times ($p < 0.001$). The ability of the placental extract to mitigate EHTM and E/K/M-induced lipid peroxidation activation demonstrated comparable effectiveness in both females without hormonal status changes and ovariectomized females (Table 2).

The assessment of oxidative stress status in the liver homogenates of male rats in the context of EHTM and the administration of E/K/M revealed that the highest level of TBA-RP was observed in rats subjected to excessive testosterone propionate administration (25.9±2.96 μmol/kg tissue), while the lowest level was in castrated male rats. This suggests a prooxidant role of male sex hormones in the indicated model pathology. The study demonstrated that in male rats, CPE exhibited a lesser ability to modulate oxidative stress activation in liver tissues under EHTM and E/K/M administration conditions. Its most pronounced antioxidant activity was observed in male rats after orchiectomy, with a TBA-RP level lower by 57.1% ($p < 0.001$) compared to male rats with EHTM (Table 2).

The assessment of catalase activity in liver tissues under EHTM and E/K/M administration revealed that in male rats administered with testosterone propionate, the activity of this parameter was statistically significantly lower ($p < 0.01$) by 20.0%, compared to rats without a hormonal status change, and it amounted to 1.2 ± 0.07 μkat/kg tissue (Table 3). However, it was also found that in male rats with EHTM after orchiectomy, the highest level of catalase activity was observed (1.9 ± 0.09 μkat/kg tissue), which was statistically significantly higher ($p < 0.01$) by 26.7% compared to animals without a hormonal status change (1.5 ± 0.06 μkat/kg tissue). These changes indicate the ability of an excess of male sex hormones to suppress the antioxidant defense system (AOS).

The administration of CPE to male rats with EHTM, to which E/K/M was administered, resulted in an increase in catalase activity by 11.8% ($p = 0.09$) in castrated animals and by 53.4% ($p < 0.001$) in male rats without a hormonal status change (Table 3). It is worth noting that in male rats with EHTM, to which CPE was administered after orchiectomy, the highest level of catalase activity was observed, which was statistically significantly higher ($p < 0.001$) by 31.6% compared to similar animals without CPE administration and by 8.6% ($p = 0.14$) compared to animals without a hormonal status change.

Table 2

Effect of Placental Extract (CPE) and E/K/M on TBA-RP Content in Liver Homogenates under Chronic Ethanol-Tetrachloromethane-Induced Liver Injury in Male and Female Rats, μmol/kg tissue (M±SE (95% CI), n=112)

The investigated parameter, units of measurement.	№ of group	Males		Females	
		♂ I group	♂ II group	♀ III group	♀ IV group
		EHTM + E/K/M	EHTM + CPE + E/K/M	EHTM + E/K/M	EHTM + CPE + E/K/M
n		7	7	7	7
Without changing hormonal status	a	22,0±2,20 (95% CI: 17,7-26,3)	10,1±1,62 (95% CI: 7,0-13,3)	31,9±2,60 (95% CI: 26,8-37,0)	11,7±2,41 (95% CI: 7,0-16,4)
			$p_{1-2} < 0,001$	$p_{1-3} = 0,01$ $p_{2-3} < 0,001$	$p_{1-4 <} 0,01$ $p_{2-4} = 0,60$ $p_{3-4 <} 0,001$
Hormone therapy	b	25,9±2,96 (95% CI: 20,0-31,7)	13,3±1,90 (95% CI: 9,6-17,0)	20,6±1,17 (95% CI: 18,3-22,9)	10,3±1,02 (95% CI: 8,3-12,3)
		$p_{а-б} = 0,32$	$p_{а-б} = 0,23$ $p_{1-2} < 0,01$	$p_{а-б} < 0,01$ $p_{1-3} = 0,12$ $p_{2-3} < 0,001$	$p_{а-б} = 0,59$ $p_{1-4 <} 0,001$ $p_{2-4} = 0,19$ $p_{3-4 <} 0,001$
Gonadectomy with combined hormone therapy	c	20,3±2,21 (95% CI: 16,0-24,6)	9,7±1,19 (95% CI: 7,4-12,0)	33,9±1,28 (95% CI: 31,3-36,4)	12,9±1,12 (95% CI: 10,7-15,1)
		$p_{а-в} = 0,59$ $p_{б-в} = 0,16$	$p_{а-в} = 0,14$ $p_{б-в} = 0,14$ $p_{1-2} < 0,01$	$p_{а-в} = 0,50$ $p_{б-в} < 0,001$ $p_{1-3 <} 0,001$ $p_{2-3} < 0,001$	$p_{а-в} = 0,67$ $p_{б-в} = 0,12$ $p_{1-4} = 0,01$ $p_{2-4} = 0,08$ $p_{3-4 <} 0,001$
Gonadectomy	d	17,0±1,72 (95% CI: 13,6-20,4)	7,3±0,84 (95% CI: 5,6-8,9)	36,1±2,79 (95% CI: 30,7-41,6)	13,3±2,29 (95% CI: 8,8-17,8)
		$p_{а-г} = 0,10$ $p_{б-г} = 0,02$ $p_{в-г} = 0,26$	$p_{а-г} = 0,14$ $p_{б-г} = 0,01$ $p_{в-г} = 0,12$ $p_{1-2} < 0,001$	$p_{а-г} = 0,28$ $p_{б-г <} 0,001$ $p_{в-г} = 0,47$ $p_{1-3} = 0,001$ $p_{2-3} < 0,001$	$p_{а-г} = 0,64$ $p_{б-г} = 0,25$ $p_{в-г} = 0,87$ $p_{1-4} = 0,22$ $p_{2-4} = 0,03$ $p_{3-4 <} 0,001$

Notes. Indices $_{1, 2, 3, 4}$ indicate the group number according to the investigated substances compared; Indices a, b, c, d indicate the group number according to the hormonal status compared; p_{2-1} – level of statistical significance of the differences in indicators.

Table 3

Effect of CPE and E/K/M on catalase activity in liver homogenates in male and female rats with chronic ethanol-tetrachloromethane-induced liver injury, mkat/kg tissue (M±m (95% CI) or Me [LQ; UQ], n=112)

The investigated parameter, units of measurement.	№ of group	Males		Females	
		I group	**II** group	**III** group	**IV** group
		EHTM + E/K/M	EHTM + CPE + E/K/M	EHTM + E/K/M	EHTM + CPE + E/K/M
n		7	7	7	7
Without changing hormonal status	a	1,5±0,06 (95% CI:1,4-1,5)	2,3±0,13 (95% CI: 2,0-2,5)	1,2±0,10 (95% CI: 1,0-1,4)	2,1±0,08 (95% CI: 2,0-2,2)
			$p_{1-2} < 0,001$	$p_{1-3} = 0,02$ $p_{2-3} < 0,001$	$p_{1-4 <} 0,001$ $p_{2-4} = 0,3$ $p_{3-4 <} 0,001$
Hormone therapy	b	1,2±0,07 (95% CI: 1,0-1,3)	1,8±0,12 (95% CI: 1,6-2,1)	1,5±0,11 (95% CI: 1,2-1,7)	2,3±0,13 (95% CI: 2,0-2,5)
		$p_{а-б} < 0,01$	$p_{а-б} = 0,03$ $p_{1-2} < 0,001$	$p_{а-б} = 0,07$ $p_{1-3} = 0,06$ $p_{2-3} = 0,06$	$p_{а-б} = 0,24$ $p_{1-4 <} 0,001$ $p_{2-4} = 0,03$ $p_{3-4 <} 0,001$
Gonadectomy with combined hormone therapy	c	1,7±0,08 (95% CI: 1,5-1,8)	1,9±0,07 (95% CI: 1,7-2,0)	0,9±0,07 (95% CI: 0,7-1,0)	2,0±0,07 (95% CI: 1,9-2,2)
		$p_{а-в} = 0,07$ $p_{б-в} < 0,001$	$p_{а-в} = 0,03$ $p_{б-в} = 0,70$ $p_{1-2} = 0,09$	$p_{а-в} = 0,04$ $p_{б-в} < 0,01$ $p_{1-3 <} 0,001$ $p_{2-3} < 0,001$	$p_{а-в} = 0,51$ $p_{б-в} = 0,13$ $p_{1-4 <} 0,01$ $p_{2-4} = 0,2$ $p_{3-4 <} 0,001$
Gonadectomy	d	1,9±0,09 (95% CI: 1,7-2,0)	2,5±0,11 (95% CI: 2,3-2,7)	0,7±0,05 (95% CI: 0,6-0,8)	1,8±0,07 (95% CI: 1,6-1,9)
		$p_{а-г <} 0,01$ $p_{б-г <} 0,001$ $p_{в-г} = 0,14$	$p_{а-г} = 0,14$ $p_{б-г <} 0,01$ $p_{в-г <} 0,001$ $p_{1-2} < 0,001$	$p_{а-г <} 0,01$ $p_{б-г <} 0,001$ $p_{в-г <} 0,05$ $p_{1-3 <} 0,001$ $p_{2-3} < 0,001$	$p_{а-г <} 0,01$ $p_{б-г <} 0,01$ $p_{в-г} = 0,03$ $p_{1-4} = 0,38$ $p_{2-4 <} 0,001$ $p_{3-4 <} 0,001$

Notes. Indices $_{1, 2, 3, 4}$ indicate the group number based on the investigated substances, between the indicators of which a comparison was conducted; Indices $_{a, b, c, d}$ indicate the group number based on the hormonal status, between the indicators of which a comparison was conducted; p_{2-1} – level of statistical significance of the differences in indicators.

Analysis of catalase activity in the liver homogenates of female rats with CCl_4-induced hepatic injury treated with E/K/M revealed that the lowest activity of the investigated indicator was observed in females after ovariectomy – catalase activity was statistically significantly ($p < 0.01$) 41.6% lower compared to the indicators of females without hormonal changes (see Table 3). The highest catalase activity was found in female rats with CCl_4-induced hepatic injury treated with E/K/M and subjected to excess estradiol propionate hormone therapy – catalase activity was 1.5 ± 0.11 μkat/kg tissue, which was 25.0% higher ($p = 0.07$) than the corresponding indicators in females without hormonal changes.

The antioxidant properties of female sex hormones are fundamental to their ability to stimulate liver regeneration. It is well known that the liver possesses a unique ability for regeneration, which involves the restoration of organ architecture and mass within a relatively short period, even when a significant portion of the organ is damaged [34, 35, 36].

It's worth noting that the observed suppression of catalase activity in the liver homogenates of female rats is consistent with literature findings regarding the greater vulnerability of the hepatobiliary system to the hepatotoxic effects of xenobiotics in females [34, 35, 36, 37].

The administration of CPE to female rats with E/K/M in the presence of ETM resulted in a more pronounced increase in catalase activity in liver homogenates compared to males. It was found that the catalase activity showed the least increase in females undergoing excessive hormone therapy – the investigated indicators statistically significantly increased by 53.4% compared to females not receiving CPE (see Table 3). In females without a change in hormonal status, the introduction of CPE caused a significant ($p < 0.001$) increase in catalase activity by 75.0%. The most significant increase in these indicators was observed in females after ovariectomy – catalase activity statistically significantly increased by 2.6 times ($p < 0.001$) compared to females not receiving CPE.

The study of TBA-RP and catalase activity in liver homogenates allowed for an integrated assessment of the oxidative stress-antioxidant defense system (OS-AOS) through the use of the AOS/OS index. It was found that in rats without a change in hormonal status, the development of ETM and the administration of E/K/M led to a 1.9 times greater reduction in AOS/OS index ($p < 0.001$) in females compared to males (see Table 4). Excessive hormone therapy resulted in a more pronounced decrease in the AOS/OS index in males compared to females and had different directions of change compared to indicators in animals without a change in hormonal status. In male rats with ETHM, to whom E/K/M and testosterone propionate were administered, the AOS/OS index statistically significantly decreased by 36.2% ($p = 0.02$), while in female rats, the administration of estradiol hemihydrate led to a statistically significant ($p = 0.01$) increase in the AOS/OS index by 2.1 times compared to animals without a change in hormonal status. It is worth noting that in male rats with ETHM after testectomy and the administration of E/K/M, the highest AOS/OS index was observed (10.0 [9.4; 12.0]), indicating the pro-oxidant properties of male sex hormones.

Table 4

Influence of CPE and E/K/M on the values of the antioxidant-prooxidant index in liver homogenates on the background of chronic ethanol-tetrachloromethane-induced liver damage in male and female rats, (M±m (95% CI) or Median [LQ; UQ], n=112)

The investigated parameter, units of measurement.	№ of group	Males				Females			
		I group		II group		I group		II group	
		EHTM + E/K/M		EHTM + CPE + E/K/M		EHTM + E/K/M		EHTM + CPE + E/K/M	
n		7		7		7		7	
Without changing hormonal status	a	6,3 [5,7; 7,8]		26,3 [16,4; 3,6]		3,4 [2,8; 3,9]		26,3 [13,3; 27,1]	
					$p_{1-2} < 0,001$		$p_{1-3} < 0,01$ $p_{2-3} < 0,001$		$p_{1-4} < 0,01$ $p_{2-4} = 0,19$ $p_{3-4} < 0,001$
Hormone therapy	b	5,0±0,77 (95% CI: 3,5-6,5)		15,7±2,71 (95% CI: 10,4-21,0)		7,2±0,50 (95% CI: 6,2-8,2)		24,0±3,52 (95% CI: 17,1-30,9)	
		$p_{а-б} = 0,02$		$p_{а-б} = 0,07$	$p_{1-2} < 0,01$	$p_{а-б} = 0,01$	$p_{1-3} = 0,04$ $p_{2-3} = 0,02$	$p_{а-б} = 0,24$	$p_{1-4} < 0,01$ $p_{2-4} = 0,09$ $p_{3-4} < 0,01$
Gonadectomy with combined hormone therapy	c	8,2 [7,6; 8,9]		20,0 [15,4; 24,2]		2,6 [2,6; 2,9]		15,0 [14,0; 16,9]	
		$p_{а-в} = 0,11$ $p_{б-в} = 0,01$		$p_{а-в} = 0,48$ $p_{б-в} = 0,20$	$p_{1-2} < 0,01$	$p_{а-в} = 0,02$ $p_{б-в} < 0,001$	$p_{1-3} < 0,001$ $p_{2-3} < 0,001$	$p_{а-в} = 0,28$ $p_{б-в} < 0,05$	$p_{1-4} < 0,01$ $p_{2-4} = 0,13$ $p_{3-4} < 0,001$
Gonadectomy	d	10,0 [9,4; 12,0]		36,3 [27,8; 45,0]		2,2 [1,4; 2,5]		12,5 [9,5; 20,4]	
		$p_{а-г} < 0,01$ $p_{б-г} < 0,01$ $p_{в-г} = 0,03$		$p_{а-г} = 0,06$ $p_{б-г} < 0,001$ $p_{в-г} = 0,01$	$p_{1-2} < 0,001$	$p_{а-г} < 0,01$ $p_{б-г} < 0,001$ $p_{в-г} = 0,06$	$p_{1-3} = 0,001$ $p_{2-3} < 0,001$	$p_{а-г} = 0,11$ $p_{б-г} < 0,05$ $p_{в-г} = 0,20$	$p_{1-4} = 0,22$ $p_{2-4} < 0,01$ $p_{3-4} < 0,001$

Notes. Indices $_{1, 2, 3, 4}$ indicate the group number based on the investigated substances, between the indicators of which a comparison was conducted; Indices a, b, c, d indicate the group number based on the hormonal status, between the indicators of which a comparison was conducted; p2-1 – level of statistical significance of the differences in indicators.

The administration of CPE led to an increase in the AOS/OS index in both males and females, but the increase was more pronounced in females. In female rats without a change in hormonal status and with ETHM to whom E/K/M and CPE were administered, the AOS/OS index statistically significantly increased by 8.5 times ($p < 0.001$) compared to animals without CPE administration (see Table 4). Meanwhile, in male rats without a change in hormonal status, a similar indicator increased by only 4.2 times due to CPE administration. In female rats with ETHM to whom E/K/M and CPE were administered, a statistically significant ($p < 0.001$) comparable increase in the AOS/OS index was observed both after ovariectomy and in the context of hormone replacement therapy. The investigated index increased by 5.7 and 5.8 times, respectively (Table 4). At the same time, in male rats with ETHM and E/K/M administration, the use of CPE resulted in a more pronounced increase in the AOS/OS index after testectomy than after testectomy with hormone replacement therapy. Accordingly, the AOS/OS index increased by 3.6 times ($p < 0.01$) and 2.4 times ($p < 0.001$), respectively, compared to males without CPE administration (Table 4).

CONCLUSIONS:

1. The administration of esomeprazole, clarithromycin, and metronidazole in cases of chronic liver damage in animals was accompanied by the suppression of the antioxidant defense system (AOS), as indicated by the reduction in catalase activity in liver tissues. It was found that the administration of CPE was associated with a statistically significant increase in catalase activity in females more prominently than in males. In females without hormonal status changes, CPE administration led to a 75.0% increase ($p < 0.001$) in catalase activity, and the most significant increase was observed in females after ovariectomy – catalase activity statistically significantly increased by 2.6 times ($p < 0.001$) compared to females not receiving CPE.

2. The combined use of anti-ulcer drugs in the context of chronic ethanol-tetrachloromethane-induced liver damage exhibited gender-specific differences. In female rats without changes in hormonal status, the AOS index (API) was found to be 1.9 times lower ($p < 0.01$). The administration of CPE under similar experimental conditions in female rats without changes in hormonal status was accompanied by a twofold greater increase in API ($p < 0.01$) compared to male rats, indicating more pronounced antioxidant properties of CPE in female rats.

Prospects for further research. The given data serve as the basis for conducting in-depth studies of the mechanisms of the hepatoprotective activity of the pasenta cryoextract.

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Резюме

СТАТЕВІ ВІДМІННОСТІ ВПЛИВУ ПРОТИВИРАЗКОВИХ ЗАСОБІВ ТА КРІОЕКСТРАКТУ ПЛАЦЕНТИ НА ІНТЕНСИВНІСТЬ ПЕРЕКИСНОГО ОКИСЛЕННЯ ЛІПІДІВ ТА АКТИВНІСТЬ АНТИОКСИДАНТНОЇ СИСТЕМИ ЗА ЕКСПЕРИМЕНТАЛЬНОГО ГЕПАТИТУ ТА ЕТАНОЛ-ІНДУКОВАНОГО ЦИРОЗУ

Ілля В. Кошурба[1,2], Федір В. Гладких[1,3], Микола О. Чиж[1], Михайло М. Марченко[4], Юрій В. Кошурба[2], Володимир Б. Грішин[5]

1 – Інститут проблем кріобіології і кріомедицини Національної академії наук України, м. Харків, Україна
2 – Комунальне некомерційне підприємство «Чернівецький обласний перинатальний центр», м. Чернівці, Україна
3 – Державна установа «Інститут медичної радіології та онкології ім. С. П. Григор'єва Національної академії медичних наук України», м. Харків, Україна
4 – Чернівецький національний університет імені Юрія Федьковича Міністерства освіти і науки України, м. Чернівці, Україна
5 – Державна наукова установа «Науково-практичний центр профілактичної та клінічної медицини» Державного управління справами, м. Київ, Україна

Вступ. Добре відомо, що продукти метаболізму лікарських засобів у печінці можуть спричинювати окислювальний стрес та мітохондріальну дисфункцію, призводячи до розвитку гепатоцелюлярного ушкодження. У якості потенційного засобу, спроможного нівелювати гепатотоксичні ефекти лікарських засобів нашу увагу привернув вітчизняний біотехнологічний препарат – кріоекстракт плаценти (КЕП).
Мета роботи. Охарактеризувати статеві відмінності впливу езомепразолу, кларитроміцину і метронідазолу (Е/К/М) та КЕП на інтенсивність перекисного окислення ліпідів та активність антиоксидантної системи при тетрахлорметановому (CCl_4) гепатиті з фоновим етанол-індукованим цирозом (ЕТХМ).
Матеріали та методи. Дослідження проведено за різного вмісту статевих гормонів на 112 самцях та самицях щурів. Хронічний ЕТХМ відтворювали шляхом введення 50,0% олійного розчину CCl_4 у дозі 8 мл/кг маси тіла тварини двічі на тиждень в комбінації з 5,0% розчином етанолу для пиття впродовж 45 днів. Вміст реактантів з тіобарбітуровою кислотою (ТБК-РП) у гомогенатах печінки визначали спектрофотометрично за методом Asakawa T. et al. Активність каталази у гомогенатах печінки визначали спектрофотометрично за методом Королюка М. А. та співав.
Результати та обговорення. Найвиразніша активація процесів перекисного окислення ліпідів відмічена у самиць на тлі хронічного ЕТХМ-індукованого ураження печінки та введення противиразкових препаратів після овариектомії, у яких вміст ТБК-РП становив 36,1±2,79 мкмоль/кг тканини. Введення Е/К/М при хронічному ураженні печінки у тварин супроводжувалось пригніченням антиоксидантної системи, на що вказувало зниження активності каталази у тканинах печінки.
Висновки. Комбіноване застосування противиразкових препаратів та КЕП на тлі хронічного ЕТХМ нівелювало активацію процесів перекисного оксиення ліпідів, на що вказувало статистично вірогідно ($p < 0,001$) нижчий вміст ТБК-РП в гомогенатах печінки у 2,7 рази. Крім того, встановлено, що введення КЕП супроводжувалось статистично вірогідним зростанням активності каталази у самиць виразніше ніж у самців. Так у самиць без зміни гормонального статусу введення КЕП викликало зростання ($p < 0,001$) активності каталази на 75,0%, а найвиразніше вказаних показник збільшився у самиць після овариектомії – активність каталази статистично вірогідно ($p < 0,001$) зросла у 2,6 рази відносно показників самиць, яким КЕП не вводили. Введення КЕП у самиць щурів без зміни гормонального статусу супроводжувалось вдвічі більшим ($p < 0,01$) зростанням антиоксидантно-прооксидантного індексу, ніж у щурів-самців, що вказує на виразніші антиоксидантні властивості КЕП у щурів-самиць.

Ключові слова: **кріоекстракт плаценти, виразкова хвороба, гепатит, гендерний детермінізм, перекисне окислення ліпідів**