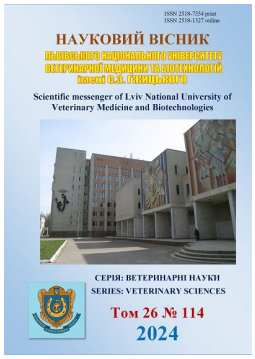

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The influence of "Butaintersyl" on the antioxidant status of rats under conditions of toxic damage caused by tetrachloromethane

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Tetrachloromethane (CCl_4) is a toxic chemical substance known in scientific research as a model for studying the damage of parenchymal liver cells. The mechanisms of CCl_4 toxicity include the activation of lipid peroxidation processes, the intensive formation of free radicals, and, as a result, the disruption of the pro-/antioxidant balance. This work aimed to study the effect of the liposomal drug "Butaintersyl" on the activity of the glutathione antioxidant protection system and the intensity of lipid peroxidation processes in the blood of rats under conditions of toxic damage caused by tetrachloromethane. The studies were conducted on white sexually mature young Wistar rats weighing 180–200 g, kept in the institute's vivarium of the State Scientific Research Control Institute of Veterinary Drugs and Feed Additives. In the blood of the rats, changes in the activity of glutathione peroxidase and the level of reduced glutathione were studied, as well as the levels of lipid peroxidation products: lipid hydroperoxides and TBA-active products. The development of oxidative stress in the first experimental group of rats was accompanied by the suppression of the glutathione antioxidant protection system, as evidenced by a decrease in blood glutathione peroxidase activity and the level of reduced glutathione. At the same time, the intoxicated animals showed an increase in lipid peroxidation processes, namely an increase in the blood levels of lipid hydroperoxides and TBA-active products throughout the study period. The studies showed that in cases of poisoning of various origins, it is advisable to use drugs that reduce the formation of reactive oxygen species and lipoperoxidation processes, exhibit antioxidant effects, and stabilize cell membranes. A special place among such drugs is occupied by the liposomal drug "Butaintersyl". This drug ensures the stabilization of biological membranes. The liposomal drug "Butaintersyl" contributed to suppressing lipid peroxidation processes and activating the antioxidant protection system, as evidenced by the high content of reduced glutathione and glutathione peroxidase activity. Due to its antioxidant properties, the drug positively affected the activity of membrane-dependent enzymes. It reduced the level of endogenous intoxication, allowing it to be recommended for inclusion in prevention schemes for toxic liver damage caused by chemical compounds.

Key words: *pharmacology, intoxication, tetrachloromethane, antioxidants, lipid peroxidation, glutathione.*

Вплив бутаінтерсилу на антиоксидантний статус організму щурів за умов токсичного ураження тетрахлорметаном

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Тетрахлорметан (CCl_4) є токсичною хімічною речовиною, відомою у наукових дослідженнях як модель для вивчення ураження паренхіматозних клітин печінки. Механізми токсичності CCl_4 включають активізацію процесів пероксидного окиснення ліпідів, інтенсивне утворення вільних радикалів і, в результаті, порушення балансу про-/антиоксидантів. Метою роботи було вивчити вплив ліпосомального препарату "Бутаінтерсил" на активність глутатіонової системи антиоксидантного захисту та інтенсивність процесів пероксидного окиснення ліпідів у крові щурів за умов токсичного ураження тетрахлорметаном. Дослідження проводилися на білих статево-зрілих молодих щурах-самцях лінії Вістар масою тіла 180–200 г, яких утримували в інститутському віварії Державного науково-дослідного контрольного інституту ветеринарних препаратів та кормових добавок. У крові щурів досліджували зміни активності глутатіонпероксидази та рівня відновленого глутатіону, а також рівні продуктів пероксидного окиснення ліпідів: гідроперекиси ліпідів та ТБК-активних продуктів. Розвиток оксидаційного стресу у щурів першої дослідної групи супроводжувався пригніченням активності глутатіонової системи антиоксидантного захисту, на що вказувало зниження у їх крові активності глутатіонпероксидази та рівня відновленого глутатіону. Водночас спостерігали в інтоксикованих тварин посилення процесів пероксидного окиснення ліпідів, а саме зростання у їх крові рівня гідроперекисів ліпідів та ТБК-активних продуктів протягом усього періоду досліджень. Дослідження показали, що при отруєннях різного походження варто використовувати препарати, які зменшують утворення активних форм кисню та процеси ліпопероксидації, а також проявляють антиоксидантну дію та стабілізують клітинні мембрани. Особливе місце серед таких препаратів займає ліпосомальний препарат "Бутаінтерсил". Даний препарат забезпечує стабілізацію біологічних мембран. Ліпосомальний препарат "Бутаінтерсил" сприяв пригніченню процесів пероксидного окиснення ліпідів та активізації системи антиоксидантного захисту, що підтверджується високим вмістом відновленого глутатіону та активністю глутатіонпероксидази. Завдяки антиоксидантним властивостям, препарат проявив позитивний вплив на активність мембранозалежних ензимів та зменшив рівень ендогенної інтоксикації, що дозволяє рекомендувати його до включення у схеми профілактики токсичних уражень печінки, викликаних хімічними сполуками.

Ключові слова: *фармакологія, інтоксикація, тетрахлорметан, антиоксиданти, пероксидне окиснення ліпідів; глутатіон.*

Introduction

The influence of anthropogenic factors on the mammalian body can lead to risks due to the activation of free radical reactions, tissue hypoxia, and impaired liver detoxification function (Kulyaba et al., 2019; Bashchenko et al., 2023; Mykhalko et al., 2023; Sidashova et al., 2024). Several circumstances determine the high sensitivity of the liver to chemical compounds. First, substances entering the body through the digestive tract primarily reach the liver, the first to encounter compounds entering the body's internal environment. Under these conditions, substances can disrupt liver cell functions up to their cytolysis through direct toxic effects (Gutyj et al., 2019; Koreneva et al., 2023; Verveha et al., 2023).

Second, the basis of toxic liver damage is the so-called "breakdown" of biotransformation systems. The animal body reacts to foreign chemical toxins and undergoes their biotransformation through various chemical reactions. According to the literature, biotransformation is the change in the physicochemical structure of substances that have entered from outside under the action of the body's enzymes. These processes mainly occur in the liver. The main direction of biotransformation is the conversion of lipophilic substances into water-soluble polar metabolites. Although biotransformation aims to eliminate the biological activity, i.e., the toxicity of the initial substances, highly reactive intermediate products with the initiation of free radical processes that can damage liver cells may occur during the metabolism of compounds (Lavryshyn & Gutyj, 2019; Gutyj et al., 2023; Sachuk et al., 2023).

Toxic liver lesions are divided into hepatotoxic substances that depend on the dose (truly toxic, directly toxic) and idiosyncratic. Dose-dependent liver damage depends on the action of a hepatotoxic substance at a specific dose. Usually, there are several hours, sometimes 1–2 days, between the substance's impact and the appearance of the first clinical symptoms. The substances or their metabolites can damage the liver. Hepatotoxic substances often affect other organs of the body as well.

Tetrachloromethane (CCl_4) is a toxic chemical known in scientific research as a model for studying the damage of parenchymal liver cells (Boll et al., 2001; Al-Rasheed et al., 2014). Structurally, it is a chlorinated hydrocarbon. The mechanisms of CCl_4 toxicity include the activation of lipid peroxidation processes, intensive formation of free radicals, and, as a result, disruption of the pro-/antioxidant balance (Deniz et al., 2019; Li et al., 2020; Martynov et al., 2023). Free radicals interact with antioxidant enzymes, such as sulfhydryl groups of GSH. This can lead to cell damage, loss of cellular ATP, hepatotoxic injuries,

calcium imbalance, inflammation, fibrosis, and other consequences (Ono et al., 2020; Dang et al., 2022; Gutyj et al., 2022).

It is known that CCl4 is metabolized in the liver with the help of cytochrome P450, converting it into the trichloromethyl radical (CCl_3). Subsequently, this radical reacts with nucleic acids, proteins, and lipids, thus affecting key cellular processes (Ernst et al., 2020; Li et al., 2021). As a result, lipid metabolism is disrupted, which can manifest as fatty dystrophy and steatosis, leading to a decrease in protein levels (Hamed et al., 2018).

The features of the molecular mechanisms of tetrachloromethane's action on subcellular membranes of hepatocytes allow using intoxication with this xenobiotic as a model of the molecular pathology of membrane structures (Ustuner et al., 2018; Pergel et al., 2019). This classic model is generally accepted for studying the mechanisms of hepatocyte membrane damage and searching for new treatment methods (Scholten et al., 2015).

Toxic substances burdening the human and animal body, causing intoxication of various origins, emphasize the need for finding new effective treatment methods in cases of multiple organ pathologies (Kovalchuk et al., 2019; Martyshuk et al., 2020; 2023; Gutyj et al., 2023). Studies have shown that in cases of poisoning of various origins, it is advisable to use drugs that reduce the formation of reactive oxygen species and lipoperoxidation processes, exhibit antioxidant effects, and stabilize cell membranes (Varkholiak et al., 2022). A special place among such drugs is occupied by “Butaintersyl”. This liposomal drug ensures the stabilization of biological membranes.

The aim of the study

The aim of the study – is to study the influence of the liposomal drug “Butaintersyl” on the antioxidant status of rats under conditions of oxidative stress.

Material and methods

The study was conducted on white sexually mature young Wistar rats weighing 180–200 g, kept in the institute's vivarium of the State Scientific Research Control Institute of Veterinary Drugs and Feed Additives. The rats were fed a balanced diet that included all necessary components throughout the experiment. They had unlimited access to drinking water from 0.2-liter glass drinkers.

The animals were divided into three groups of 10 animals each: the 1st group (C) – intact animals; the 2nd group (E_1) – rats affected by tetrachloromethane; the 3rd group (E_2) – rats affected by tetrachloromethane and treated with the liposomal drug “Butaintersyl” (Fig. 1). Toxic damage to the rats was induced by intramuscular injection of a 50 % oil solution of tetrachloromethane at a dose of 0.25 ml/100 g body weight on the first and third days of the study. The animals of group E_2 were additionally administered the liposomal drug “Butaintersyl” intramuscularly at a dose of 2 ml/kg body weight one hour before the tetrachloromethane injection on the first and third days of the study. This drug contains the following substances: butaphosphan, interferon, milled fruits of milk thistle, and vitamins A, E, and D_3.

Blood for biochemical and hematological studies was collected from the jugular vein of the rats under ether anesthesia on the second, fifth, tenth, and fourteenth days of the experiment.

The reaction with ammonium thiocyanate determined the content of lipid hydroperoxides in blood plasma according to the L. A. Romanova and method I. D. Stalnoy. The intensity of the color was measured colorimetrically at 480 nm. The content of lipid hydroperoxides was expressed in extinction units per 1 ml of blood plasma (Vlizlo, 2012).

The content of TBA-active products in blood plasma was determined by the method based on the reaction between malondialdehyde and thiobarbituric acid. The intensity of the color of the formed trimethine complex was measured colorimetrically at wavelengths 535 and 580 nm. Double measurement of absorption allows for the exclusion of absorption of colored TBA complexes by non-lipid substances. The content of TBA-active products was expressed in nmol of malondialdehyde per ml of blood plasma (Vlizlo, 2012).

The activity of glutathione peroxidase (EC 1.11.1.9) was determined in blood plasma by the rate of oxidation of the reduced form of glutathione in the presence of tert-butyl hydroperoxide in a color reaction with 5,5'-dithiobis-(2-nitrobenzoic) acid, measured at a wavelength of 412 nm (Vlizlo, 2012).

The reduced glutathione content in erythrocyte hemolysate was determined by E. Butler's method using Ellman's reagent and spectrophotometric measurement at a wavelength of 412 nm (Vlizlo, 2012).

All manipulations with animals were conducted by the European Convention for the Protection of Vertebrate Animals, and “Butaintersyl” used for Experimental and Scientific Purposes (Strasbourg, 1986).

Statistical processing of the data was performed using standard computer programs (Statistica Version 6, StatSoft, Inc., SPSS Statistics 17.0) with the determination of the arithmetic mean (M) and the standard error of the mean (m). Group differences were considered significant if the probability value was $p < 0.05$ (ANOVA).

Results and discussion

Free radical oxidation is a biochemical process involving the transformation of oxygen, lipids, nucleic acids, proteins, and other compounds under the action of free radicals, and lipid peroxidation (LPO) is one of its consequences (Gutyj et al., 2023). According to the data in Figure 1, after the intramuscular injection of tetrachloromethane into laboratory animals in the experimental group E_1, the level of lipid hydroperoxides (intermediate products of LPO) in their blood was significantly higher in all periods of the study compared to the control group rats. On the 2nd day of the experiment, the highest level of this indicator was recorded in the blood of rats in the E_1 group, which increased by 3.49 times compared to the control. Subsequently, the level of lipid hydroperoxides in the blood of rats in the E_1 group slightly decreased, reaching 0.631 ± 0.0138 extinction units per ml on the 10th

day. On the 14th day of the study, the level of intermediate lipid peroxidation products increased again, and compared to the control group animals, it was 2.91 times higher ($P < 0.001$).

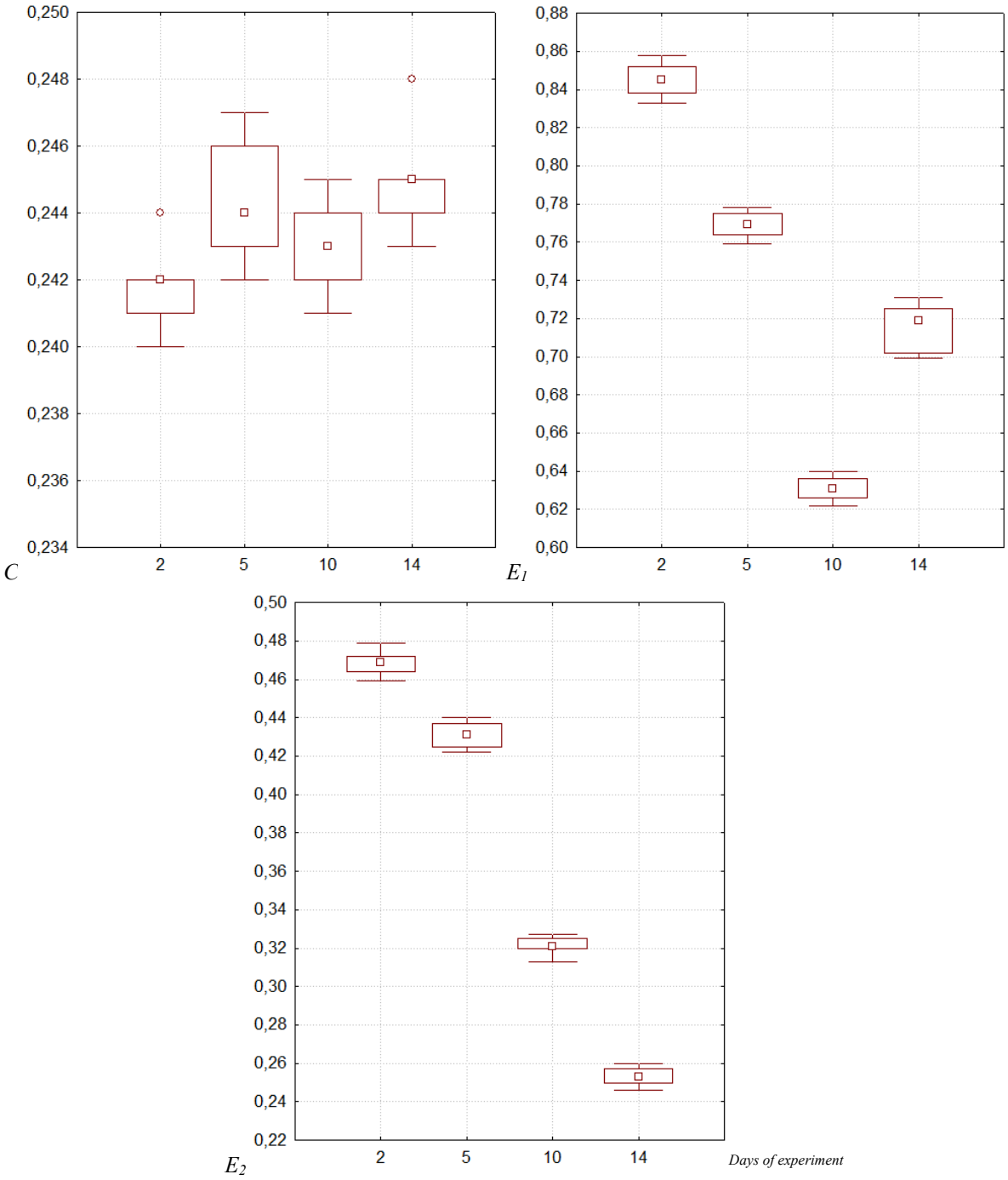

Fig. 1. The effect of butaintersil on the level of lipid hydroperoxides in the blood plasma of rats under conditions of oxidative stress, units/ml

The same differences were found in the blood plasma of rats when studying the final products of LPO, namely TBA-active products (Fig. 2). It was found that on the second day of the experiment, the content of TBA-active products in the blood of rats of experimental group E_1 increased by almost 1.89 times compared to the control. On the fifth day of the experiment, the content of TBA-active products in the blood plasma of rats injected with tetrachloromethane increased twice. On the 10th and 14th days of the experiment, a slight decrease in the content of the studied indicator was found in the blood plasma of rats of experimental group E_1; however, in comparison with the control group, it was found that this indicator was 1.93 and 1.96 times higher, respectively.

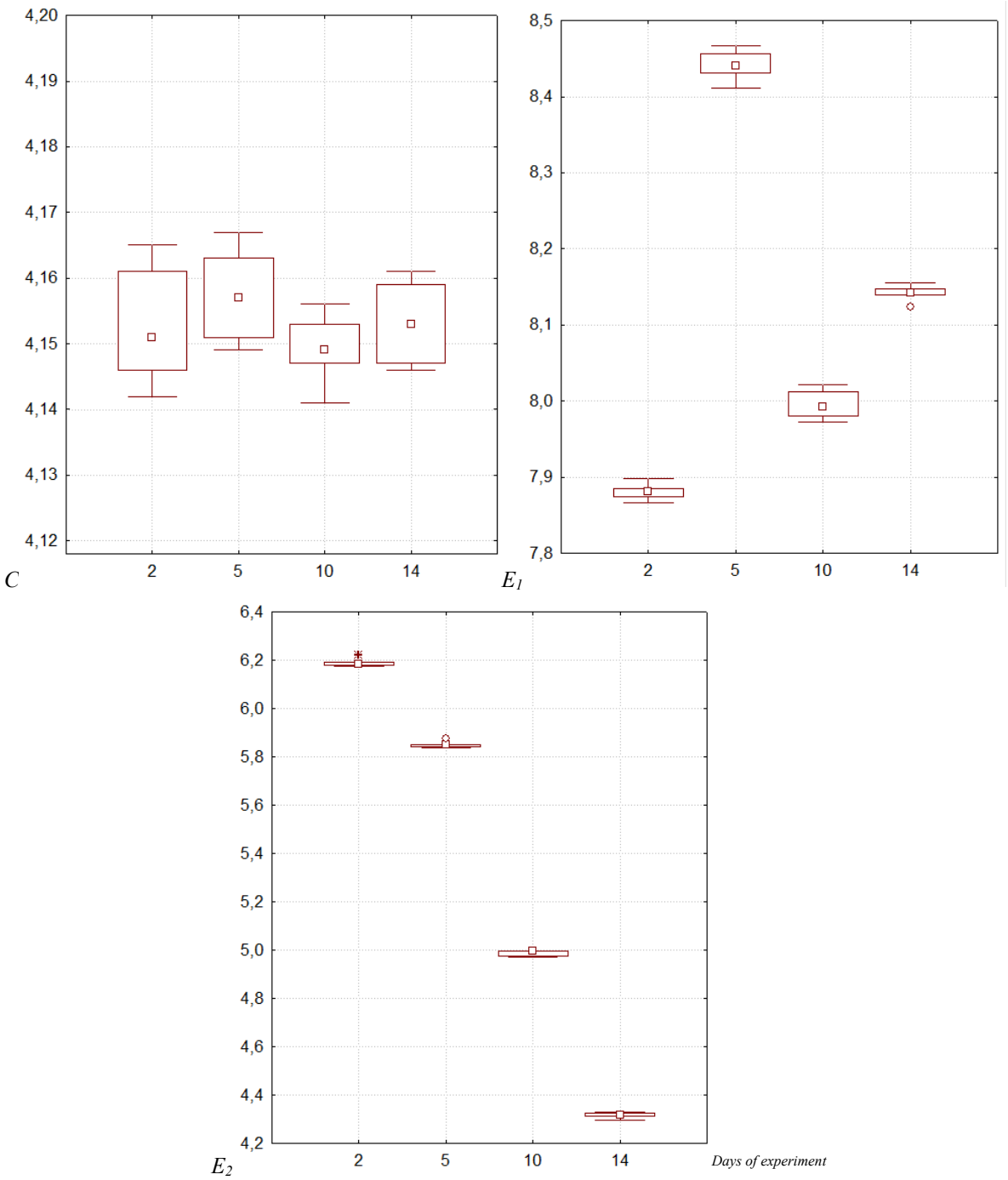

Fig. 2. The influence of butintersil on the level of TBK-active products in the blood plasma of rats under conditions of oxidative stress, nmol/ml

Overall, our obtained results indicate that the development of oxidative stress leads to significant and reliable ($P < 0.001$) formation and accumulation of lipid hydroperoxides and TBA-active products in the blood plasma of rats throughout the study period.

In recent years, antioxidant drugs have been increasingly used to combat manifestations of toxic liver damage. These drugs correct the antioxidant defense system and neutralize the products of free radical oxidation (Gutyj et al., 2017). The search for active components with antioxidant properties is a promising research direction, although it requires careful consideration of the compatibility of natural and synthetic antioxidants. Important factors for the effectiveness of an antioxidant drug include the total amount of antioxidants in its composition, the diversity of the antioxidant spectrum (particularly the presence of vitamins, vitamin-like substances, and metal trace elements), and the overall quantitative content of substances with antioxidant properties.

Under the action of the liposomal drug “Butaintersyl” in rats of the experimental group E_2 under oxidative stress conditions, it was found that on the second day of the experiment, the content of both intermediate and final LPO products decreased compared to the animals of the first experimental group, but remained higher than in the control group. On the fifth day of the experiment, the

content of lipid hydroperoxides and TBA-active products in the blood of animals in the experimental group E_2 continued to decrease, reducing by 8.1 % and 5.5 %, respectively, compared to the previous day. On the tenth day of the experiment, the content of lipid hydroperoxides in the blood of rats treated with the liposomal drug was 32% higher compared to the control group. During this period, the content of TBA-active products in the blood of rats in the E_2 experimental group exceeded the control by 20.2 %.

On the 14th day of the experiment, the level of intermediate and final LPO products in the blood of rats treated with the liposomal drug reached physiological values.

Thus, the liposomal drug “Butaintersyl” administered to rats during the development of oxidative stress, suppressed the formation of lipid oxidation products, as evidenced by the low levels of lipid hydroperoxides and TBA-active products in their blood. This is likely due to two powerful antioxidants in the drug's composition.

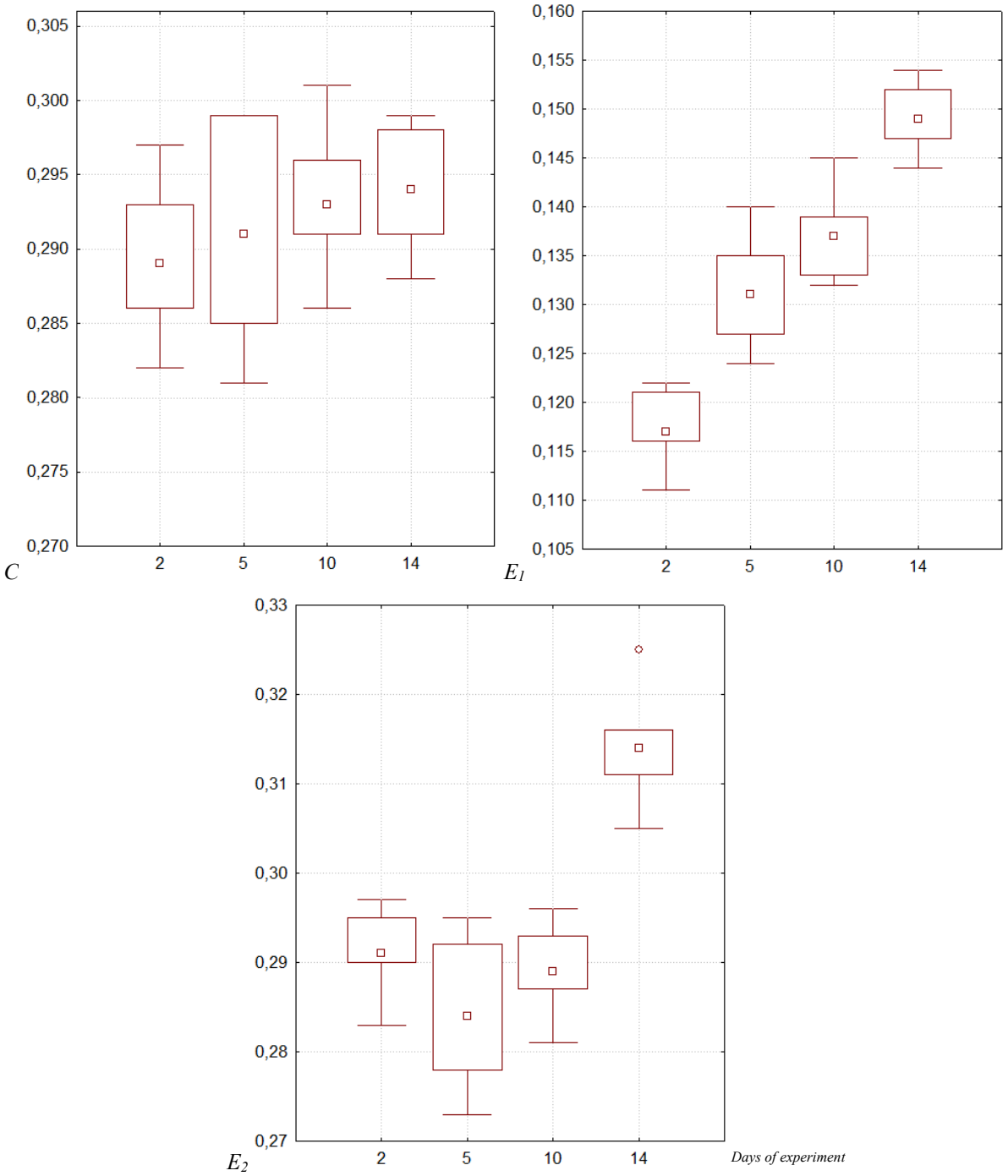

Fig. 3. Effect of butaintersil on glutathione peroxidase activity in blood serum of rats under conditions of oxidative stress, nmol GSH/min×mg protein

The suppression of the glutathione antioxidant protection system accompanied the development of oxidative stress in rats of the experimental group E_1. In rats of the experimental group E_1, a decrease in the activity of gluta-

thione peroxidase, an enzyme that protects cell membranes from the harmful effects of peroxide radicals, was observed. This enzyme catalyzes the breakdown of hydrogen peroxide and oxidizes glutathione. On the second day of the study, the activity of glutathione peroxidase in the blood of rats in the experimental group E_1 was the lowest, decreasing by 59.5 % compared to the control group. Subsequently, the activity of this enzyme in the blood of rats under further development of oxidative stress slightly increased but remained 55 % lower compared to the control group. On the 10th and 14th days of the experiment, the activity of glutathione peroxidase in the blood of rats in the first experimental group ranged from 0.137 to 0.149 nmol GSH/min×mg protein (Fig. 3). Comparing these values with the control group, the activity of this enzyme was reduced by 53.2 % and 49.3 %, respectively.

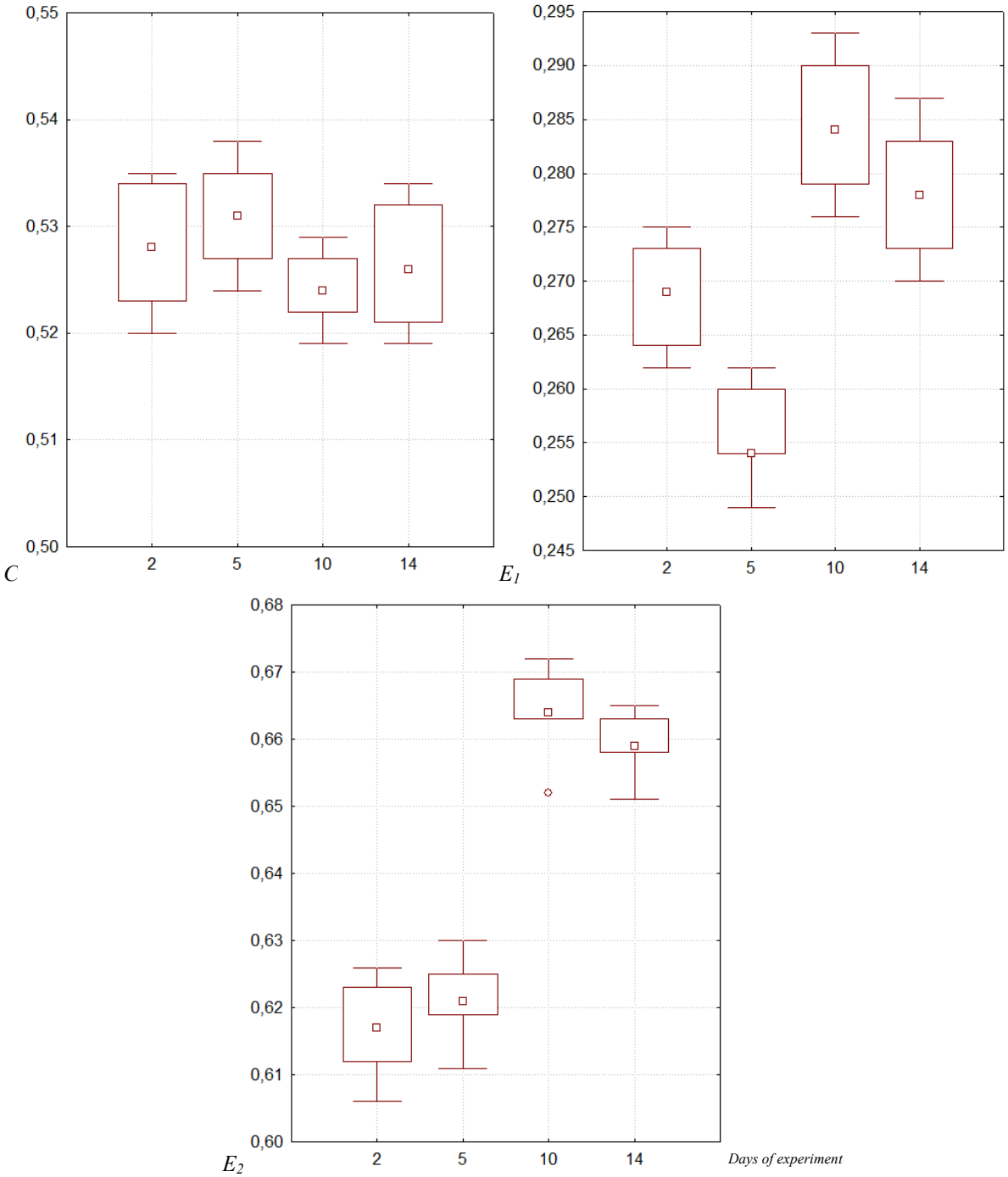

Fig. 4. The influence of butintersil on the content of reduced glutathione in the blood of rats under conditions of oxidative stress, µmol/ml

Reduced glutathione is the primary sulfur-containing antioxidant in the animal body. It protects the sulfhydryl groups of globin, erythrocyte membranes, and divalent iron from oxidizers. Glutathione is a central element of the antioxidant defense system for almost all cells and organs. Its antioxidant action involves the transfer of

sulfhydryl groups. Under oxidative stress conditions, on the second day of the experiment, the level of reduced glutathione in the blood of the experimental group of rats decreased by 49.1 % compared to the control group (Fig. 4). The minimum content of reduced glutathione was observed in the blood of the experimental group rats on the fifth day of the experiment. On the 10th and 14th days of the experiment, the level of this indicator was lower by 45.8 % and 47.1 %, respectively, compared to the control group.

The study data indicate that a slight increase in the activity of glutathione peroxidase and reduced glutathione content may result from the increased formation of radical metabolites and the accumulation of lipid oxidation products caused by the toxic effect of tetrachloromethane. This activates animal protective response and stimulates the body's antioxidant defense system.

Overall, our research results indicate that the development of oxidative stress disrupts the balance between the “Antioxidant Defense System” and “Lipid Peroxidation”.

In the experimental group E_2 rats, which were administered the liposomal drug “Butaintersyl”, a significant increase in the activity of glutathione peroxidase and the content of reduced glutathione was observed on the 2nd day of the experiment, by 149 % and 129 %, respectively, compared to the first experimental group. On the 5th day, the activity of glutathione peroxidase in this group decreased but remained at a level 117 % higher than the intoxicated rats that were not treated.

On the 10th day, the reduced glutathione content in the blood of rats receiving the liposomal drug was 2.34 times higher than in those not treated. The activity of glutathione peroxidase was 0.289 ± 0.0211 nmol GSH/min×mg protein.

On the 14th day, the content of reduced glutathione and glutathione peroxidase activity in the blood of rats treated with the liposomal drug were at their highest levels.

Thus, the liposomal drug “Butaintersyl” suppresses lipid peroxidation processes and activates the antioxidant defense system, as evidenced by the high content of reduced glutathione and glutathione peroxidase activity. This may be related to the presence of milk thistle in the preparation, which, according to the literature, also has antioxidant properties. It contains vitamins B, A, E, and K, vitamin D precursors, carotenoids, macroelements - potassium, calcium, magnesium, iron, and microelements - copper, zinc, manganese, and iodine. The combined action of these biologically important elements provides high hepatoprotective and antioxidant effects.

Conclusions

The series of studies revealed significant disruptions in the balance between oxidation and antioxidant processes in animals under oxidative stress conditions. This is primarily manifested in enhanced free radical lipid oxidation processes and suppressed activity of the glutathione system, which is responsible for antioxidant defense.

Due to its antioxidant properties, the liposomal preparation “Butaintersyl” has positively influenced the activity of membrane-bound enzymes and reduced the level of endogenous intoxication. This allows it to be recommended for inclusion in schemes for preventing toxic liver damage caused by chemical compounds.

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Conflict of interest

The authors declare that there is no conflict of interest.

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