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THE EFFECT OF L-GLUTAMIC ACID AND N-ACETYLCYSTEINE ADMINISTRATION ON BIOCHEMICAL BLOOD PARAMETERS IN RATS TREATED WITH CCl_4

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A toxic organic substance CCl_4 is a well known model compound for studying detoxification function of the liver and developing oxidative stress. The goal of the study was to estimate the effect of L-glutamic acid (L-Glu) and N-acetylcysteine (NAC) administration on rat blood parameters upon the toxic effects of CCl_4. Experimental male Wistar rats were injected intraperitoneally with CCl_4, the rats of CCl_4/L-Glu group were additionally injected with L-Glu (750 mg/kg), of CCl_4/NAC group – with NAC (150 mg/kg), of CCl_4/L-Glu/NAC group – with L-Glu (750 mg/kg) and NAC (150 mg/kg). The duration of the experiment was 24 h. Increased level of lipid peroxides, TBARS, triacylglycerols, cholesterol and decreased glutathione peroxidase, glutathione reductase, glutathione-S-transferase activity and GSH content were observed in the blood of the CCl_4 treated animals compared to the control untreated group. When animals were additionally injected with L-Glu or L-Glu/NAC, the most of the studied indicators were shown to be close to the control level. These results suggest that the mentioned above aminoacids attenuated CCl_4-induced oxidative stress in the blood of rats.

K e y w o r d s: tetrachloromethane, L-Glutamic acid, N-acetylcysteine, glutathione dependent enzymes, oxidative stress, biochemical blood parameters.

The potential danger for people and animals from the influence of man-made factors is caused, in particular, by the activation of free radical reactions, the occurrence of tissue hypoxia and the violation of the detoxification function of the liver. Tetrachloromethane (CCl_4) is a toxic organic substance of artificial origin, well known in scientific practice, as a model compound for studying damage to liver parenchymal cells [1-3]. The mechanisms of CCl_4 toxicity largely consist in the activation of lipid peroxidation processes, the intensive formation of free radicals and, as a result, a violation of the pro-oxidant balance [4, 5]. Free radicals bind to antioxidant enzymes, including sulfhydryl groups of GSH. Ultimately, they lead to cell damage, depletion of cellular ATP, hepatotoxic damage, disruption of calcium homeostasis, inflammation, fibrosis, etc [6-8].

Amino acids play an important role in controlling various cellular functions. In particular, L-Glu plays a central role in amino acid metabolism, takes an active part in the metabolic and biosynthetic pathways of all living organisms [9,10]. This amino acid is a key metabolite of cellular energy metabolism. L-Glu is necessary for removing excess ammonia, modulating gene expression, immune responses, regulating blood glucose levels, cell signaling, and removing excess nitrogen. On the other hand, glutamic acid is the precursor of a large number of biologically active components, such as purine and pyrimidine nucleotides, γ-aminobutyric acid, reduced glutathione. Reduced glutathione (GSH) homeostasis is vital for cellular protection against oxidative stress, as GSH regulates the redox state of cells and is involved in the detoxification process in all cell types. Many clinical research have evaluated the efficacy of NAC administration [11-14]. NAC has antioxidant properties and, along with L-Glu, are substrates in the biosynthesis of GSH. Despite the large number of studies and new data obtained on various aspects of the action of L-Glu, many aspects of the effects of this amino acid remain unexplored. There-

fore, the study of the effect of L-Glu on the animal body in order to mitigate the effects of CCl_4 was the subject of our research.

Materials and methods

Chemicals. All reagents used were obtained from Sigma-Aldrich (USA).

Animals. Male rats came from the Research Center of the Institute of Animal Biology NAAS (Ukraine). They were housed in cages under standard laboratory conditions with a 12-h light/12-h dark cycle. All rats had free access to a standard rodent diet and water *ad libitum*. This study was carried out in accordance with ethical standards of the European Convention for the protection of vertebrate animals used for Experimental and Other Scientific Purposes (Strasbourg, 2005) and General ethical principles of experiments using animals adopted by the First National Congress of Bioethics (Kyiv, 2001). All experimental procedures were performed according to the Bioethical Committee for Animal Experimentation of the Institute of Animal Biology NAAS of Ukraine

Experimental design. The studies were performed on male Wistar rats (180-200 g). A total of five experimental groups of rats (CCl_4, CCl_4/L-Glu, CCl_4/L-Glu/NAC, CCl_4/NAC, control) were created after 1 week of acclimatization. The duration of the experiment was 24 h. Four groups of experimental rats (CCl_4, CCl_4/L-Glu, CCl_4/L-Glu/NAC and CCl_4/NAC) were administered CCl_4 as an emulsion in corn oil at a dose of 3 ml/kg intraperitoneally. Then, the rats of the CCl_4/L-Glu group were injected with L-Glu (aqueous solution) at a dose of 750 mg/kg, the CCl_4/L-Glu/NAC experimental group was injected with L-Glu (750 mg/kg) and NAC (150 mg/kg) and the experimental group CCl_4/NAC – (150 mg/kg). Appropriate amounts of saline and corn oil were administered to the Control group of rats. After all these actions, the animals of all groups were killed. Euthanasia was carried out by removing the cervical vertebrae, after which the animals were decapitated. To obtain red blood cells and plasma, blood was centrifuged in a refrigerated centrifuge MLWT23D, angular rotor - LCD 8×90 at 3000 g for 15 min. Plasma was collected and red blood cells were washed three times with 0.85% NaCl solution, followed by centrifugation of the cell suspension at 3000 g for 5 min.

Biochemical indices determination. GPx (glutathione hydrogen-peroxide oxidoreductase, EC 1.11.1.9) activity was studied by measuring the rate of GSH oxidation before and after incubation with tertiary butyl hydroperoxide, as described earlier [15]. The color reaction is based on the interaction between SH-groups and 5,5-dithiobis-2-nitrobenzoic acid (DTNBA), resulting in the formation of colored product – dinitrophenyl anion. Enzyme activity was expressed as nmol GSH/min/mg protein. GR (glutathione NADP + oxidoreductase, EC 1.6.4.2) activity was measured as described previously [15]. This method is based on the catalytic NADPH-dependent reduction of the oxidized form of GSH. The enzyme activity was expressed in μmol NADPH/min/mg protein. GST (glutathione-S-transferase EC 2.5.1.18) activity was assessed by reaction between GSH and CDNB (1-chloro-2,4-dinitrobenzene) at 340 nm, the content of formed adduct was measured as described previously. The enzyme activity was expressed in μmol/min/mg protein. GSH content was determined by the level of thionitrophenyl anion formation as described previously [15]. This colour reaction is based on the interaction between SH-groups of GSH and DTNBA. GSH content was measured using the calibration graph and expressed in mmol GSH per gram of tissue. Glucose-6-phosphate dehydrogenase (G6PDH) activity was measured at 340 nm in 50 mM KPi-buffer (pH 7.0), 0.5 mM EDTA, 5 mM $MgCl_2$, 0.2 mM NADP, 2 mM G6P with supernatant were measured as described by [15]. The enzyme activity was expressed in μmol $NADP^+$/min·mg of protein. The activity of superoxide dismutase (SOD, EC 1.15.1.1) was evaluated by the level of inhibition of the rate of nitroblue tetrazolium (NBT)-reduction in the presence of NADH and phenazine methosulfate. The enzyme activity was expressed in conventional units CU/mg protein. The activity of catalase (CAT, EC 1.11.1.6) was measured by monitoring the formation of a stable colored complex of hydrogen peroxide and molybdenum salts. The enzyme activity was expressed in μmol/min/mg protein. The content of lipid hydroperoxides (LOOH) in tissues were determined as described previously [16]. The content of LOOH was calculated by the difference between experimental and control values, and expressed in standard units per gram of tissue. The concentration of thiobarbituric acid reactive substances (TBARS), characterizing the rate of lipid peroxidation based on the reaction between malondialdehyde (MDA) and thiobarbituric acid (TBA) was measured as described previously [16]. The values are expressed as nmol of TBARS per ml. The concentrations of triacylglycerol and cholesterol were

determined in the blood plasma, which was carried out using a biochemical analyzer "Humalyzer 2000".

Statistical methods. The obtained experimental data were analyzed with multifactorial statistics methods ANOVA using Statistica 12 software (StatSoft Inc., Tulsa, OK, USA)

Results and Discussion

The GPx, GR, GSH, GST, SOD, CAT and TBARS levels are critical indicators used to evaluate the degree of oxidative stress. A decrease in the activity of antioxidant enzymes can be caused by compensatory reactions occurring in the body as a result of metabolic disorders. As shown by the research results (Fig. 1), the GSH content decreased in the red blood cells of the first and fourth experimental groups compared to the control group of rats. A decrease in the GSH level may be associated with intensive use of reduced glutathione for metabolic processes. As shown in Fig. 2-4, the activity of GPx, GR, GST in the rat red blood cells of the CCl_4-treated groups were significantly lower ($P < 0.05$). L-Glu and NAC treatment reversed the changes in GSH, GPx levels induced by CCl_4.

The activity of GPx, a key enzyme of antioxidant protection, was probably lower in the blood erythrocytes of the first experimental group ($P < 0.05$) compared to the control. It should be noted that in the animals of the second and third experimental groups, this indicator reached the level of control values, and in the animals of the fourth experimental group, it was probably higher than in the control (Fig. 2). This indicator reached the level of control values in the second and third experimental groups of rats. It should be specified, the activity of GPx was higher ($P < 0.05$) in the animal of the fourth experimental group compared to the control.

GR maintains sufficient levels of active glutathione by reducing its disulfide form. The activity of GR in the erythrocytes was lower in the animals of the first experimental group ($P < 0.05$) compared to the control (Fig. 3). GST activity was also lower in the animals of the first and fourth experimental groups in comparison to this indicator in the control (Fig. 4). Catalase plays a key role in protecting cells from H_2O_2. However, as shown by the results (Fig. 5), catalase activity in the rat red blood cells did not differ. SOD activity (Fig. 6) also did not undergo significant changes in any of the studied groups.

In our studies, the intensification of lipid peroxidation under the action of carbon tetrachloride was revealed. In particular, the content of lipid hydroperoxides in the animals of the first and fourth experimental groups increased compared to the control group (Fig. 7). The content of TBARS in blood also changed. Namely, the level of this indicator increased ($P < 0.05$) in the first, third and fourth experimental groups of rats compared to the control (Fig. 8).

The chemical properties of triacylglycerols are due to the presence of higher fatty acids, aldehydes and alcohols. They are present in the largest amount

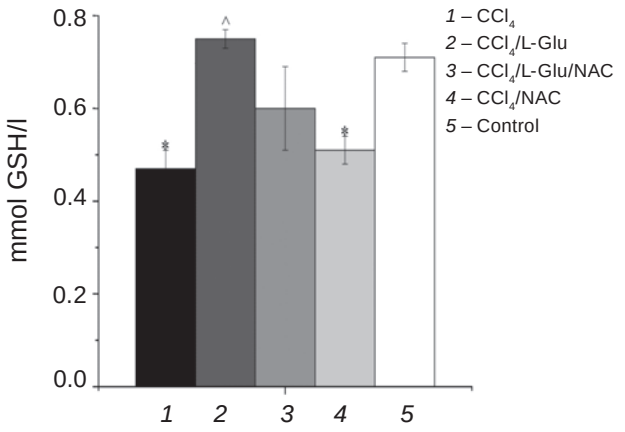

*Fig. 1 Effect of L-Glu, L-Glu/NAC and NAC on the content of GSH in rat red blood cells. Data are means ± SEM. *Significantly different from the control group with (P < 0.05); ^Significantly different from the first experimental group with (P < 0.05)*

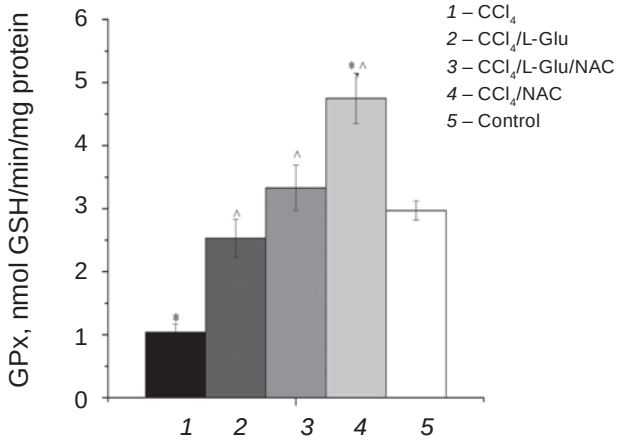

*Fig. 2 Effect of L-Glu, L-Glu/NAC and NAC on GPx activity in rat red blood cells. Data are means ± SEM. *Significantly different from the control group with (P < 0.05); ^Significantly different from the first experimental group with (P < 0.05)*

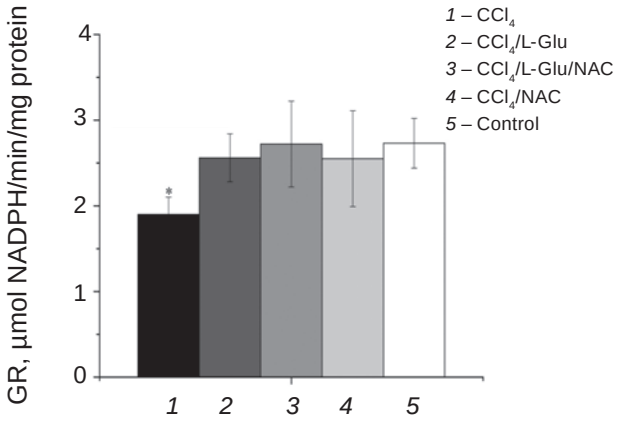

*Fig. 3 Effect of L-Glu, L-Glu/NAC and NAC on GR activity in rat red blood cells. Data are means ± SEM. *Significantly different from the control group with (P < 0.05); ^Significantly different from the first experimental group with (P < 0.05)*

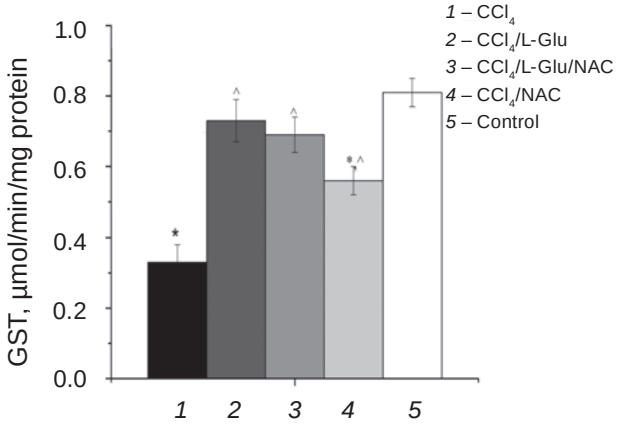

*Fig. 4 Effect of L-Glu, L-Glu/NAC and NAC on GST activity in rat red blood cells. Data are means ± SEM. *Significantly different from the control group with (P < 0.05); ^Significantly different from the first experimental group with (P < 0.05)*

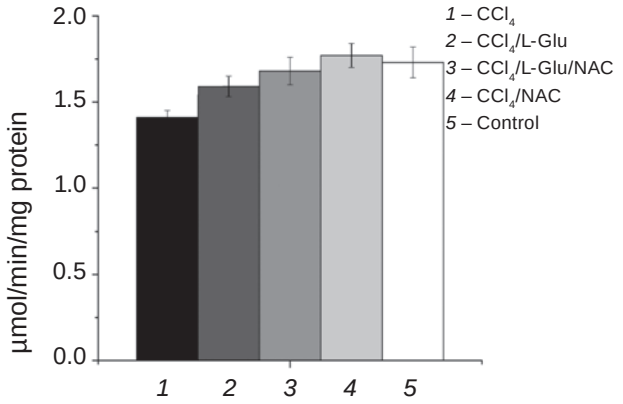

*Fig. 5 Effect of L-Glu, L-Glu/NAC and NAC on CAT activity in rat red blood cells. Data are means ± SEM. *Significantly different from the control group with (P < 0.05); ^Significantly different from the first experimental group with (P < 0.05)*

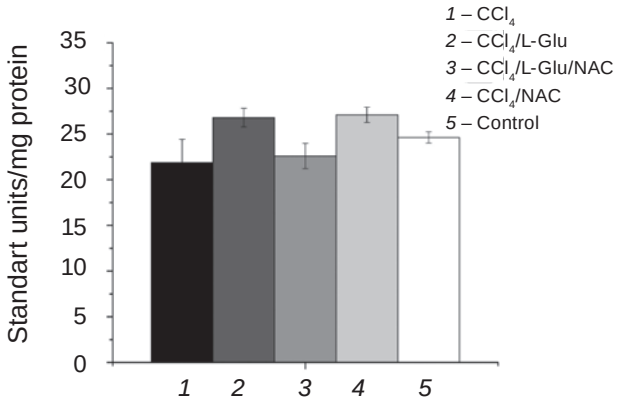

*Fig. 6 Effect of L-Glu, L-Glu/NAC and NAC on SOD activity in rat red blood cells. Data are means ± SEM. *Significantly different from the control group with (P < 0.05); ^Significantly different from the first experimental group with (P < 0.05)*

in adipocytes. As shown by the results of our research (Fig. 9), the concentration of triacylglycerol in the blood plasma of the first, third and fourth experimental groups of rats that were exposed to CCl_4 intoxication was higher ($P < 0.05$) compared to animals of the control group.

The largest amount of cholesterol is synthesized in the liver. The precursor in the biosynthesis of cholesterol is acetyl-CoA, which is formed during the oxidation of glucose due to the oxidative decarboxylation of pyruvate or during the β-oxidation of fatty acids. Cholesterol is subject to biotransformation, as a result of which biologically active compounds of a steroid nature are formed. The cholesterol concentration increased ($P < 0.05$) in the blood plasma of the first (CCl_4) and fourth (CCl_4/NAC) experimental groups compared to the control group of rats (Fig. 10).

Researching the mechanisms of GSH level regulation, especially under conditions that contribute to its depletion, is an important problem. Long-term activation of glutathione-dependent enzymes is possible only under the condition of maintaining a sufficiently high level of intracellular GSH, which acts as a substrate for reactions [17]. Analyzing the results of our research, it can be assumed that the

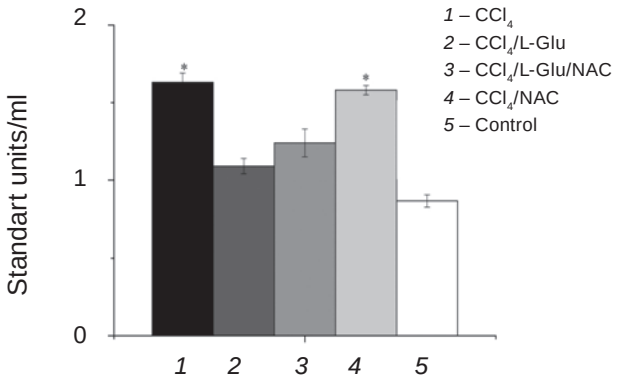

Fig. 7 Effect of L-Glu, L-Glu/NAC and NAC on the content of LOOH in rat blood

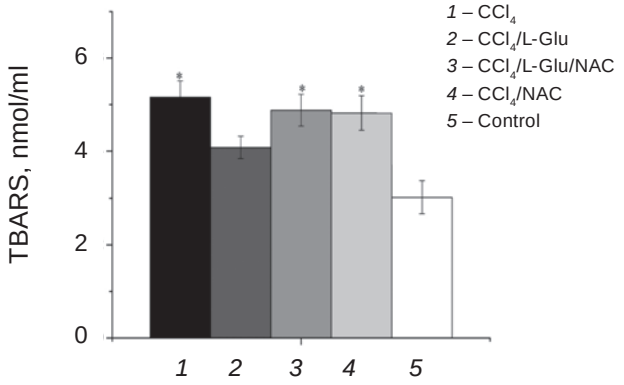

Fig. 8 Effect of L-Glu, L-Glu/NAC and NAC on the content of TBARS in rat blood

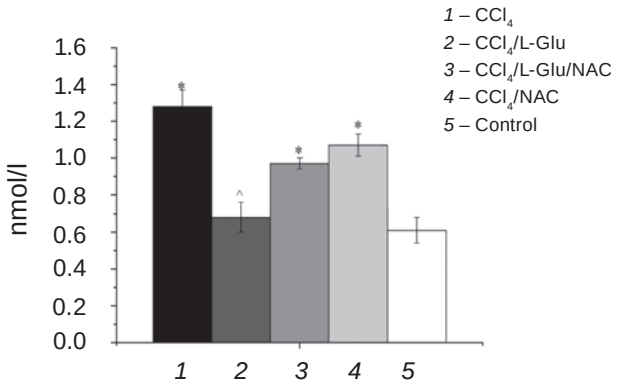

Fig. 9 Effect of L-Glu, L-Glu/NAC and NAC on triacylglycerol concentration in rat blood

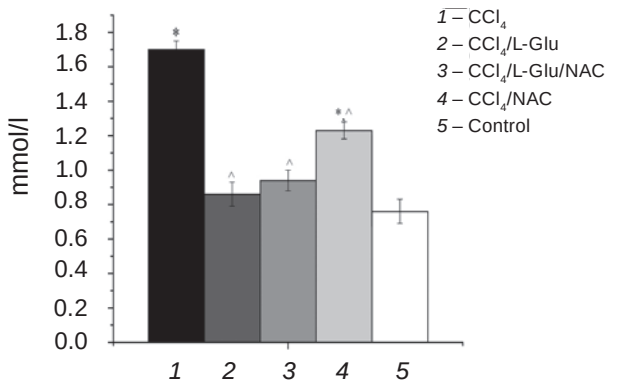

Fig. 10 Effect of L-Glu, L-Glu/NAC and NAC on cholesterol concentration in rat blood

intracellular GSH content in the red blood cells of the second and third experimental groups, which additionally received amino acids L-Glu and L-Glu in combination with NAC, is provided not only by the regeneration of oxidized glutathione under the action of the enzyme glutathione reductase and NADPH (H^+). The GSH content in the CCl_4/L-Glu was at the level of the control group (CCl_4/L-Glu) or slightly below the control level in the CCl_4/L-Glu/NAC, in contrast to the animals of the CCl_4 experimental group. As the results showed, the effect of the CCl_4 was accompanied by the activation of lipid peroxidation processes. The content of triacylglycerol and cholesterol in the blood plasma of white rats under the conditions of CCl_4 intoxication, it should be noted increase in the studied parameters in the animals of the CCl_4 (cholesterol, triacylglycerol), CCl_4/L-Glu/NAC (triacylglycerol) and CCl_4/NAC (cholesterol, triacylglycerol) experimental groups compared to the control. There were no changes in the above-mentioned indicators in the animals of the CCl_4/L-Glu experimental group when compared to the control. Summarizing the obtained results, the CCl_4 treatment significantly reduced the content of GSH and GPx, GR, GST activities and increased LOOH and TBARS levels in the blood of rats. The cholesterol and triacylglycerol concentrations were also increased under the introduction of carbon tetrachloride. Similarly, these metrics all improved to one degree or another after the L-Glu, L-Glu/L-Cys, L-Cys treatment. These results suggest that the mentioned above amino acids attenuated CCl_4-induced oxidative stress in the blood of rats.

Conflict of interest. Authors have completed the Unified Conflicts of Interest form at http://ukrbiochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

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ВПЛИВ L-ГЛУТАМІНОВОЇ КИСЛОТИ І N-АЦЕТИЛЦИСТЕЇНУ НА БІОХІМІЧНІ ПОКАЗНИКИ КРОВІ У ЩУРІВ, ЯКІ ОТРИМУВАЛИ CCl_4

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Токсична органічна речовина CCl_4 є добре відомою модельною сполукою для вивчення детоксикаційної функції печінки та розвитку оксидативного стресу. Метою дослідження було оцінити вплив введення L-глутамінової кислоти (L-Glu) і N-ацетилцистеїну (NAC) на параметри крові щурів за токсичної дії CCl_4. Піддослідним самцям щурам Вістар внутрішньоочеревинно вводили CCl_4, щурам групи CCl_4/L Glu додатково вводили L-Glu (750 мг/кг), щури групи CCl_4/NAC отримували NAC (150 мг/кг), а група CCl_4/L-Glu/NAC – L-Glu (750 мг/кг) і NAC (150 мг/кг). Тривалість експерименту становила 24 год. У крові тварин, які отримували CCl_4, спостерігали підвищений рівень пероксидів ліпідів, TBARS, триацилгліцеринів, холестерину та зниження активності глутатіонпероксидази, глутатіонредуктази, глутатіон-S-трансферази та вмісту GSH порівняно з контрольною групою без лікування. Показано, що після додаткового введення тваринам L-Glu або L-Glu/NAC, більшість досліджуваних показників була близька до контрольного рівня. Ці результати свідчать про те, що вищезазначені амінокислоти послаблювали CCl_4-індукований оксидативний стрес у крові щурів.

Ключові слова: тетрахлорметан, L-глутамінова кислота, N-ацетилцистеїн, глутатіонзалежні ензими, оксидативний стрес, біохімічні показники крові.

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