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Ameliorative effect of L-Glutamic acid against CCl_4-induced oxidative stress in rat's brain and spleen

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ABSTRACT: Carbon tetrachloride (CCl_4) causes tissues damage by inducing a state of oxidative stress. This research was arranged to evaluate the role of L-glutamic acid (L-Glu) against oxidative stress induced by CCl_4 in brain and spleen tissues of rats. Antioxidant profiles were evaluated by estimating the activities of antioxidant enzymes; superoxide dismutase (SOD), catalase (CAT), glutathione-S-transferase (GST), glutathione peroxidase (GPx), glutathione reductase (GR) and reduced glutathione (GSH). In addition to this, the lipid peroxidation products content (TBARS and LOOH) was monitored. Rats were divided into three groups: two experimental (CCl_4, CCl_4/L-Glu) and Control. The rats of CCl_4 group were treated with CCl_4 only, CCl_4/L-Glu group was treated with CCl_4 and L-Glu. Administration of CCl_4 reduced the antioxidant enzymes activity in the spleen and brain tissues of rats. Co-treatment of L-Glu restored the activities of antioxidant enzymes GPx (brain), GST (spleen), GR (spleen, brain) compared to control and CCl_4 experimental group of animals. Increased spleen TBARS content was observed in rats treated with CCl_4. But this index didn't change in the CCl_4/L-Glu group, which additionally received L-Glu. Results of the present study revealed that L-Glu could protect the brain and spleen tissues against oxidative stress induced by CCl_4.

KEYWORDS: L-glutamic acid; carbon tetrachloride; antioxidant system; enzymes; oxidative stress.

1. INTRODUCTION

In recent years, more and more attention has been paid to the protective effect of antioxidants against chemically induced toxicities [1-5]. CCl_4 is widely used to induce tissues toxicity. It is the animal model for studying xenobiotic- and oxidative stress-mediated toxicity in experimental animals [6-8]. CCl_4 is metabolized by cytochrome P-450 enzymes (CYP 2E1 and CYP3A), with the production of the trichloromethyl radical (CCl_3*) [9-12]. This free radical can be fixed in particular to lipids, thus altering their metabolism or form DNA adducts. CCl_4-induced tissues injury results from bioactivation of CCl_4 into free-radical metabolites and lipid peroxidation [13-17]. The cellular calcium homeostasis dysfunction may be also an important factor in CCl_4-induced toxicity.

Oxidative stress results in a disproportion between reactive oxygen species (ROS) and antioxidant defenses. CCl_4 exposure causes tissue damage due to the generation of ROS [18-21]. GSH is the most important antioxidant [22]. Depleting cellular antioxidants such as GSH tends to increase the toxicity of tissues because CCl_4 causes injury through oxidative pathways [23-26].

The motivation of the current study was to search for effective antioxidants which could prevent or reduce the oxidative stress effects caused by xenobiotics. In particular, in this research, we focused our attention on aminoacid L-Glu. Glutamic acid plays a central role in the metabolic and biosynthetic pathways of the organism. L-Glu is a building block of proteins and, accordingly, a key cell energy metabolite [27-31]. Furthermore, it is a substrate to maintain tricarboxylic acid cycle intermediates level [32-38]. L-Glu is a precursor of the GSH which homeostasis is essential to protect cells from oxidative stress [39]. Our previous research studies have demonstrated that L-Glu has the antioxidant properties and can suppress free radicals' generation [25,40,41]. These findings are consistent with those of other authors on ameliorative and protective effects of L-Glu supplementation under exposure to toxicants [42-44]. These results suggested that a natural product such as glutamine prevents glutathione depletion and consequently heme oxygenase induction [45].

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The central objective of this research was to investigate the effect of L-Glu under the action of a higher dose of CCl_4 on changes in the enzymatic activity of the antioxidant system in the brain and spleen tissues of rats.

2. RESULTS AND DISCUSSION

2.1. Indexes of the antioxidant system.

The use of CCl_4 led to a change in the GSH activity in the tissues of two experimental groups of animals. GSH can participate in reactions with hydroperoxides without the participation of any enzymes. It can inhibit free radical oxidation when initiating a chain process, capable of reacting with free radicals as actively as tocopherol [46-48]. From the data presented in Figure 1(a) it can be seen that the level of GSH in the spleen is significantly reduced ($p \leq 0.05$) in the first (CCl_4) and second (CCl_4/L-Glu) experimental groups compared with the control group of animals. Depletion of cellular antioxidants, such as glutathione, tends to increase organ toxicity because CCl_4 causes oxidative injury. It can be assumed that the depletion of GSH in the spleen tissue occurs due to the GST reaction. In this reaction, GSH is conjugated to foreign compounds. Some authors have linked a decrease of GSH level with ascorbic acid and lipid peroxidation [49]. In brain, on the contrary, the content of the studied tripeptide elevated ($p \leq 0.05$) in the CCl_4 and CCl_4/L-Glu experimental groups of animals compared to the control group.

One of the components of the antiperoxide complex is GPx, which including GR and GSH. GR restores oxidized glutathione formed during the operation of the glutathione-dependent antiperoxide system. The effectiveness of the GPx mechanism of hydroperoxide reduction depends on the level of the main hydrogen donor for this reaction - glutathione. As shown by the results of studies (Figure 1(b)), the activity of GPx decreased ($p \leq 0.05$) in the spleen of both groups of animals. The studied enzyme activity in the brain tissue (CCl_4 experimental group) decreased ($p \leq 0.05$) compared to the second experimental groups and control. It can be seen that the GST activity in the spleen of the CCl_4 group animals decreased ($p \leq 0.05$) (Figure 1(c)). These data are consistent with the data[50] on the reduction of GPx activity under the action of CCl_4 in the tissues of the spleen and brain.The activity of GST in brain tissue did not change in CCl_4 and CCl_4/L-Glu groups compared to the control group.

Maintenance of a sufficient level of the reduced form of glutathione, which is oxidized during the functioning of glutathione-dependent antiperoxide systems, is carried out by a special enzyme - GR. The glutathione reductase converts the oxidized form of glutathione (GSSG) to reduced form (GSH). GR and GPx form an antiperoxide complex (peroxidase neutralizes peroxides to hydrogen and water, and GR restores GSSG, turning it into a substrate for GPx activity). The activity of GR reduced in the spleen and brain tissues of the CCl_4 group animals comparing to the control and CCl_4/L-Glu experimental group (Figure 2(a)). The spleen and brain tissues are very sensitive to the action of CCl4. As shown by [51] the spleen and brain tissues had the highest fold expression of NF-κB, while the other mediators in the target organs had varying degrees of fold expressions. It is worth noting that the GR activity in rats of the CCl_4/L-Glu group did not change and remained at the level of control values. G6PDH activity in the spleen tissue of both study groups was slightly reduced (Figure 2(b)). The activity of G6PDH in the brain of the CCl_4 and CCl_4/L-Glu experimental groups was significantly reduced ($p \leq 0.05$).

The CAT enzymatic activity in the brain is only a few percent of its level in the liver. As shown by our results (Figure 3(a)), the activity of CAT in brain tissue decreased very significantly ($p \leq 0.05$) in the CCl_4 and CCl_4/L-Glu groups of animals, respectively, by 3.70 and 3.59 times compared to the control group of rats. Zargar et al. reported a significantly decrease in CAT activity and GSH level in brain under theCCL_4 exposure [52]. No changes in the test enzyme were found in the spleen tissue of rats. SOD plays a major role in the enzymatic chain of the endogenous antioxidant defense system. The most important highly active metabolite of the dismutation reaction is hydrogen peroxide, which is formed as a result of biochemical reactions occurring in the endoplasmic reticulum, peroxisomes, mitochondria, cell cytosol. As shown by the results (Figure 3(b)), the SOD activity decreased in the brain of the first and second experimental groups by 1.44 and 1.26 times.

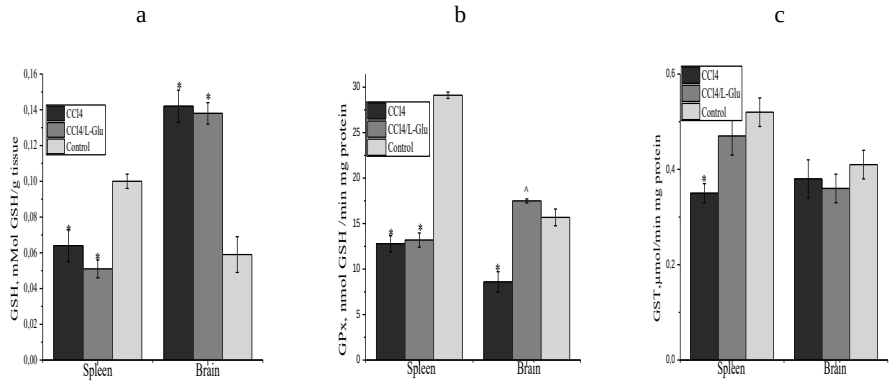

Figure 1. Effect of L-Glu on the GSH content (a), GPx (b) and GST (c) activities in rat tissues. Data are means ± S.E.M; n=10.
* Significantly different from the control group with ($P < 0.05$); ^Significantly different from the first experimental group with ($P < 0.05$).

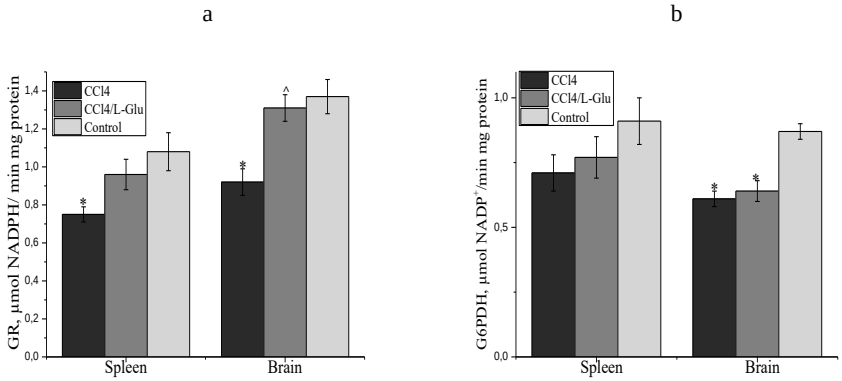

Figure 2. Effect of L-Glu on GR(a) and G6PDH(b) activities in rat tissues. Data are means ± S.E.M; n=10.
* Significantly different from the control group with ($P < 0.05$); ^ Significantly different from the first experimental group with ($P < 0.05$).

2.2. Indexes of free radical processes.

As a result of the body's vital activity, about 65% of the total amount of hydrogen peroxide is formed, which is considered a necessary metabolite and participates in the implementation of various physiological functions of the body. In addition, hydrogen peroxide as a strong oxidant has a toxic effect on the cell. Therefore, it is very important to maintain a normal level of hydrogen peroxide and prevent its

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accumulation in the body. As shown by the results of studies (Figure 4(a)), the content of LOOH did not change in any of the studied groups of animals.

In this study, we have shown that oxidative stress induced by CCl_4, manifested by increase TBARS content in the spleen tissue ($p \leq 0.05$) by 1.57 times (Figure 4(b)). The TBARS activity in the second experimental group (CCl_4/L-Glu), which additionally received L-Glu, did not change.

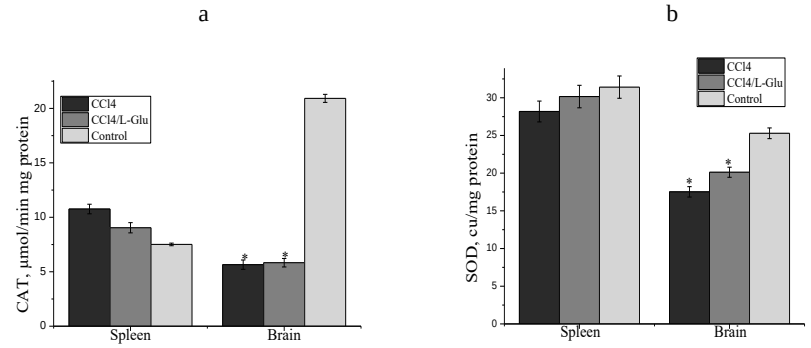

Figure 3. Effect of L-Glu on CAT (a)and SOD (b)activities in rat tissues. Data are means ± S.E.M; n=10.
* Significantly different from the control group with ($P < 0.05$); ^Significantly different from the first experimental group with ($P < 0.05$).

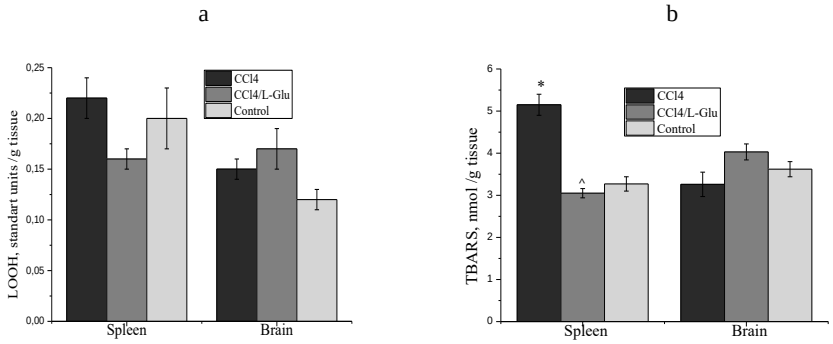

Figure 4. Effect of L-Glu on the LOOH (a) and TBARS (b) content in rat tissues. Data are means ± S.E.M; n=10.
* Significantly different from the control group with ($P < 0.05$); ^Significantly different from the first experimental group with ($P < 0.05$).

ROS plays also a crucial role in the pathogenesis of various human organs, including the liver, brain, heart, lung, spleen, and kidney. The liver is the main organ exposed to CCl4 toxicity. Other organs can also be affected by its toxicity. Free radicals' production will increase the ROS level in the liver. As a result, oxidative stress, inflammation, fibrosis, and necrosis and, in turn, hepatic damage. This damaging effect in the liver will lead to changes and inflammatory response in the other organs, in particular, brain and spleen [53-55]. In this paper, we focused on studying the effects of L-Glu against CCl4-induced toxicity in the spleen and brain tissues (systemic toxicity). Various reports revealed that CCl_4 plays a determinative role in spleen and brain injury [56-58].

The current study detected decrease in the redox state markers GPX, GST, GR, G6PDH, CAT and SOD in brain tissue compared to those in the control group. The high level of ROS, leading to membrane lipids peroxidation (high spleen TBARS level), which resulted in the exhaustion of the spleen GSH, GPX, GR. The

administration of L-Glu to improve the spleen oxidative stress condition caused by CCl4 injection. L-Glu exhibited an ameliorating impact on the CCl4-induced toxicity, particularly, in the spleen tissue. The antioxidant defense system of brain is more sensitive to oxidative stress compared to other organs. The CCl4 crosses the blood-brain barrier, because it is lipophilic. Then CCl4 accumulate in the brain and lead to neurotoxicity [57]. Ritesh et al. have found that the single hepatotoxic dose of CCl4 causes more severe oxidative stress in the brain compared to the liver [58].

3. CONCLUSION

The present study demonstrated that CCl_4 leads to oxidative stress by depleting the antioxidant enzymes activity in the spleen and brain tissues of animals. The treatment with L-Glu partially attenuated the CCl_4-induced tissues toxicity. L-Glu protects cells against CCl_4 intoxication by improving the antioxidant enzymes such as GPx (brain), GR (brain, spleen), and GST (spleen). The experimental group of rats treated with L-Glu showed a significant reduction in spleen TBARS levels when compared with that of the CCl_4-induced group. Our results indicate that L-Glu supplementation might reduce and facilitate problems caused by oxidative stress. However, additional research is required to find out the exact mechanisms the L-Glu efficacy.

4. MATERIALS AND METHODS

4.1. Chemicals

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

4.2. Experimental animals

Male Rats came from the Research Center of the Institute of Animal Biology NAAS (Ukraine). The animals were kept under standard vivarium conditions (t ° 22 ± 2 ° C, light / dark cycle – 12/12 h). All rats had ''*ad libitum*'' access to feed and water. This study was carried out according to the ethical standards of the European Directiveon the protection of animals used for Scientific Purposes (Strasbourg, 2010) and Law of Ukraine "On Protection of Animals from Cruel Treatment" (2006) [59,60].

4.3. Experimental design

Studies were carried out on male Wistar rats (weighing 200–220 g). The rats were divided into three groups: two experimental (CCl_4, CCl_4/L-Glu) and Control, 10 animals in each group. The duration of the experiment - 24 h. Two groups of experimental rats (CCl_4 and CCl_4/L-Glu) were intraperitoneally received CCl_4, administrated as emulsions in corn oil (ratio 1:1) at the dose of 3,5 ml/kg. After that, the CCl_4/L-Glu experimental group of rats was injected intraperitoneally with L-Glu (an aqueous solution) at the dose of 750 mg/kg. The control group of rats were administered the appropriate amount of saline and corn oil. After that animals of all groups were sacrificed. Before euthanasia by cervical dislocation rats were anesthetized by inhalation of isofurane (2%) for 2 min. After that the animals were decapitated. The brain and spleen tissues were frozen in liquid nitrogen and stored at −80 °C for further analysis.

4.4. Tissues preparation

The brain and spleen tissue samples were homogenized (Tris HCl buffer pH 7.4). The ratio of tissues and medium was 1:10 (weight: volume).The homogenate is then centrifuged at 15000×g (15 min at 4°C). The supernatant was used for determining the antioxidant enzymes activities, reduced glutathione and lipid peroxidation products content.

4.5. Biochemical indices determination

Glutathione peroxidase activity (GPx, EC 1.11.1.9) was determined by the glutathione recovery rate in the presence of NADPH, as described earlier [61]. The activity of GPx was reportedas nmolGSH/min/mg protein. Glutathione reductase activity (GR, EC 1.6.4.2) was determined by catalyzing the NADPH-dependent reduction of GSSG, as described previously [61] and expressed in μmol NADPH/ min/ mg protein. Glutathione transferase activity (GST, EC 2.5.1.18) was determined in the reaction of 1-chloro-2,4-dinitrobenzene with GSH [61]. The activity of GST was measured in μmol/min/mg protein. The reduced glutathione content (GSH) was quantified in the reaction between GSH SH-groups and 5,'5'-dithio-bis(2-

nitrobenzoic acid) [61]. GSH content was reported in mmolGSH/per gram of tissue. Glucose-6-phosphate dehydrogenase activity (G6PDH) was determined as described by [61]. The activity of G6PDH was finally reported in μmol NADP+/min·mg of protein. The superoxide dismutase activity (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 catalase activity(CAT, EC 1.11.1.6) was evaluated by monitoring the formation of a stable colored complex of hydrogen peroxide and molybdenum saltsandmeasured in μmol/min/mg protein. The lipid hydroperoxides concentration (LOOH) was calculated by the difference between experimental and control values, as described previously[62] and expressed in standard units/per gram of tissue. The content of thiobarbituric acid reactive substances products (TBARS) was measured by reaction between malonic dialdehyde and thiobarbituric acid, as described previously [62]. The TBARS content was expressed in nmol of TBARS/ per gram of tissue.

4.6. Statistical Analysis

In the present study, the experimental data were analyzed with multivariate statistical methods ANOVA using Statistica 12 software (StatSoft Inc., Tulsa, OK, USA).

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Conflict of interest statement: The authors declare no conflict of interest.

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