

Original Research

# Glutathione-Related Enzyme Activity in Liver and Kidney of Rats Exposed to Cadmium and Ethanol

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## Abstract

The activity of glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione S-transferase (GST) was investigated in liver and kidney of rats exposed to cadmium (Cd) and ethanol (EtOH) alone and in combination. Rats were treated with 50 mg Cd/dm<sup>3</sup> in drinking water and/or 5 g of EtOH/kg body wt/24 h intragastrically, for 12 weeks.

Exposure to Cd led to an increase in GPx and GST activity with a simultaneous decrease in GR activity in the liver. In the kidney of rats treated with Cd, an increase in the activity of GPx and GR was noted. In the EtOH-exposed rats, GPx activity decreased in the liver, but increased in the kidney. Exposure to EtOH caused a reduction in GR activity only in the liver. The co-exposure to Cd and EtOH led to an increase in the liver and kidney GPx activity compared to control. In the rats simultaneously exposed to Cd and EtOH liver activity of GR decreased compared to control, whereas the kidney GR activity increased compared to control as well as to the groups treated with Cd and EtOH separately. The co-exposure to Cd and EtOH led to an increase in the liver activity of GST compared to the control and EtOH groups. Analysis of variance (ANOVA/MANOVA) revealed that the changes noted in the activity of investigated enzymes in the Cd + EtOH group resulted from the independent action of both Cd or EtOH as well as from their interactive action. Numerous correlations (negative or positive) were noted between the activity of GPx, GR and GST, and the concentration of GSH, Cd and MDA in the liver and kidney.

On the basis of our results it can be concluded that changes in the activity of GPx, GR and GST in the liver and kidney may be involved in the mechanism leading to a decrease in GSH concentration in these organs due to exposure to Cd and EtOH alone and in conjunction with each other.

**Keywords:** cadmium, ethanol, glutathione peroxidase, glutathione reductase, glutathione S-transferase

## Introduction

Literature data give evidence that oxidative stress is one of the main mechanisms of toxic action of cadmium (Cd) [1-4] and ethanol (EtOH) [5-7]. Liver and kidneys, being organs which play a vital role in the metabolism of Cd and EtOH, are at special risk of damage due to the oxidative action of these xenobiotics. Our own results [8, 9] and findings of other investigators [2, 4, 10] confirm

that the Cd- and EtOH-induced lipid peroxidation of cellular membranes plays a crucial role in the hepato- and nephrotoxic action of these substances. We have noted [8] enhanced concentration of malondialdehyde (MDA), being an indicator of lipid peroxidation and disturbances in the activity of antioxidant enzymes (superoxide dismutase – SOD and catalase – CAT) in the liver and kidney of rats exposed to Cd and EtOH alone and in conjunction with each other. We also revealed [9] that a decrease in the concentration of reduced glutathione (GSH) in the liver and kidney of rats exposed to Cd or/and EtOH plays a crucial

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role in the mechanism of the peroxidative action of these substances in the two organs.

The endogenous GSH, synthesized mainly in the liver, plays an important role in the cell defense system. GSH, being a thiol compound, acts in cells as an antioxidant [11, 12]. GSH, as a co-factor of glutathione peroxidase (GPx), participates in the reduction of peroxides (hydrogen peroxide –  $H_2O_2$  and organic peroxides formed during lipid peroxidation) with concomitant formation of oxidized glutathione disulfide (GSSG) [12, 13]. At normal physiological conditions GSSG is reduced to GSH by glutathione reductase (GR) at the expense of reduced nicotinamide adenine dinucleotide phosphate (NADPH), thereby forming a redox cycle [12]. The peroxidase/glutathione reductase redox cycle is responsible for the maintenance of proper GSH concentration [14].

GSH is also involved in detoxication of many xenobiotics through the formation of S-conjugates with toxic metabolites in the second phase of biotransformation [15, 16]. GSH forms S-conjugates also with products of lipid peroxidation [17, 18]. The reaction of S-conjugation can be significantly accelerated by glutathione S-transferase (GST) [16].

Changes in the activity of GPx, GR and GST can cause disturbances in the concentration of GSH. Both Cd [19, 20] and EtOH [21–23] may lead to changes in activities of these enzymes. However, there is no data estimating the influence of combined exposure to these substances on the activity of GPx, GR and GST.

Our own studies [9] have related that one of the mechanisms leading to the lipid peroxidation in the liver and kidney of rats exposed to Cd and EtOH alone and in conjunction involve a decrease in GSH concentration in these organs [9]. That is why, for further explanation of these mechanisms of peroxidative action of Cd and EtOH, the present work was aimed at investigating the activities of GPx, GR and GST in the liver and kidneys of those rats.

## Experimental Procedures

A total of 32 adult male Wistar rats (8-week-old weighing approximately 170 g) were used. The animals throughout the experimental period were kept under controlled conventional conditions ( $22\pm 2^\circ C$ , relative humidity of  $50\pm 10\%$ , natural day/night cycle) and had free access to drinking water (redistilled water free of contaminants but not completely deprived of necessary bioelements) and a standard LSM dry chow (Agropol, Motycz, Poland). The energetic value of the diet was 12.2 MJ/kg. Cd concentration (assessed in our laboratory) in the food was 0.122  $\mu g/g$ .

The experiment lasted for 12 weeks. The rats were randomly divided into four groups of 8 animals in each:

1. Control group – divided into two subgroups, of which one received redistilled water free of Cd and EtOH; the animals of the other were additionally given physiological saline (0.9% NaCl) p.o. (intragastrically through a tube);

2. Cd group – the animals were administered an aqueous solution of cadmium chloride at the concentration of 50 mg Cd/dm<sup>3</sup> as the only drinking fluid;
3. EtOH group – the rats drank redistilled water and received EtOH p.o. in a total dose of 5 g/kg body wt./24 h divided into two equal doses of 2.5 g/kg body wt. each (the first dose was administered at 8 a.m., the other 6 h later) for 5 consecutive days a week during the whole experimental period;
4. Cd + EtOH group – the animals were exposed to Cd in drinking water (like the Cd group) and received EtOH (like the EtOH group).

The experimental design was approved by the Local Ethic Committee for Animal Experiments in Białystok (Poland) for care and use of laboratory animals.

EtOH was administered as 40% solution. The volume of 40% EtOH solution, corresponding to a dose of 5 g/kg body wt./24 h, was calculated individually for each rat from the EtOH and Cd + EtOH groups, depending on changes in body weight throughout the experiment. The rats were weighed on the first day of each week to modify the volume. The body weight gain, 24-hour consumption of drinking water in each of the four experimental groups and Cd intake in the Cd and Cd + EtOH groups during the whole experiment have been presented in our previous report from studies in this model [8]. The experimental model used here ensured equal EtOH and Cd intake in the groups of rats exposed to both substances alone and in conjunction with each other. This is very important under the evaluation of mutual interactions between two substances.

The applied model of rats' exposure to Cd and EtOH reflects the exposure to these xenobiotics that may occur in human life. Cd concentration in the blood of the rats continuously intoxicated with 50 mg Cd/dm<sup>3</sup> [8, 24] is within a range of values noted in inhabitants of areas heavily contaminated with Cd or in Cd workers [25, 26]. The dose of 5 g EtOH/kg body wt./24 h is equivalent to consumption of about 0.7 l/day of 40% vodka by men [6]. Since the rate of EtOH oxidation in rats is three times faster than in humans (0.1 g/kg body wt./h), the animals need a higher dose of EtOH than humans to produce a comparable toxic effect [6]. Thus the level of EtOH treatment used in this study may be tantamount to its misuse by people.

After the termination of the experiment, following overnight starvation (under barbiturate anaesthesia with Vetbutal, 30 mg/kg body wt., i.p.), liver and kidneys were collected for investigation. The organs were immediately washed in ice-cold 0.9% NaCl, weighed and frozen at  $-80^\circ C$  for further analysis.

The activity of GPx, GR, GST in the liver and kidney was determined using commercial diagnostic kits. Known-weight slices of these organs were homogenized and centrifuged, according to procedures given by producers. In received supernatants the activities of GPx, GR and GST were measured using the Bioxytech GPx-340 (Oxis-Research, USA), Bioxytech GR-340 (OxisResearch) and

Cayman GST Assay (Cayman Chemical Company, USA) kits, respectively. In the supernatants the concentration of total protein was determined, according to the Lowry method modified by Peterson [27] as well.

### Statistical Analysis

Since there were no differences in any of the studied parameters between the two control subgroups the results have been presented together as one control group. Values are mean  $\pm$  S.E. of eight rats in each group. The calculations were made using Statistica 5.0 package (StatSoft, Tulsa, OK, USA). To evaluate statistically significant differences between experimental groups the Kruskal-Wallis one-way ANOVA was used. Spearman rank correlation analysis was performed to investigate the relationship between the activity of GST, GPx and GR. Moreover, the activities of GPx, GR and GST were correlated with GSH, Cd and MDA concentrations reported previously in these animals [6, 7]. Differences and correlations were considered statistically significant at  $p < 0.05$ . To discern the occurrence of interactions between Cd and EtOH, two-way analysis of variance (ANOVA/MANOVA, test F) was used. F values having  $p < 0.05$  were considered statistically significant. In order to show the statistically significant difference between the group of rats exposed to Cd and EtOH simultaneously and the groups receiving these xenobiotics separately, we did additional mathematical calculations to specify the character of the interaction. In order to do this we summed up the effect of exposure of Cd and EtOH separately (Cd effect + EtOH effect) and the obtained result was compared to the effect of Cd and EtOH action at the co-exposure (Cd + EtOH effect) received in this experiment. The effect was presented as a percentage of change of the studied parameters compared to the control group at the separate and simultaneous exposure to Cd and EtOH. On the basis of the obtained results it was estimated whether the interaction had an antagonistic (Cd + EtOH effect < Cd effect + EtOH effect), synergistic (Cd + EtOH effect > Cd effect + EtOH effect) or other character [28].

## Results

### Activity of GPx, GR and GST in Liver

In the rats exposed to Cd alone the liver activity of GPx and GST increased by 11% ( $p < 0.01$ ) and 17% ( $p < 0.01$ ), respectively, compared to control group, whereas the activity of GR decreased by 23% ( $p < 0.01$ ) (Fig. 1). The exposure to EtOH alone resulted in a decrease in liver activity of GPx (by 10%,  $p < 0.01$ ) and GR (by 17%,  $p < 0.01$ ), but had no influence on GST activity compared to the control group (Fig. 1). After simultaneous exposure to Cd and EtOH, the liver GPx activity

increased by 5% ( $p < 0.05$ ) and 17% ( $p < 0.001$ ) compared to the control and EtOH groups, respectively, but it decreased by 6% ( $p < 0.05$ ) compared to the Cd group. The activity of GR in the Cd + EtOH group was lower when compared to control group (by 17%,  $p < 0.05$ ) and did not change compared to the Cd and EtOH groups. The liver GST activity in the Cd + EtOH group increased compared to the control (by 11%,  $p < 0.05$ ) and EtOH (by 19%,  $p < 0.01$ ) groups (Fig. 1).

The analysis of variance revealed that changes in the activity of investigated enzymes in the liver of the rats co-exposed to Cd and EtOH resulted from an independent action of Cd (GPx and GST) or EtOH (GPx) (Table 1).

The liver activity of GPx negatively correlated with the concentration of GSH ( $r = -0.453$ ,  $p = 0.046$ ), whereas it positively correlated with the activity of GST ( $r = 0.662$ ,  $p = 0.000$ ), concentration of MDA ( $r = 0.397$ ,  $p = 0.025$ ) and concentration of Cd ( $r = 0.734$ ,  $p = 0.000$ ) in this organ. A positive correlation was noted between the liver activity of GR and the concentration of GSH ( $r = 0.370$ ,  $p = 0.037$ ). Moreover, the activity of this enzyme negatively correlated with MDA ( $r = -0.461$ ,  $p = 0.008$ ) and Cd ( $r = -0.388$ ,  $p = 0.028$ ) concentration. The liver activity of GST negatively correlated with the concentration of GSH ( $r = -0.524$ ,  $p = 0.041$ ). A positive correlation occurred between the GST activity and MDA ( $r = 0.495$ ,  $p = 0.004$ ) and Cd ( $r = 0.629$ ,  $p = 0.000$ ) concentration.

### Activity of GPx, GR and GST in Kidney

The exposure to Cd alone led to an increase in the kidney activity of GPx and GR by 21% ( $p < 0.01$ ) and 9% ( $p < 0.05$ ), respectively, compared to control (Fig. 2). In the EtOH group only an increase (by 31%  $p < 0.001$ ) in the kidney activity of GPx was noted compared to the control group (Fig. 2). The co-exposure to Cd and EtOH led to an increase (by 10%  $p < 0.05$ ) in the kidney activity of GPx compared to the control group and its decrease (by 16%  $p < 0.01$ ) compared to the EtOH group (Fig. 2). Moreover, the kidney GR activity increased by 17% ( $p < 0.01$ ), 7% ( $p < 0.05$ ) and 13% ( $p < 0.01$ ), respectively, compared to the control, Cd and EtOH groups (Fig. 2). The kidney GST activity was unchanged by any treatment (Fig. 2).

The ANOVA/MANOVA analysis revealed that the change in the kidney GPx activity in rats co-exposed to Cd and EtOH resulted from an independent action of EtOH and its interaction with Cd. The observed interaction had an antagonistic character compared to the animals exposed to EtOH alone (Table 2). The change in GR activity was caused by an independent action of Cd and EtOH (Table 2).

A negative correlation was noted between GR activity and in the kidney GSH concentration ( $r = -0.711$ ,  $p = 0.000$ ). Moreover, a positive correlation occurred between GR activity and the concentration of MDA ( $r = 0.533$ ,  $p = 0.007$ ) and Cd ( $r = 0.680$ ,  $p = 0.007$ ) in this organ.

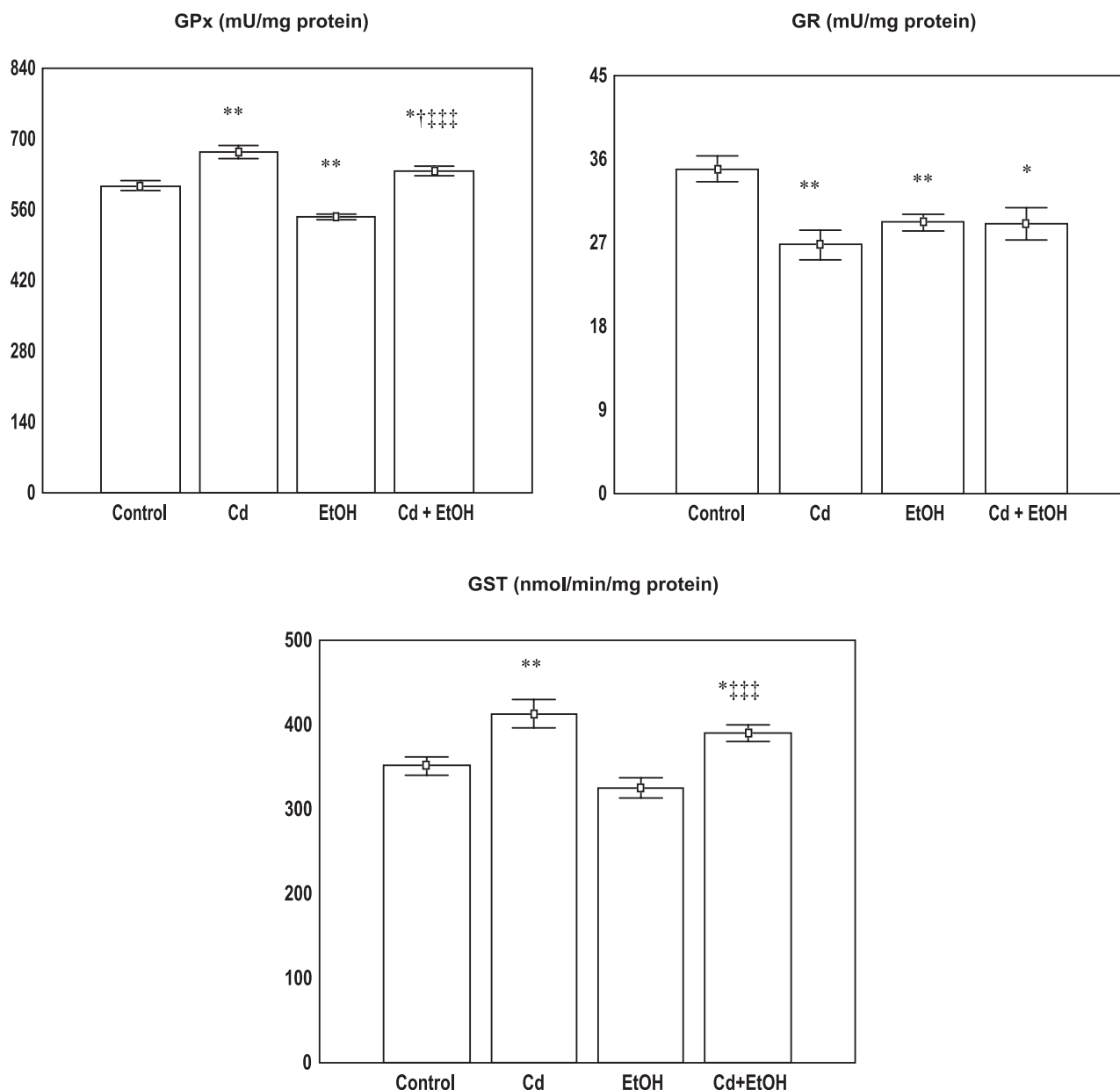


Fig. 1. Effects of cadmium (Cd), ethanol (EtOH) and their co-exposure on glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione S-transferase (GST) activity in the liver.

Values are means  $\pm$  S.E. for 8 rats. Statistically significant differences (Kruskal-Wallis one-way ANOVA) are indicated by \*  $p < 0.05$  and \*\*  $p < 0.01$  vs. control; †  $p < 0.05$  vs. Cd group; ‡‡‡  $p < 0.001$  vs. EtOH group.

## Discussion

We recently reported [9] that exposure to Cd and EtOH, both alone and in conjunction, resulted in a decrease in GSH concentration in the liver and kidney of rats. Our findings allowed for the conclusion that the Cd- and EtOH-induced decrease in this tripeptide concentration can be one of the mechanisms of peroxidative action of both xenobiotics in the liver and kidney. As a result, in the present study the activity of enzymes responsible for GSH homeostasis, such as GPx, GR and GST was estimated in the liver and kidney of rats exposed to Cd and EtOH alone and in conjunction.

The correlations noted between the liver activity of GPx and GR, and GSH concentration in this organ allow for the conclusion that a decrease in the concentration of this tripeptide might result from its utilization as an electron donor in the enzymatic system, which serves as a free radicals scavenger. Although relatively resistant to "spontaneous oxidation," GSH reacts rapidly and non-enzymatically with hydroxy radical, nitrogen oxide (III) and peroxynitrite [11, 29]. In addition to its action as a chemical antioxidant, GSH also acts in the enzymatic first line antioxidant defense as a co-factor in GPx-mediated reduction of peroxides [12, 13].



Table 1. Interactive effects of cadmium (Cd) and ethanol (EtOH) action regarding the activity of glutathione peroxidase (GPx) and glutathione S-transferase (GST) in the liver.

	GPx	GST
ANOVA/MANOVA analysis of interactive effect of Cd and EtOH		
Main effect of Cd	F = 64.296 p = 0.000	F = 25.200 p = 0.000
Main effect of EtOH	F = 25.808 p = 0.000	NS
Interactive effect of Cd and EtOH	NS	NS
Estimation of the character of the interaction between Cd and EtOH		
Character of interaction	Independent action	Independent action

F – the coefficient calculated with ANOVA/MANOVA; NS, not statistically significant effect

Table 2. Interactive effects of cadmium (Cd) and ethanol (EtOH) action regarding the activity of glutathione peroxidase (GPx) and glutathione reductase (GR) in kidneys.

	GPx	GR
ANOVA/MANOVA analysis of interactive effect of Cd and EtOH		
Main effect of Cd	NS	F = 34.058 p = 0.000
Main effect of EtOH	F = 5.580 p = 0.025	F = 9.320 p = 0.005
Interactive effect of Cd and EtOH	F = 26.348 p = 0.000	NS
Estimation of the character of the interaction between Cd and EtOH		
Character of interaction	Cd + EtOH effect < Cd effect + EtOH effect Antagonism	Independent action

F – the coefficient calculated with ANOVA/MANOVA, NS, not a statistically significant effect.

The decreased liver activity of GPx due to EtOH administration might result from the involvement of this enzyme in the dismutation of peroxides generated during EtOH biotransformation [5, 30, 31]. Because GSH is utilized in the GPx-mediated reactions, an enhanced intensity of these reactions might also be a cause of the previously reported decrease in GSH concentration in the liver of the EtOH-treated rats [9]. Decreased GPx activity after chronic exposure to EtOH was also noted by other authors [31, 32, 33]. In a reaction catalyzed by GPx, an oxidized form of GSH – GSSG is formed. GSSG under the influence of GR and reduced nicotinamide adenine dinucleotide phosphate (NADPH) is reduced to GSH [34, 35]. The decrease in liver GR activity in the rats treated with EtOH might result from this enzyme utilization to GSSG reduction or from NADPH deficiency. Glucose-6-phosphate dehydrogenase (G6PD) is

responsible for maintenance of proper NADPH concentration. It was noted that in conditions of chronic exposure to EtOH the activity of this enzyme was reduced and may lead to a decrease in NADPH concentration, which is necessary for proper functioning of GR [21, 36]. On the basis of the results received we hypothesize that in the liver of rats exposed to EtOH the peroxidase/glutathione reductase cycle did not function properly. The increase in GPx activity with the simultaneous decrease in GR activity in the liver of Cd-exposed rats suggests that the peroxidase/glutathione reductase system functioned normally. The changes in the liver activity of GPx and GR in these rats might be a protective response to peroxides generated under the Cd-induced lipid peroxidation. Despite the proper functioning of the peroxidase/glutathione reductase cycle, the concentration of GSH in the liver of rats exposed to Cd was decreased [9]. The increase in GST activity in the liver after exposure to Cd suggests that GSH might be utilized in the reaction of conjugation. GST catalyzes reactions of toxic substances conjugation with GSH [16-18]. GST, like metallothionein (Mt), can be induced in cells by various factors including Cd [37]. Complexes of GSH with other metals can also be formed in non-enzymatic reactions. GSH is one of the essential ligands, which play a vital role in transport, storing and metabolism of metals, including Cd [38, 39]. Formation of the GSH complexes in the enzymatic and non-enzymatic reactions leads in consequence to a decrease in GSH concentration.

Similar changes in the liver activities of GPx, GR and GST in rats co-exposed to Cd and EtOH as in those treated with Cd alone seem to indicate the main effect of Cd on the activity of these enzymes. The ANOVA/MANOVA analysis confirmed the independent and dominant influence of Cd on the antioxidant enzymes under co-exposure with EtOH, particularly in the case of change in GST activity. The change in the liver activity of GPx in animal co-exposure to Cd and EtOH was caused by the independent action of Cd or EtOH, Cd in particular. However, the independent action of EtOH, as opposed to Cd, might be a reason why the observed increase in GPx liver activity in animal co-exposure to both xenobiotics was lower than after exposure to Cd alone.

The negative correlations between GPx, GR and GST activity, and GSH concentration in the liver suggest that the decrease in the liver GSH concentration due to co-exposure to Cd and EtOH might result from its utilization in the reactions of free radicals scavenge and S-conjugation.

The changes of GPx and GR activity and the unchanged GST activity in the kidney of rats exposed to Cd alone suggest that the reduction of GSH concentration in this organ [9] might result from GSH utilization in anti-oxidative reactions. The increase in GPx and GR activity after exposure to Cd can be an adaptative response of this organ to the lipid peroxidation enhanced by Cd [8]. The influence of EtOH on the kidney activity of the examined antioxidative enzymes was rather minor. It might result from a different participation of the kidney in Cd and EtOH metabolism. In spite of this, the increase in GPx ac-

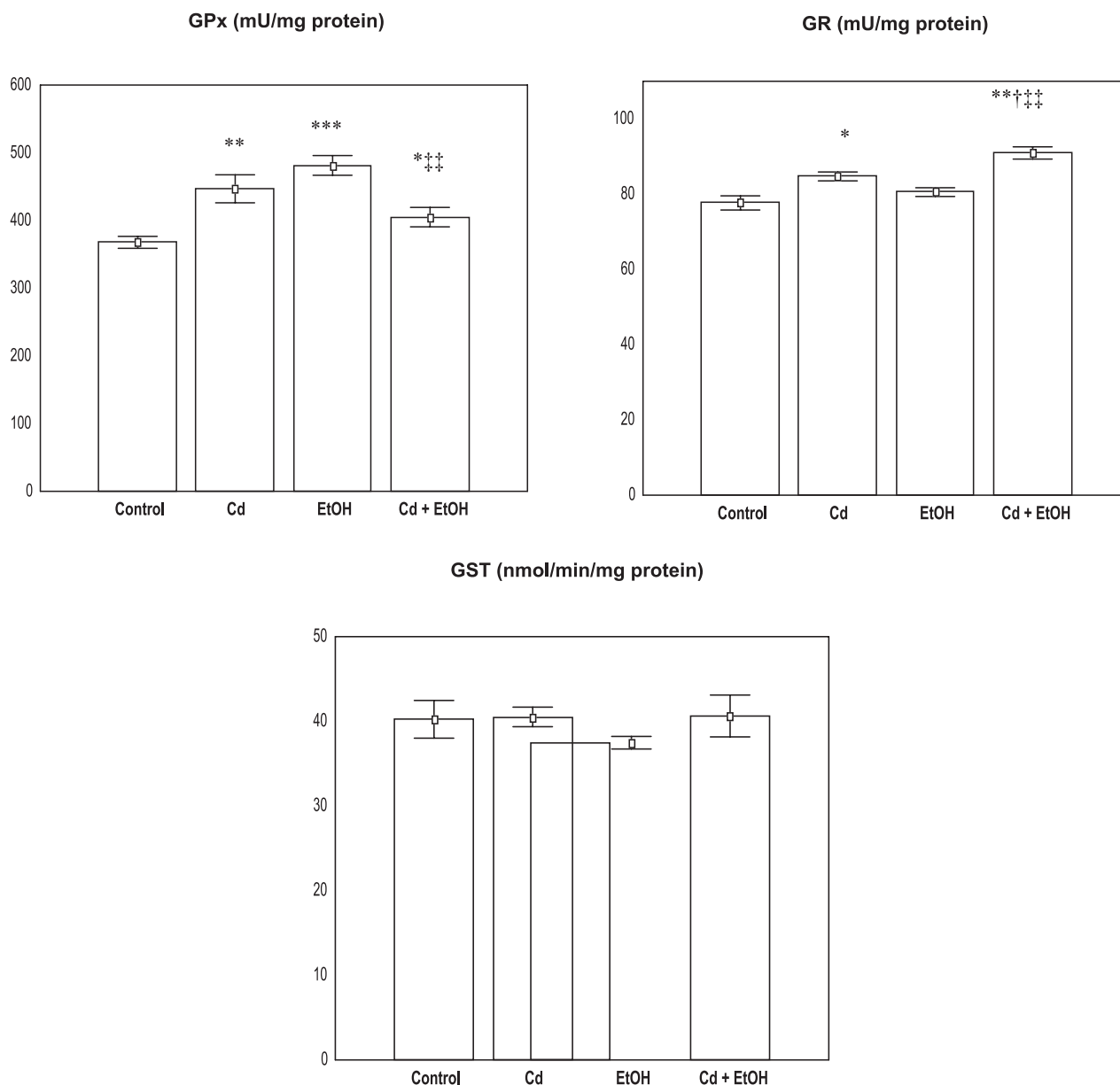


Fig. 2. Effects of cadmium (Cd), ethanol (EtOH) and their co-exposure on glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione S-transferase (GST) activity in the kidney.

Values are means  $\pm$  S.E. for 8 rats. Statistically significant differences (Kruskal-Wallis one-way ANOVA) are indicated by \*  $p < 0.05$ , \*\*  $p < 0.01$  \*\*\*  $p < 0.001$  vs. control; †  $p < 0.05$  vs. Cd; ††  $p < 0.01$  vs. EtOH group.

tivity in the kidney of the EtOH-exposed rats together with an unchanged MDA concentration noted in these animals [8] can suggest that the defensive antioxidative system of the kidney might act properly and prevent EtOH-induced oxidative stress [22].

The increase in kidney activity of GPx at the co-exposure to Cd and EtOH resulted mainly from the interactive action of both xenobiotics. The observed interaction had an antagonistic character. This might be why the increase in the Cd + EtOH group was lower than in the EtOH group. On the other hand, the increase in GR activity at the simultaneous exposure to Cd and EtOH was caused by an independent action of the two substances.

On the basis of the received results it can be supposed that the changes of activities of GPx, GR and GST may be one of the crucial factors contributing to the decrease in GSH concentration in these organs after exposure to Cd and EtOH, both alone and in conjunction with each other. The estimation of changes of the antioxidative enzyme activities cannot completely explain the mechanisms leading to the decrease in GSH concentration at the exposure to Cd and/or EtOH. The reduction of GSH might result not only from its utilization in redox reactions or from disorders of its regeneration but also from its improper synthesis. Further studies are necessary to better estimate the mechanisms of Cd and EtOH influence, especially at co-exposure, to GSH concentration.

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