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ORIGINAL RESEARCH

The Toxic Effects of Ethylene Glycol Tetraacetate Acid, Ferrum Lek and Methanol on the Glutathione System: correction Options

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ABSTRACT

Objectives: The purpose of this study was to measure the level of lipid peroxidation and investigate the response of the glutathione system to toxic doses of ethylene glycol tetraacetate acid (EGTA), Ferrum Lek, methanol, and Depakine (valproate sodium).

Methods: This study focused on analyzing the toxic effects of EGTA, Ferrum Lek and methanol on lipid peroxidation processes and glutathione levels in animals. The study involved 375 outbred adult mice, of both sexes, weighing 28–31 g, and 100 outbred rats, weighing 180–200 g.

Results: After 14 days of valproate sodium/ademethionine treatment, the GR (glutathione reductase) activity in experimental animals continued to be higher than in controls. Using EGTA enhanced glutathione reductase and glutathione S transferase activities in the liver and kidney. The activity of glutathione peroxidase, however, increased only in the kidney (2.1-fold, $p \leq 0.001$), while in the liver, a 31% drop was observed ($p \leq 0.05$). The 15-mg and 30-mg doses of Ferrum Lek caused the liver level of thiobarbituric acid reactive substances to grow 3- and 3.5-fold, respectively ($p \leq 0.001$).

Conclusion: The results of the study indicate that poisoning affected practically all components of the glutathione system. The oxidative stress was likely to result from an increased generation of reactive oxygen species against the background of inhibited antioxidant protection.

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KEYWORDS
Xenobiotics; EGTA; ferrum Lek; methanol; depakine; glutathione

1. Introduction

Continuous exposure to various oxidants (such as production waste, stabilizers, thickeners, flavoring agents, drugs, and household chemicals) can increase the annual incidence of acute poisoning. At present, the incidence is about 600.000 cases in the U.S. and 570.000 cases in Russia. At the same time, the rate of lethal poisonings shows no dramatic decline.

Using pro-oxidant substances, such as xenobiotics, may intensify the process of lipid peroxidation [1,2]. The oxidative degradation of lipids can in turn, disrupt the oxidant/antioxidant balance in favor of oxidants. When this happens, a human body (primarily liver) experiences a rise in the levels of lipoperoxide, fatty acid radicals, ketones, aldehydes, and keto acids. The consequences include damage to cell membranes, increase in their permeability, and oxidative modification of structural proteins, enzymes, and biologically active substances. One of the essential antioxidants in a defense system is glutathione. Among other functions, it supports xenobiotic and metabolic detoxification reactions and influences enzyme activity [3,4]. From this perspective, glutathione metabolism may affect the body's resistance to toxicants. Currently, there are some glutathione-based drugs to avoid toxicity and treat affected tissues [5]. Thus, measuring glutathione levels in the blood is a high-potential solution to assess poisoning severity [6]. Previous studies were concerned with the combined effect of glutathione and vitamin E on ethanol toxicity in liver cells or hepatocytes of laboratory rats [7]. Other studies dealt with the antioxidant potential of natural products [8] and medicinal plants [9]. Another topic area includes the effect of carbofuran toxicity on redox states in rat brain and heart [10–12].

Antioxidants are crucial in treating poisoning due to various toxicants, but their effect on the formation of glutathione remains poorly understood. The findings of this study may help expand our understanding of the lipid peroxidation processes, glutathione system, and glutathione-dependent metabolism. The results may also provide an opportunity to deeper understand the antioxidant activity and differentially decide on the antioxidant therapy.

The purpose of this study was to measure metabolite levels and activities of glutathione metabolism enzymes in organs of experimental animals after the intracardiac administration of EGTA. The objectives of the study were to investigate the effect of protective agents that counteract the examined medicines and develop methods for the correction of revealed abnormalities.

The research hypothesis is that agents, such as EGTA, Ferrum Lek, and methanol, diminish the glutathione system of the mouse liver. Hypothetically, high doses of Ferrum lek will activate glutathione reductase, glutathione transferase and glutathione peroxidase.

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Article Highlights

- EGTA enhances free radical processes in rat and mice tissues.
- Decreased GP activity and decreased GSH together diminish the antioxidant defense system.
- MDA after CPA is within the normal range, but GSH continues to be low.
- L normalizes MDA, but causes GSH to grow.
- Synergistic action of CPA+ L +EGTA reverts the GSH and enzyme activities to normal.

2. Materials and Methods

2.1. Materials

The study on 375 outbred adult mice, of both sexes, weighing 28–31 g, and 100 outbred rats, weighing 180–200 g, investigates a dose-dependent effect of medication, such as EGTA, lipoic acid, N6-cyclopentyladenosine (CPA), and lidocaine (L), on the concentration of xenobiotic compounds. A dose exhibiting the desired effect in half of the studied population is considered to be the most effective one. In regards to xenobiotics, the most effective dosage is also a semi-lethal one, which kills 50% of animals in the study sample.

2.2. Animals and Regimens

For EGTA experiments, 90 mice were divided into six groups consisting of 15 mice each, weighing 28 to 31 grams. The first group (the control) received no medication. Mice in the second group were injected with EGTA intracardially (1.9 mg/100 µL). Mice in the third group were administered 100 mg/kg of lipoic acid once peritoneally 72 h after the EGTA challenge. The fourth grouped received CPA subcutaneously at a dose of 2.4 mg/kg 3 h before EGTA. Group 5 was subcutaneously administrated with lidocaine (50 mg/kg) 15 min after the EGTA challenge. Group 6 mice were given CPA, followed 3 h later by lidocaine, which was, in turn, followed 15 min later by EGTA. All mice were anesthetized by inhalation of fluorotan. For the assays, the supernatant from mouse liver and kidney homogenates was used.

For Ferrum Lek, and Ferrum Lek/lipoic acid experiments, 135 mince were divided into nine groups. The first group (the control) received no medication. Other eight groups received different doses of Ferrum Lek (5, 15, 30, 75 mg/day) subcutaneously for a week. Doses of Ferrum Lek were given alone and in combination with the intraperitoneal injection of lipoic acid (25 mg/kg per day).

Glutathione responses to methanol, alone and in combination with lipoic acid and ethanol, were investigated on a sample of 170 mice divided into 15 groups with each group containing ten mice. The first group (the control) received no medication. Mice injected with methanol at different doses were followed for different intervals of time: groups 2 to 5 (40 and 80 mg) 2 and 4 h, groups 6 to 7 (62 mg) 4 and 7 h, groups 8 and 9 (324 mg) 4 h and 7 h. Mice in group 10 were administered 162 mg of ethanol plus 162 mg of methanol and followed for 4 h. Mice in group 11 were given 162 mg of ethanol in combination with 324 mg of methanol and followed for 7 h. Mice in group 12 were injected with 162 mg of ethanol plus 324 mg of methanol and followed for 10 h. Mice in group 13 were administered 100 mg/kg of lipoic acid in combination with 162 mg of methanol and followed for 7 h. Mice in group 14 received lipoic acid plus 324 mg of methanol and were followed for 4 h. Finally, mice in group 15 were administered lipoic acid in combination with 324 mg of methanol and followed for 16 h. Methanol was administered intraperitoneally via a 0.9% NaCl injection. Ethanol and lipoic acid were also given intraperitoneally.

The Depakine (valproate sodium) experiments were carried out on 100 laboratory rats. A challenge dose of Depakine (600 mg/kg/day) was administered intragastrically for 14 and 28 days. Possible side effects were counteracted with intraabdominal administration of ademethionine (1000 mg/kg/day) given during the whole period of Depakine intake. All rats were divided into five groups of 20 each. Group 1 rats did not receive an anticonvulsant treatment. Rats in the second group were given anticonvulsants daily for 14 days. Rats in the third group received anticonvulsants daily for 28 days. Finally, rats in groups 4 and 5 received Depakine in combination with ademethionine daily for 14 and 28 days, respectively. The average pH of blood was 6.4 ± 0.5.

2.3. Procedures

Before spectrophotometric enzyme assay, all tests were carried out at 4°C. Animals underwent euthanasia, and their livers and kidneys were harvested using the cervical dislocation technique. The study samples (blood, liver, and kidney) were obtained from decapitated animals. The decapitation procedure was carried out under ether and chloroform anesthesia in accordance with the European Directive 86/609/EEC and the Helsinki Declaration.

The liver and kidney were perfused with cold saline solution and then homogenized in an isolation medium containing 0.005 N Tris and 0.1 N KCl in 1:100 (liver) and 1:50 (kidney) ratios. The resulting homogenate was centrifuged. All samples were blotted on filter paper, weighed, and then homogenized in a test tube immersed in an ice bath. Homogenization was carried out in a mortar with the help of a pestle, sterile sand, and Hanks solution. Homogenates made up 10% of the total volume of organs harvested. This study employed standard spectrophotometric assays to measure glutathione (GSH) levels [13] and enzyme activities, namely glutathione S transferase or GST [14], glutathione reductase or GR [15], glutathione peroxidase or GPx [16] and gamma-glutamyl transferase or GGT. Lipid peroxidation was measured using the thiobarbituric acid reactive substances (TBARS) assay [17,18].

2.4. Data Analysis

Findings were statistically processed via Fisher's LSD test, Student's t-test, and Welch's t-test [19]. Welch's t-test was applied for assessing the equality of mathematical expectations. Fisher's LSD test was employed to identify samples with equal means, and Student's t-test was applied to compare the means of two

Table 1. Drug Trial Layout.

Poisoner	Dosage	Active-Treatment Period	Correction Substances	Animals
EGTA	1.9 mg per 100 µL	-	ALA, CPA, Lidocaine	90
Ferrum Lek Iron (III) hydroxide polymaltosatum	5, 15, 30, and 75 mg	7 days	ALA	135
Methanol	40.5, 81, 162, and 324 mg	2, 4, 7, 10 and 16 hours	Ethanol, ALA	150
Depakine	600 mg/kg	14 and 28 days	Ademethionine	100

Note: EGTA – ethylene glycol tetraacetate acid; CPA – N6-Cyclopentyladenosine; ALA – α-lipoic acid

populations. Differences were considered significant at $p < 0.05$. The drug trial layout is presented in Table 1.

2.5. Ethical Statement

All procedures performed in studies involving animal participants were in accordance with the International Guiding Principles for Biomedical Research Involving Animals, the European Directive 86/609/EEC, the Helsinki Declaration, and with standards and bioethical norms approved by the Ethics Committee of the I.M. Sechenov First Moscow State Medical University.

3. Results

A comparative analysis showed that poisoning due to toxicants has led to elevated lipid peroxidation and a decline in the reduced GSH level. These findings were not toxicant-dependent. Hence, one can assume that the above trends can occur no matter which toxicant affects the body. Nevertheless, the enzyme response to drug toxicity varied between enzymes, and the intensity of oxidative stress (OS) thus altered.

Injecting EGTA caused the level of TBARS to increase by 75% ($p \leq 0.01$) in the kidney and decrease by 26% in the liver ($p \leq 0.05$). The GSH level fell by 30% and 54% in the liver and kidney, respectively ($p \leq 0.05$). Using EGTA enhanced GR (by 51% and 62%, $p \leq 0.01$) and GST (by 41%, $p \leq 0.05$; and 5.3-fold, $p \leq 0.001$) activities in the liver and kidney. GPx activity was only found to increase in the kidney (2.1-fold, $p \leq 0.001$), while in the liver, it declined by 31% ($p \leq 0.05$) (Figure 1).The kidney GPx activity was 54% lower ($p \leq 0.01$) when compared to normalized GR and GSTs activities. The liver GR activity continued to be elevated and the liver GSTs activity increased by 131% ($p \leq 0.001$).

When lidocaine was administrated in combination with EGTA, no alterations in the TBARS level were detected. However, there was a 21% increase in the liver GSH level ($p \leq 0.05$). The liver GPx activity sharply increased (2.3-fold, $p \leq 0.01$), while the kidney GPx activity did not alter and continued to be elevated (2.1-fold, $p \leq 0.01$). The liver GR activity decreased (by 31%) and the kidney GR activity was reverted to normal (Figure 1). The kidney GST activity decreased slightly but remained elevated.

The synergistic action of CPA, lidocaine, and EGTA prevented the activation of lipid peroxidation, as evidenced by

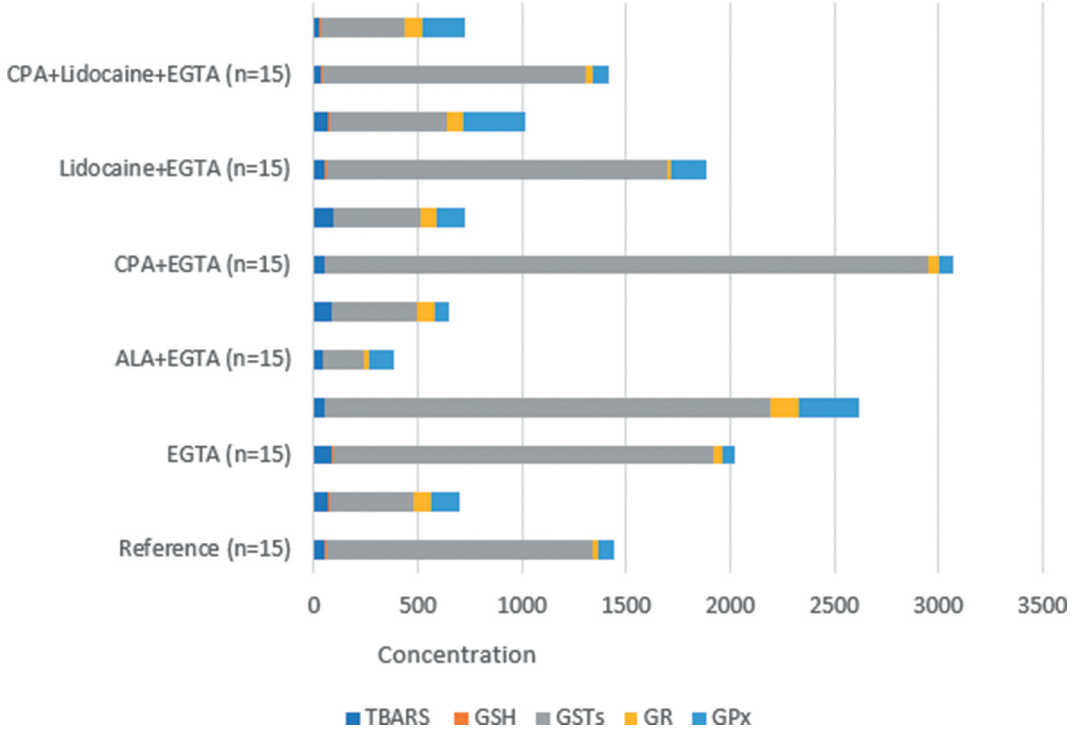

Figure 1. Effect of EGTA on glutathione concentrations, TBARS levels and enzyme activities in the mouse liver (upper bar) and kidney (lower bar).
Note: enzyme activity is expressed as nmol/min/mg of protein; GSH level is expressed as µmol per gram of tissue; TBARS level is expressed as nmol per gram of tissue.

a decrease in the liver and kidney TBARS levels (declined by 34% and 58%, respectively, $p \leq 0.05$). The kidney GPx activity has increased by 44% ($p \leq 0.05$). By contrast, administrating CPA alone normalized the liver and kidney GPx activities, and the lidocaine-only treatment caused a sharp increase in the liver GPx activity (Figure 1).

A 5-mg dose of FL increased the liver and kidney TBARS levels by 57% and 26%, respectively ($p \leq 0.05$). It also reduced the kidney GSH level by 30% ($p \leq 0.05$). Higher doses were associated with the elevation of the TBARS level and with the lowering of the GSH level. For instance, 15-mg and 30-mg doses of FL caused TBARS levels to grow 3- and 3.5-fold in the liver ($p \leq 0.001$) and by 60% and 72% in the kidney ($p \leq 0.05$). Furthermore, GSH levels were induced to decrease by 33% and 62% in liver ($p \leq 0.05$) and by 44% and 50% in kidney ($p \leq 0.05$). After a 75-mg dose of FL, the liver and kidney TBARS levels were found to be on a dramatic rise (increased by 393% and 141%, respectively, $p \leq 0.01$). The liver and kidney GSH levels declined by 77% and 64%, respectively ($p \leq 0.05$).

Administrating FL increased the liver GR activity to different extents depending on the dose: 5-mg dose by 39%; 15–30-mg dose by 62% ($p \leq 0.05$); and 75-mg dose by 125% ($p \leq 0.01$). The kidney GR activity increased by 76% ($p \leq 0.05$) with a minimum dose of FL, but with a 15-mg dose of FL and higher, it gradually decreased (by 27%, 34% and 39%, respectively, $p \leq 0.05$).

The liver GSTs activity increased by 56% after a 75-mg dose of FL, while the kidney GSTs activity increased by 55% after a minimum dose of medication ($p \leq 0.05$). The 15-mg dose of FL caused GSTs activity to increase but it was lower than after a 5-mg dose. A 30-mg dose of FL caused a 100% increase in the GSTs activity, but the most prominent change (a 185% increase) was detected after the maximum dose of 75-mg ($p \leq 0.01$).

The liver GPx activity increased by 29% and 42% with higher doses of 30 mg and 75 mg, respectively ($p \leq 0.05$), whereas the kidney GPx activity dropped immediately after the minimum challenge dose and remained reduced with higher doses ($p \leq 0.05$). The activity of liver GGT, the only enzyme that breaks sown GSH, increased sharply (3.1-fold, $p \leq 0.001$) with a 15-mg dose of FL and continued to increase with higher doses. With a 75-mg dose, it became higher 4.6-fold as compared to controls ($p \leq 0.001$). The kidney GGT activity demonstrated a decreasing trend after the minimum dose of Ferrum Lek and continued to fall with higher doses (Figure 2).A moderate increase in liver GSTs and GPx activities could result from the induction caused by oxidative stress. Such a trend may be considered a favorable factor. A significant increase in the kidney GSTs activity (55% to 185%, $p \leq 0.01$) undoubtedly was a result of induction.

A 15-mg dose of Ferrum Lek co-administrated with ALA normalized the liver GSH level. When 30-mg and 75-mg doses of FL were administrated in combination with ALA, liver GSH levels were found to be higher than after the FL-only treatment. They remained reduced through. This has influenced the liver TBARS level, resulting in the smaller increase in all cases: 74% vs 198% after the 15-mg dose ($p \leq 0.01$), 117% vs 249% after the 30-mg dose ($p \leq 0.001$), and 271% vs 393% after the 75-mg dose ($p \leq 0.001$).

Using methanol diminished lipid peroxidation, decreased the level of GSH, and reduced the liver and kidney enzyme activities (Figure 3). Data showed that these changes depend on the drug dose and exposure time.The first marker decreased by 29%, whereas the second one demonstrated a 34% increase ($p \leq 0.05$). With 80 mg of methanol, alterations detected within the 2- and 4-h intervals were more pronounced than with the lower dose of methanol. The liver TBARS levels grew by 76% and 139% ($p \leq 0.01$), respectively, whilst the kidney TBARS levels increased by 48% and 75% ($p \leq 0.05$).

Higher doses of methanol (162 mg and 324 mg) were associated the symptoms of active oxidative stress. For

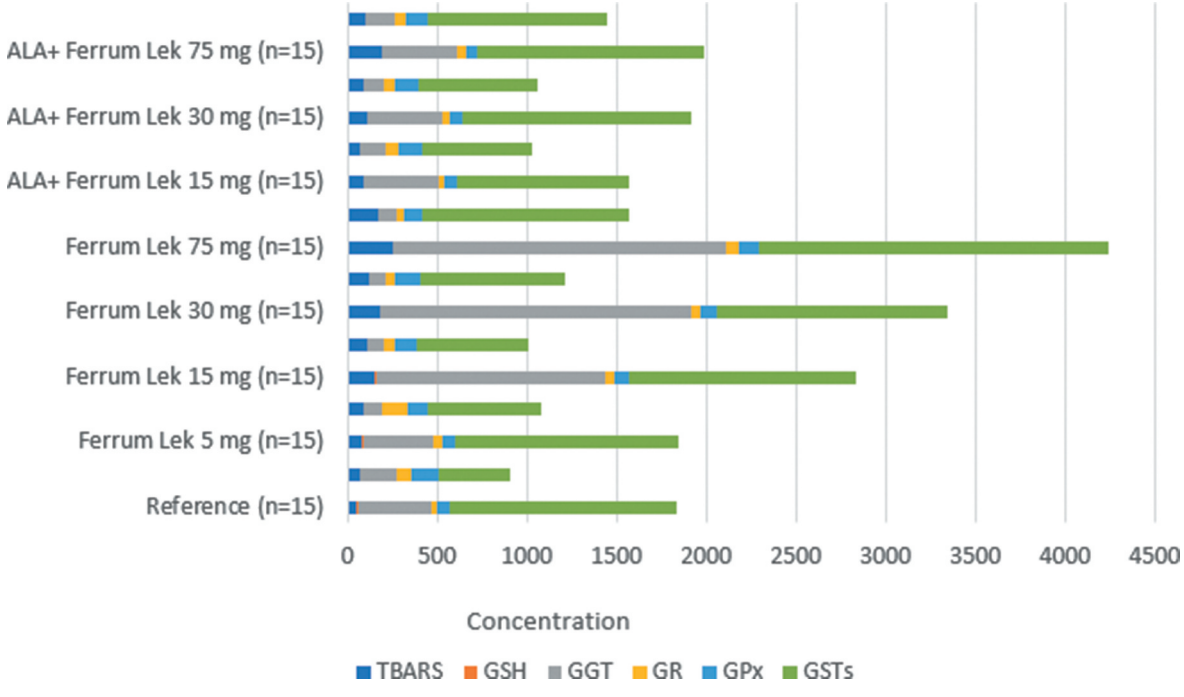

Figure 2. Effect of Ferrum Lek on glutathione concentrations, TBARS levels and enzyme activities in the mouse liver (upper bar) and kidney (lower bar).

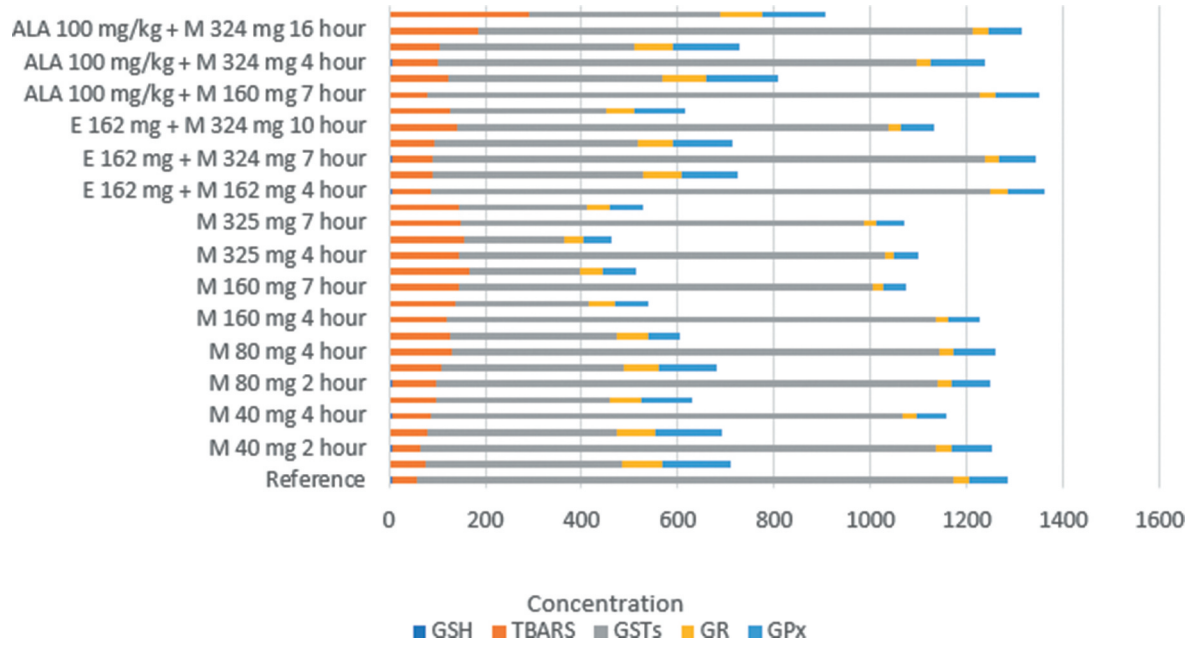

Figure 3. Effect of methanol on glutathione concentrations, TBARS levels and enzyme activities in the mouse liver (upper bar) and kidney (lower bar).

instance, liver GSH levels declined to an extent of 29% and 54%, respectively ($p \leq 0.05$), and kidney GSH levels decreased to an extent of 50% and 75% ($p \leq 0.05$). Liver TBARS levels increased 119% and 177% ($p \leq 0.05$), whereas kidney TBARS levels increased 88% and 144% ($p \leq 0.01$) (Figure 3).

Four h after the ethanol administration, liver and kidney TBARS levels remained elevated ($p \leq 0.05$). Furthermore, liver and kidney GSH levels continued to be below the norm (by 12% and 60%, respectively, $p \leq 0.05$).

Seven h after the lipoic acid administration, liver TBARS level was found normalized, but the kidney TBARS level continued to be reduced by 51% ($p \leq 0.05$). The enzyme activities were close to those in the control group.

Mice exposed to a lethal dose of methanol (324 mg) died 7 h after the challenge. Those mice that additionally received ethanol had a longer lifespan and were around for 10 h. However, the liver and kidney TBARS levels continued to be elevated, by 134% ($p \leq 0.01$) and 66% ($p \leq 0.05$), respectively (Figure 2). The liver and kidney GSH levels were lower by 34% and 52% as compared to controls ($p \leq 0.05$).

Administrating lipoic acid prolonged the mouse life to 16 h. However, the liver and kidney TBARS levels were caused to rise

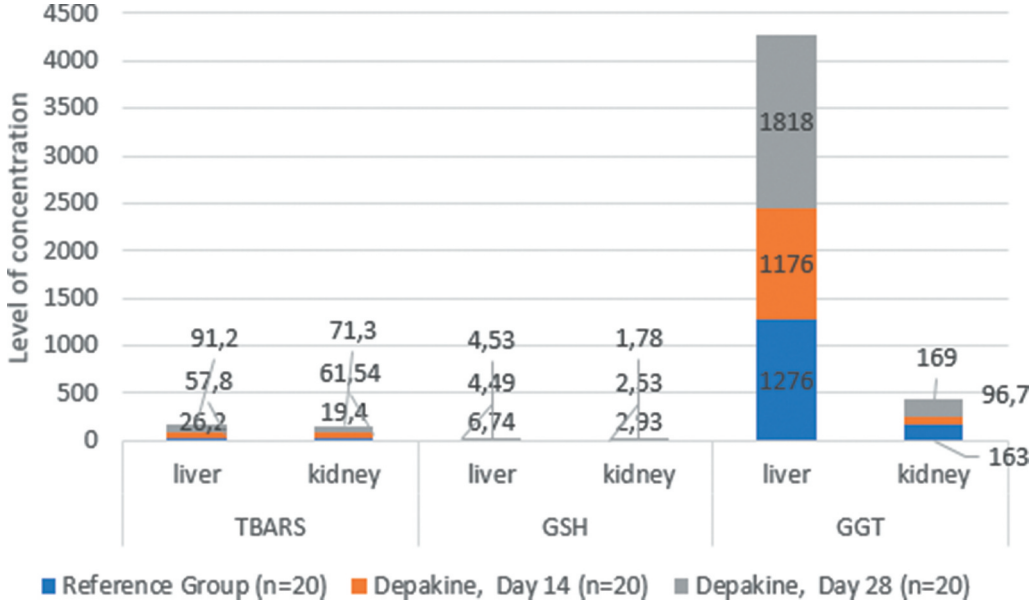

Figure 4. TBARS, GSH and GGT Activity in Tissues on Days 14 and 28 after Depakine Administration.

dramatically by 236% and 314%, respectively ($p \leq 0.001$). The enzyme activity returned to normal by contrast. In combination with lipoic acid, high doses of methanol (162 mg and 324 mg) increased the level of TBARS in liver (by 30% and 62%, respectively) and kidney (by 46% and 51%, respectively).

A 162-mg dose of methanol was toxic but did not kill the mice. A dose of 324 mg was very toxic and killed the animals 7 h after administration. Injecting methanol in combination with ethanol increased the lifespan of mice by 42%, up to 10 h, whilst the α-lipoic acid increased their lifespan 2.2-fold, up to 16 h ($p \leq 0.001$).

The liver and kidney GSH levels in mice were found to fall when compared to controls (Figure 4). The longer intake was followed by 17% and 16.4% decrease in the liver and kidney GSH levels, respectively. With a daily intake of valproate sodium, rats showed prominent shifts in the enzyme activities. The 14-day treatment with 600 mg/kg of anticonvulsant agent caused the liver GR activity to increase by 99.3% ($p \leq 0.01$). The liver GPx activity became higher by 72% on the 28th day of the challenge as compared to that in controls ($p \leq 0.05$).The most dramatic change of enzyme activity was detected in the kidney. For instance, on day 14 of the anticonvulsant treatment, the GR activity reached its peak and showed a 115% increase when compared to controls ($p \leq 0.01$).

The findings showed that valproate sodium has induced a pronounced oxidative stress, resulting in high level of TBARS. It also caused liver and kidney GSH levels to fall and liver GGT activity to increase (Figure 4).

Administrating valproate sodium in combination with ademethionine influenced the activity of GR stronger when compared to other enzymes (Figure 5). On day 7 of intake, for example, the liver and kidney GR activities increased as compared to controls (by 85% and 80.3%, respectively, $p \leq 0.01$). Significant changes were also found on day 14. The GR activities in liver and kidney continued to be higher (by 94.6% and 129.6%, respectively, $p \leq 0.01$). By contrast, the kidney GPx activity decreased (by 43.1%) on day 7 and continued to decline, dropping 46.3% on day 14 as compared controls ($p \leq 0.05$).The above results indicate that poisoning affected practically all components of the glutathione system. The oxidative stress was likely to result from an increased generation of reactive oxygen species against the background of inhibited antioxidant protection.

The glutathione response aside, toxicity was found to disturb the enzymatic (catalase, superoxide dismutase) and non-enzymatic antioxidants in the defense systems.

4. Discussion

It is important to find a correlation between glutathione levels and acute exposure to toxic chemicals because, with this information, healthcare specialists will be able to offer better pharmacological treatment and give more accurate indications. There are two universal markers of intoxication-induced disturbances to glutathione system: an increase of peroxidation products and a drop in reduced glutathione. EGTA has the potential to reduce glutathione peroxidase activity, but the side effect is the increase in the activity of glutathione reductase and glutathione transferase in the mouse liver. If combined with N6-cyclopentyladenosine or lidocaine, EGTA is projected to normalize the reduced glutathione content and three enzyme activities (glutathione reductase, glutathione transferase, and glutate peroxidase). That may also prevent the activation of lipid peroxidation.

Additionally, the use of this drug may dramatically increase the gamma-glutamyl transferase activity. The Ferrum lek/lipoic acid combination is expected to significantly reduce the activation of lipid peroxidation, to smooth the reduced glutathione decrease, and to normalize glutathione peroxidase and gamma-glutamyl transferase activities in the liver. Methanol poisoning is a source of strong stress affecting the glutathione system, which, as expected, will lead to a decrease in enzyme activity both in the liver and the kidney. Ethanol and lipoic acid prevent negative changes, but the lipoic acid is expected to perform better. Finally, Depakine may activate gamma-glutamyl transferase and reduces glutathione transferase activity when alone and prevent the activation of lipid peroxidation if co-administered with ademethionine.

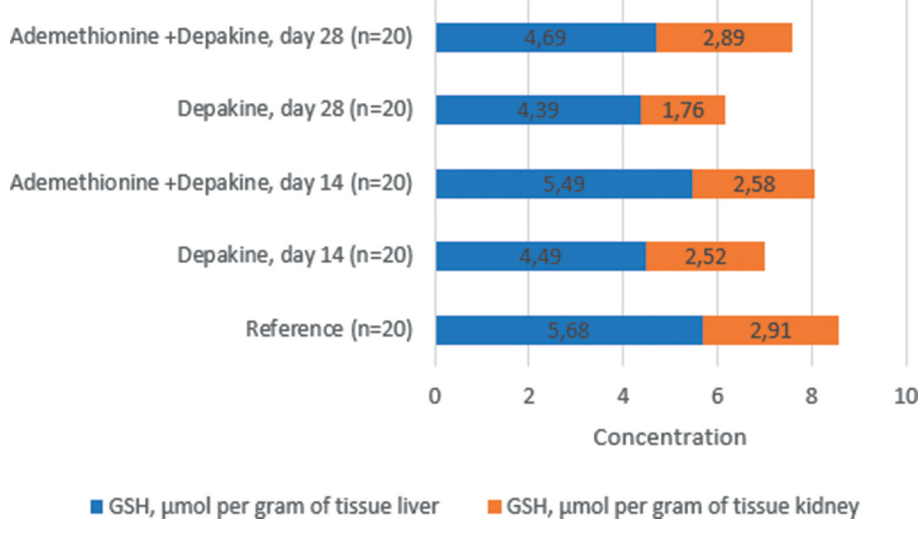

Figure 5. Liver GSH Level in Rats on Days 14 and 28 after ademethionineco-administration with Depakine.

The EGTA administration was fraught with side effects, such as cardiac arrest and global cerebral ischemia. Golapel reported that EGTA has reduced the lifespan of mice to 34 sec after administration [20]. The combination of EGTA with CPA and lidocaine increased the life expectancy of animals to 2 min and 20 sec. However, only 37% of the mice survived. These data prove that N6-cyclopentyladenosine has a high protective effect.

ALA failed to completely prevent disturbances to the glutathione system during the EGTA treatment. CPA, on the other hand, was found to be the best protector. Adenosine is a widely recognized inhibitory neurotransmitter and CPA acts as a selective adenosine A1 receptor agonist. Therefore, the glutathione system can be influenced either by adenylate cyclase inhibition or by phospholipase C activation, which enhances the production of inositol trisphosphate, diacylglycerol and calcium mobilization. The CPA/lidocaine combination was more effective in protecting against toxicants than CPA or lidocaine alone. Using EGTA in combination with CPA and lidocaine helped prevent the activation of lipid peroxidation and normalize the GSH level and enzyme activities. When administrated alone, CPA, lidocaine, and ALA failed to provide such an effect.

Iron therapy is a standard for treating patients with iron deficiency anemia. Among other functions, iron promotes the formation of reactive oxygen; it stimulates cellular defenses when in low concentrations and causes oxidative stress when in high concentrations. The oxidative modification in many cellular structures causes damage to the genetic apparatus of the cell [21,22]. Studies exploring the properties of iron salts and iron-containing medicine provide data on their ability to activate free radical processes and thus cause oxidative stress [23–26].

Methanol poisoning most often occurs when methanol is used instead of ethanol by mistake. Industrial poisoning with methanol vapors is less common. During alcohol intoxication, free radical oxidation intensifies primarily due to suppression of the antioxidant system [27–29].

In this study, kidney GGT activity was initially high. Therefore, its decrease can be viewed as a compensatory response to preserve the initial GSH level. A decrease in GR and GPx activities may indicate the minimization of redox imbalance and adaptation.

Thus, differences in the effect of Ferrum Lek on enzyme activities between tissues are evident. Higher doses of Ferrum Lek caused marked oxidative stress and a decrease in the liver and kidney GSH levels. In the liver, this effect was enhanced by high GGT activity and in kidney, it was aggravated by a decrease in GR and GPx activities. Hodkova proved iron (III) inductive to lipid peroxidation [24].

The GSH deficiency may indicate a weakening of the antioxidant system and be the earliest marker of increased oxidation processes taking place in cells. Such a sharp increase in TBARS concentration indicates that Depakine produces oxidative stress when administrated. The literature knows cases of sodium valproate-induced oxidative stress (decline in GSH) [30–32].

The present findings allow for a better understanding of the lipid peroxidation processes, the glutathione system response, and glutathione metabolism that occur during the xenobiotic intoxication. These findings will allow deciding upon an appropriate antioxidant therapy.

These data indicate the potential of ALA to positively affect the antioxidant system during methanol intoxication. There were also evidences on the protective effect of melatonin [33].

This study identified the mechanism of the protective action of lipoic acid, which appeared to reduce toxic products of methanol metabolism, increase the NADH/NAD ratio, decrease the level of lipid peroxidation, and enhance glutathione synthesis, resulting in the predominance of antioxidant activity over the processes of free radical oxidation. This helps to stabilize and improve the functioning of important organs, such as liver and kidney.

Epilepsy is one of the most common diseases of the nervous system, which requires the use of specific anticonvulsants for therapy. There are many reports on adverse reactions to various anticonvulsants. Given the long intake period, often high dosages, complex drug-drug interactions, and side effects, some adverse reactions and complications are naturally expected to appear.

Valproate sodium is a common broad-spectrum antiepileptic drug used to treat various forms of epilepsy. Approximately 11% of patients, who took sodium valproate, demonstrated an asymptomatic increase in transaminase activity, which decreased with lower doses or drug withdrawal. However, it may cause severe to lethal hepatic reactions, mostly in children and young people aged 2.5 months to 34 years. Sixty-nine percent of cases involved patients under the age of 10. First symptoms appear within 1–2 months after the intake period starts and do not occur after 6–12 months of treatment. The first manifestation includes vomiting and impaired consciousness alongside hypoglycemia and blood clotting disorders. Additionally, one may detect other signs. Aside from that, the electron microscopy revealed mitochondrial damage [34,35].

Verrotti discusses in detail the mechanisms of valproic acid toxicity in encephalopathy [36]. In his work, toxicity results from inhibition of glutamate uptake by astrocytes, which may lead to a rise in intracellular osmolarity and perhaps to cerebral edema. Thus, clinical observations describe cases of very severe encephalopathy, including with valproate-induced life-threatening conditions.

Different substances have varying toxicity. Since it manifests itself when a xenobiotic interacts with a biological system, its level depends on the properties of both the toxicant and the biosystem and is ultimately determined by: (1) the ability of the toxic substance to reach a target site; (2) the nature and strength of toxicant–target interaction; (3) and the target's role in maintaining homeostasis. The structure of the biological system and its morpho-functional organization underwent relatively little change throughout history. Hence, a particular toxic substance with its specific properties will have a unique effect on the organism (biological system). The change of the toxicant will entail qualitative and/or quantitative alterations in that effect. One of the most important principles of toxicology holds that qualitative and quantitative characteristics of toxicity are dependent upon the structure of the toxicant.

The structure of the toxic substance determines its properties, such as molecular size and mass, solubility, volatility, and state of

aggregation under both normal conditions and chemical stress. Although all of these properties affect toxicity, none of them has a significant impact alone. Chemically inert substances with low molecular weight, such as gas or solutions, normally can easily make their way into the bloodstream through the lungs, gastrointestinal tract, and sometimes skin, and are rapidly distributed into tissues, as they perfectly pass through the histohematological barriers. However, the ability of low molecular weight compounds to penetrate barriers is largely determined by solubility. Hydrophilic molecules, even those with a molecular weight of 50–100 D, for example, have a limited ability to penetrate through mucous membranes. Compounds with high molecular weight are likely to encounter more challenges when passing through the barriers. On the one hand, lipophilic substances can sometimes make their way through biological barriers relatively easily, despite their large molecular size. On the other hand, large molecules of substances that are poorly soluble in water and lipids (artificial and natural polymers) practically do not penetrate into the internal environment of a body and thus have no general toxic effect.

5. Conclusion

The use of Ferrum Lek is accompanied by oxidative stress and disturbance to the glutathione system in the liver and kidney, manifested by a decrease in GSH concentration and changes in enzyme activities (GR, GPx, GT, and GGT). Lipoic acid when administered together with Ferrum Lek significantly reduces lipid peroxidation and normalizes GGT activity in the liver. Lipoic acid smoothed the decrease and/or increase in the activities of glutathione metabolism enzymes, potentiating the function of the glutathione system.

Administrating EGTA diminished the glutathione system; it reduced the GSH level, increased enzyme activities (glutathione reductase, glutathione S-transferase and glutathione peroxidase), and caused the TBARS level to rise.

Ferrum Lek caused changes in enzyme activities (GR, GPx, and GGT) that were more pronounced with higher doses. When 30-mg and 75-mg doses of FL were administrated in combination with ALA, liver GSH levels were found to be higher than after the FL-only treatment. They remained reduced through. For instance, the liver GSH levels dropped by 42% and 61% ($p \leq 0.05$), rather than 62% and 77% ($p \leq 0.05$), respectively. This influenced the liver TBARS levels, resulting in the smaller increase in all cases: 74% vs 198% after the 15-mg dose ($p \leq 0.01$), 117% vs 249% after the 30-mg dose ($p \leq 0.001$), and 271% vs 393% after the 75-mg dose ($p \leq 0.001$).

When co-administered with ALA, Ferrum Lek significantly diminished lipid peroxidation and normalized the liver GGT activity. The liver and kidney TBARS levels were caused to dramatically rise (by 236% and 314%, respectively, $p \leq 0.001$). Methanol toxicity led to stress: the GSH level dropped, enzyme activities decreased in both the liver and kidney, and the TBARS level elevated. Using methanol in combination with ethanol increased the lifespan of mice by 42%, up to 10 h, whereas the α-lipoic acid increased their lifespan 2.2-fold, up to 16 h ($p \leq 0.001$). The use of ethanol allowed mice to live the longest (10 h). However, their liver and kidney TBARS levels continued to be elevated, by 134% ($p \leq 0.01$) and 66% ($p \leq 0.05$), respectively.

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