Original Article

Effect of Taurine on Ethanol-Induced Oxidative Stress in Mouse Liver and Kidney

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Abstract

The purpose of this study was to investigate the effect of alcohol exposure on liver and kidney antioxidant systems in taurine exhibition during different time periods. Mice were divided into groups: I – control; II – alcohol (2.5 g/kg b.w.); III – taurine (42.84 mg/kg b.w.); and IV – alcohol + taurine. Treatments were provided for 24 h, 14 days, and 56 days. In the liver and kidney of the alcohol group, antioxidant enzyme (superoxide dismutase, catalase, and glutathione peroxidase) activities, reduced glutathione (GSH), and malondialdehyde (MDA) levels were decreased, as compared to the control group in all time periods. Taurine was found to be effectively inhibiting oxidative action of alcohol and increasing all the tested parameters in the liver (after 24 h) and kidney (after 24 h and 14 days). Moreover, the positive effect of taurine administration on GSH and MDA levels persisted in the kidneys of mice exposed to alcohol for 56 days. In conclusion, alcohol administration led to a significant influence on antioxidant system in the liver and kidney, but simultaneous intake of taurine, along with ethanol, partly attenuated the antioxidant changes in these organs.

Keywords: Antioxidant enzymes, ethanol, lipid peroxidation, reduced glutathione, taurine

Introduction

Ethanol, a substance commonly abused by society, has been shown to produce a wide variety of pathological disturbances affecting a number of organs. Ethanol reacts comparatively nonspecifically, in this way, giving itself the capacity to influence a wide field of cellular targets instead a singular site. Being a small molecule, soluble in both lipids and water, ethanol permeates all tissues of the body and affects the most vital functions of all organs including brain, liver, kidney, and heart.[1] Data disclose that the metabolism of ethanol provides growth to the generation of over numbers of free radicals.[2,3] Intense ingestion of ethanol has been linked to the production of reactive oxygen species (ROS) such as superoxide anion, hydrogen peroxide, hydroxyl radical, and lipid peroxides which are becoming involved in a variety of human affections.[4-6] Moreover, the situation becomes worse as the intake of new substances with alcohol grows increasingly more general. Among young people, the trend of mixing alcohol with energy drink (ED) (e.g., Red Bull® and vodka or other super caffeinated cocktails) has become widespread.[7,8]

EDs (e.g., Red Bull®, Monster®, and Rockstar®) include a variation of compounds including plant-based excitants (e.g., guarana), simple sugars (e.g., glucose, fructose), herbs (e.g., ginseng), and amino acids (e.g., taurine).[9] Taurine (2-aminoethanesulfonic acid) is the principal intracellular sulfur containing free amino acid present in most mammalian tissues which is engaged in growth and development.[10,11] Due to its actions as an antioxidant, neuromodulator, osmoregulator, and intracellular calcium flux regulator, it possesses an amount of cytoprotective properties.[12,13] The favorable effect of taurine as an antioxidant has been ascribed to its ability to steady biological membrane, scavenge ROS, and minimize the peroxidative damage.[14-16]

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Although the current dramatic rise in the consumption of alcohol mixed with ED,[17] very little studies have examined how these beverages modify the objective and subjective measures of poisoning. It is likely that intake of ED with alcohol may be more hazardous than alcohol use alone. Mixing alcohol and other beverage with powerful stimulant qualities may modify the perception of poisoning and give a sensation that more and for longer times can be drunk, thus extending drinking activities. Certain interest regarding the combined use of alcohol and EDs has occurred together with the recent data. They indicate that the consumption of EDs induces more alcohol intake, due to the ED properties reducing the alcohol depressant effects and the causes of the physiological problems.[18,19] The results suggested that the chronic consumption of alcohol in combination with ED causes an inflammatory response and oxidative stress, which induces cell death via apoptosis in the brain of the adult rats.[20] Moreover, there are studies indicating that such combination leads to kidney and liver alterations in rats.[21] Different effects of EDs on tissues' functions could be attributed to the different mixture of ED ingredients which are characterized by psychostimulant effects in humans and animals.[22] However, the toxicological impact of this excessive consumption is unknown. Therefore, the association between alcohol and EDs was considered not safe by the Food and Drug Administration in 2010.[23] Considering these, it seems reasonable to study the relationship between alcohol and individual components of EDs and their influence on the selected organs during a long-term exposure to these substances in doses compared to average consumption. There are few reports on taurine and alcohol administered orally at doses corresponding to the current intake in the population, for an oxidation system in the liver and kidney. The objective of this study was, therefore, to demonstrate and compare the effect of the connection of taurine and alcohol on the activities of the most important elements of the antioxidant barrier such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), reduced glutathione (GSH), and the main marker of oxidative stress (malondialdehyde [MDA]) in the liver and kidney of mice in different time intervals.

Materials and Methods

Animals

Adult male Swiss mice (n = 60; age 3 months; Experimental Research Laboratory of the Institute of Biology, Pedagogical University of Cracow, Poland) weighing 27.0 ± 0.3 g were used for this research. Mice were maintained on a 12-h light: 12-h dark cycle (lights on from 08:00 to 20:00 h) at a constant room temperature of 20°C ± 2°C. The Animal Care and Utilization Committee approved the procedures used in this study (32/2016).

Protocol of the experimental groups

The research was conducted in three series. In each of them, the mice were randomly divided into 4 groups (5 mice/group): (I) the control group (distilled water); (II) the alcoholic group (2.5 g/kg b.w. ethanol); (III) the taurine group (42.84 mg/kg b.w. taurine); and (IV) the alcoholic + taurine group (2.5 g/kg b.w. ethanol + 42.84 mg/kg b.w. taurine). The doses for the treatment were chosen or calculated on the basis of previous reports and information on the average consumption of these substances, especially among young drinkers.[20,24-26] The taurine was administered in a dose equivalent to 3 cans (250 ml/can) of commercially available ED – Red Bull® for a 70 kg human being. A dose of alcohol was adjusted to this amount of taurine. For the alcohol dose, a 0.91 g/kg of vodka has been previously shown to elicit the priming effects of alcohol in social drinkers.[26] All drugs were administered daily to the animals by oral gavage at 10 am. After 24 h from the exposure, the first series of animals was killed, second series of animals was killed after 14 days of everyday exposure, and third series of animals was killed after 56 days. The animals were housed individually and had free access to water and food. After an appropriate time, the mice were sacrificed by decapitation; liver and kidney were quickly removed and stored at −80°C until examination.

Preparation of tissue extract

Tissues were sliced, weighed, and homogenized in cold 50 mM sodium phosphate buffer (pH 7.0) containing 0.1 mM EDTA. The homogenates were then centrifuged at 14,000 ×g for 10 min at 4°C. The supernatants were separated and used for protein determination and enzyme assays. For MDA determination, tissues were homogenized in cold RIPA buffer concentrate with protease inhibitors, centrifuged at 1600 ×g for 10 min at 4°C, while to determine the GSH, the liver and kidney tissues were homogenized in 0.1 M sodium phosphate buffer (pH 7.4) containing 10 mM EDTA and then centrifuged 14,000 ×g for 10 min at 4°C.

Biochemical studies

The measurements were based on a colorimetric reaction of the target substance and further with the help of the ultraviolet/visible spectrophotometric detection at a specific wavelength. MDA content was assessed using the Multiskan FC microplate photometer. Protein concentration was determined by the method of Bradford[27] with the use of bovine serum albumin as a standard. SOD activity was determined at room temperature according to the method of Rice-Evans *et al*.;[28] the absorbance was registered for 2 min in a spectrophotometer at λ = 550 nm. CAT activity was determined at room temperature by a slightly modified version of the Aebi method[29] with hydrogen peroxide as a substrate. Absorbance was measured at 25°C and λ = 240 nm for 1 min in a spectrophotometer. GPx activity was determined at room temperature with the modified method of Lück,[30] which consists in measuring the amount of the oxidation products of p-phenylenediamine with an H_2O_2. The measurement was carried out with spectrophotometer at λ = 460 nm for 1 min. The activity of the studied antioxidative enzymes in tissue extracts was calculated in U/mg of protein. The concentration of the GSH in liver and kidneys was determined with the use of the Ellman method[31] and was estimated in μM/g of tissue. The extent of lipid peroxidation was calculated as the concentration of thiobarbituric acid

reactive products (MDA) with the use of TBARS Assay Kit (Cayman chemical) and expressed in μM.

Statistical analysis

Results are shown as mean ± standard deviation for five animals in each group. Comparisons between multiple groups were made by an analysis of variance (MANOVA) followed by Turkey's *post hoc* test. Values of $P < 0.05$ were considered statistically significant.

Results

Liver

Statistical analysis showed significant influence of ethanol ($F = 53.22$; $P < 0.00001$) on SOD activity in the liver of mice in comparison to control in each period of the experiment. The SOD activity significantly decreased by 29.5% after 24 h ($P = 0.0001$), 23.0% after 14 days ($P = 0.0005$), and 18.9% after 56 days ($P = 0.019$). MANOVA test showed significant influence of taurine on SOD activity ($F = 60.70$; $P = 0.0001$). Moreover, there were some interactions between ethanol and taurine, which significantly influenced the activity of SOD ($F = 23.56$; $P = 0.00001$). In general, the interaction resulted in the increased SOD activity in relation to mice exposed to ethanol exclusively by 52.8% ($P = 0.0001$), 22.77% ($P = 0.017$), and 24.6% ($P = 0.010$) after all series. Furthermore, there were some interactions between taurine and time of the experiment which significantly influenced the SOD activity ($F = 23.56$; $P = 0.00001$) [Table 1].

Administration of ethanol has significant influence on CAT activity ($F = 94.26$; $P < 0.00001$) in the liver of mice in comparison to control in each tested periods. CAT activity decreased by 28.1% ($P = 0.001$), 32.5% ($P = 0.0001$), and 37.7% ($P = 0.0001$) in relation to control after 24 h, 14 days, and 2 months. The analysis also showed the significant influence of taurine administration on CAT activity ($F = 21.96$; $P = 0.00002$) and significant interactions between ethanol and taurine ($F = 19.36$; $P = 0.000$). Only 24 h after the administration of ethanol and taurine, the CAT activity in the liver of mice was higher (36.64%; $P = 0.002$) in relation to mice exposed to ethanol exclusively [Table 1].

Statistical analysis showed significant influence of alcohol ($F = 38.59$; $P < 0.00001$) on GPx activity in the liver of mice in comparison to control in each period of the experiment. The GPx activity decreased after ethanol

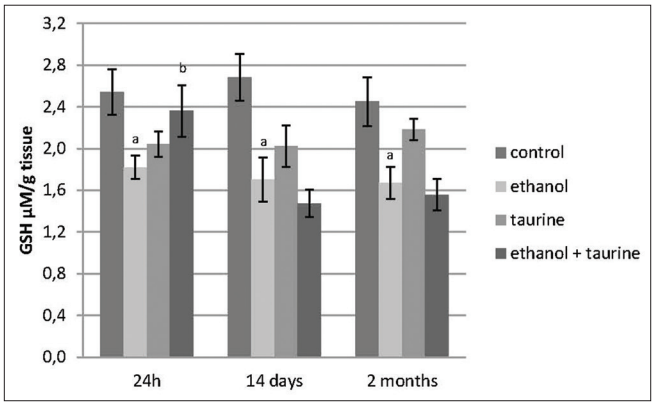

Figure 1: Level of reduced glutathione in the liver of control and experimental animals. Values are mean ± standard deviation. [a]Significant as compared to control ($P < 0.05$); [b]Significant as compared to alcohol ($P < 0.05$)

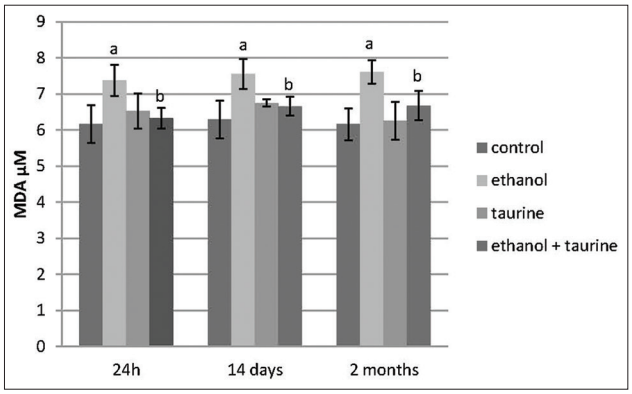

Figure 2: Level of malondialdehyde (μM) in the liver of control and experimental animals. [a]Significant as compared to control ($P < 0.05$); [b]Significant as compared to alcohol ($P < 0.05$)

Table 1: Activities of superoxide dismutase, catalase, and glutathione peroxidase in the liver of control and experimental animals

	Parameters	Group I	Group II	Group III	Group IV
24 h	SOD	11.434±1.33	8.065±1.02[a]	13.525±1.01	12.323±1.71[b]
	CAT	2.723±0.37	1.957±0.12[a]	2.825±0.33	2.674±0.27[b]
	GPx	0.034±0.00	0.026±0.00[a]	0.037±0.00	0.035±0.00[b]
14 days	SOD	12.164±0.57	9.365±0.35[a]	12.174±0.91	11.498±0.33[b]
	CAT	2.891±0.32	1.950±0.08[a]	2.985±029	2.502±0.24
	GPx	0.035±0.00	0.026±0.00[a]	0.040±0.01	0.035±0.00[b]
2 months	SOD	11.159±0.66	9.048±0.23[a]	11.062±0.26	11.277±0.75[b]
	CAT	2.871±0.33	1.788±0.11[a]	2.731±0.16	2.316±0.22
	GPx	0.034±0.00	0.025±0.00[a]	0.033±0.01	0.033±0.00[b]

[a]Significant as compared to control ($P<0.05$), [b]Significant as compared to alcohol ($P<0.05$). SOD, CAT, GPx: U/mg protein. Values are mean±SD. Group I: Control, Group II: Ethanol treated, Group III: Taurine treated, and Group IV: Ethanol and taurine treated. SD: Standard deviation, SOD: Superoxide dismutase, CAT: Catalase, GPx: Glutathione peroxidase

administration by 25.4% after 24 h ($P = 0.006$), 26.6% after 14 days ($P = 0.002$), and 25.7% after 56 days ($P = 0.013$). Furthermore, taurine significantly influenced the GPx activity ($F = 42.84$; $P < 0.0001$). Moreover, there were interactions between ethanol and taurine, which influenced the GPx ($F = 16.54$; $P < 0.00001$). This interaction resulted in the increased GPx activity in Group IV in relation to the ethanol-treated mice by 38.1% ($P = 0.001$), 34.0% ($P = 0.005$), and 35.3% ($P = 0.007$) after 24 h, 14 days, and 56 days respectively [Table 1].

MANOVA analysis showed significant influence of ethanol ($F = 139.02$; $P < 0.00001$) on GSH concentration in livers of mice in comparison to control in each period of the experiment. The level of GSH decreased by 28.4% after 24 h ($P = 0.00001$), 36.5% after 14 days ($P = 0.0001$), and 31.9% after 56 days ($P = 0.0001$). The test also showed significant influence of taurine administration on GSH level ($F = 20.705$; $P = 0.00004$) and interactions between ethanol and taurine ($F = 26.7$; $P = 0.000$). Only 24 h after the injection of these two substances, the GSH level in the liver of mice was higher (29.6%; $P = 0.002$) in relation to mice exposed to ethanol exclusively. Furthermore, time of the experiment influenced the interactions between ethanol and taurine ($F = 8.8$; $P = 0.01$) [Figure 1].

Completed analysis showed significant influence of ethanol ($F = 40.33$; $P < 0.00001$) on MDA concentration in the liver of mice in comparison to control in each tested periods. MDA level increased 19.6% ($P = 0.001$), 20.0% ($P = 0.000$), and 23.5% ($P = 0.000$) after 24 h, 14 days, and 2 months, respectively. Analysis showed significant influence of taurine ($F = 9.40$; $P < 0.004$) on lipid peroxidation. Furthermore, there were interactions between ethanol and taurine, which influenced the MDA concentration ($F = 35.35$; $P < 0.00001$). The MDA level decreased in the combine group in relation to the ethanol-treated mice by 14.2% ($P = 0.01$), 11.9% ($P = 0.04$), and 12.2% ($P = 0.034$) after 24 h, 14 days, and 56 days, respectively [Figure 2].

Kidney

MNOVA analysis showed significant influence of ethanol ($F = 26.51$; $P < 0.00001$) on SOD activity in kidneys of mice in comparison to control in each period of the experiment. The SOD activity significantly decreased by 15.5% after 24 h ($P = 0.001$), 16.7% after 14 days ($P = 0.006$), and 13.8% after 56 days ($P = 0.009$). MANOVA test showed also significant influence of taurine on SOD activity ($F = 12.97$; $P = 0.001$) and interactions between ethanol and taurine ($F = 36.75$; $P = 0.00001$). In general, the interaction resulted in increased SOD activity in relation to mice exposed to ethanol exclusively by 20.9% ($P = 0.0004$) after 14 days [Table 2].

Statistical analysis showed significant influence of ethanol ($F = 87.90$; $P < 0.00001$) on CAT activity in kidneys of mice in comparison to control in each tested period. The CAT activity decreased 27.9% ($P = 0.0004$), 37.3% ($P = 0.0001$) and 41.1% ($P = 0.0001$) of control after 24 h, 14 days and 2 months. MANOVA analysis also showed significant influence of taurine ($F = 17.85$; $P < 0.00001$) and interactions between ethanol and taurine ($F = 36.57$; $P < 0.00001$) regarding CAT activity. In general, interaction

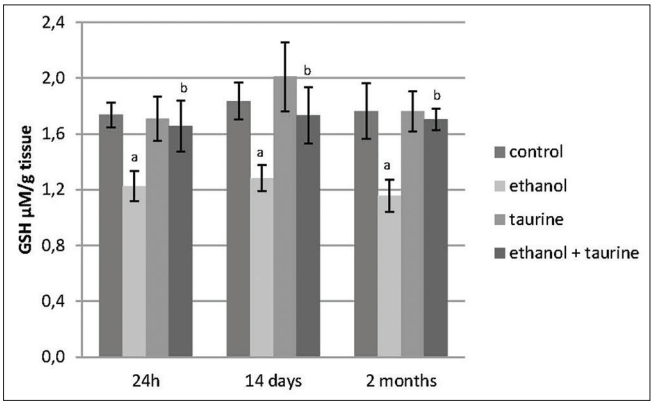

Figure 3: Level of reduced glutathione in the kidney of control and experimental animals. Values are mean ± standard deviation. [a]Significant as compared to control ($P < 0.05$); [b]Significant as compared to alcohol ($P < 0.05$)

Table 2: Activities of superoxide dismutase, catalase, and glutathione peroxidase in the kidney of control and experimental animals

	Parameters	Group I	Group II	Group III	Group IV
24 h	SOD	5.327±0.13	4.499±0.30[a]	5.048±0.39	5.105±0.28[b]
	CAT	2.653±0.07	1.910±0.18[a]	2.467±0.37	2.485±0.29[b]
	GPx	0.026±0.00	0.014±0.00[a]	0.024±0.00	0.019±0.00[b]
14 days	SOD	5.230±0.39	4.353±0.14[a]	5.004±0.12	5.261±0.11[b]
	CAT	2.534±0.21	1.587±0.22[a]	2.590±0.24	2.449±0.33[b]
	GPx	0.022±0.00	0.013±0.00[a]	0.022±0.00	0.019±0.00[b]
2 months	SOD	5.221±0.26	4.497±0.21[a]	5.194±0.43	5.078±0.32
	CAT	2.540±0.21	1.495±0.10[a]	2.347±0.21	1.878±0.07
	GPx	0.024±0.00	0.015±0.00[a]	0.020±0.00	0.002±0.00

[a]Significant as compared to control ($P<0.05$), [b]Significant as compared to alcohol ($P<0.05$). SOD, CAT, GPx: U/mg protein. Values are mean±SD. Group I: Control, Group II: Ethanol treated, Group III: Taurine treated, and Group IV: Ethanol and taurine treated. SD: Standard deviation, SOD: Superoxide dismutase, CAT: Catalase, GPx: Glutathione peroxidase

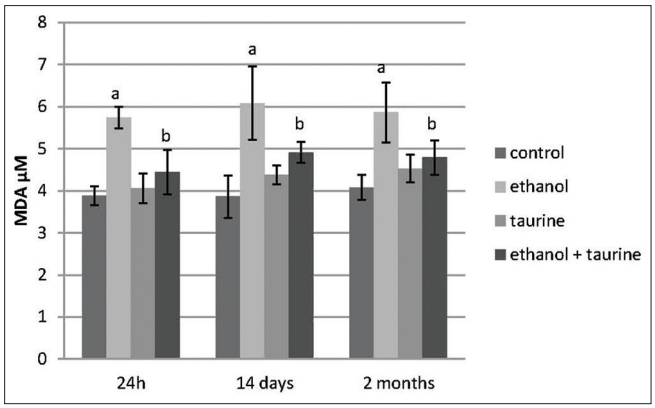

Figure 4: Level of malondialdehyde (µM) in the kidney of control and experimental animals. [a]Significant as compared to control ($P < 0.05$); [b]Significant as compared to alcohol ($P < 0.05$)

resulted in the increased activity of this enzyme in the combine group in relation to ethanol-treated mice by 30.1% ($P = 0.01$) and 54.4% ($P = 0.0001$) after 24 h and 14 days, respectively [Table 2]. Furthermore, statistical analysis showed significant influence of time on CAT activity ($F = 9.96$; $P < 0.00001$) [Table 2].

Statistical analysis indicated significant influence of alcohol on GPx activity ($F = 97.20$; $P < 0.00001$) in kidneys of mice in comparison to control in each period of the experiment. The GPx activity significantly decreased after ethanol administration by 46.9% after 24 h ($P = 0.0001$), 39.1% after 14 days ($P = 0.0001$), and 36.8% after 56 days ($P = 0.0001$). Furthermore, taurine significantly influenced GPx activity ($F = 8.41$; $P < 0.006$). Moreover, there were interactions between ethanol and taurine, which influenced GPx ($F = 33.95$; $P < 0.00001$) and resulted in increased GPx activity in Group IV, in relation to ethanol-treated mice by 40.8% ($P = 0.0261$), 42.0% ($P = 0.0317$) after 24 h, and 14 days, respectively. In addition, statistical analysis showed significant influence of time on this enzyme activity ($F = 3.33$; $P < 0.044$) [Table 2].

MANOVA analysis showed significant influence of ethanol ($F = 74.59$; $P < 0.00001$) on GSH concentration in kidneys of mice in comparison to control in each period of the experiment. The level of GSH decreased by 29.4% after 24 h ($P = 0.0002$), 30.1% after 14 days ($P = 0.0001$), and 34.4% after 56 days ($P = 0.0001$). Test also showed significant influence of taurine on GSH level ($F = 43.66$; $P = 0.00001$) and interactions between ethanol and taurine, which influenced concentration of GSH ($F = 29.17$; $P = 0.00001$). In this group, GSH level increased in relation to mice exposed to ethanol exclusively by 35.1% ($P = 0.002$), 35.1% ($P = 0.001$), and 47.3% ($P = 0.0001$) after 24 h, 14 days, and 2 months, respectively. Time of the experiment also had significant influence ($F = 4.58$; $P < 0.015$) on the GSH level in kidneys of mice [Figure 3].

The test showed significant influence of ethanol ($F = 98.27$; $P < 0.00001$) on MDA concentration in kidneys in comparison to control in each tested periods. The MDA level increased 48.1% ($P = 0.000$), 57.6% ($P = 0.000$), and 43.6% ($P = 0.000$) after 24 h, 14 days, and 2 months. Furthermore, the analysis showed significant influence of taurine ($F = 11.36$; $P < 0.001$) and interactions between ethanol and taurine ($F = 43.79$; $P < 0.00001$) which influenced the MDA concentration. The MDA level decreased in combine group in relation to ethanol-treated mice by 22.7% ($P = 0.002$), 19.2% ($P = 0.009$), and 18.3% ($P = 0.02$) in all three series [Figure 4].

Discussion

Alcohol beverages are widely consumed throughout the world. However, excessive alcohol consumption may cause several pathological conditions such as liver failure, brain damage, and various forms of cancer. It was reported that alcohol consumption constitutes evaluated 3.8% of global mortality. Alcoholic liver disease (ALD) is one of the most important causes of liver-related death, which is related with the increased dose and time of alcohol intake.[32] Although pathogenesis of ALD has not been fully compiled, the straight consequence of ethanol metabolism appears to be related to the ROS production and the development of oxidative stress which are the general qualities of acute and chronic alcohol exposure.[33,34] It is well known that the liver is highly susceptible to the oxidative events associated with the toxicity of ethanol.[35] Exogenous ethanol is metabolized by different pathways in liver, with CYP2E1 catabolism generating ROS that injures liver.[36] Kidney expresses about a tenth of the body's CYP2E1 content, and so, kidney, similar to liver, metabolizes circulating ethanol with local generation of damaging ROS.[37]

Our study has demonstrated that enzymatic as well as nonenzymatic systems which provide cellular homeostasis are highly influenced by alcohol in the used model. Particularly, the activities of SOD, CAT, GPx, and GSH contents as well as the levels of lipid peroxidation were changed in animals treated with alcohol. These enzymatic antioxidants are the first line of defense versus oxidative injury. In the present study, we observed a significant decrease in the SOD activity in the tissues of ethanol-treated mice. It has been reported that variation in the SOD activity in any of trend may relate to the presence of ROS rinse. Therefore, the decrease in enzymes' activity after a long treatment of ethanol indicates an oxidative stress response by the liver defense system and shows the inability of a tissue to scavenge surplus superoxide anions leading to oxidative stress.[38] Due to ethanol intake, enzymes are inactivated, on account of alpha-hydroxyethyl radical generation. The nature of the reaction of these radicals with SOD may consist of an alkylation of nonessential amino acids of the side chain or electron transfer leading to deactivation.[39] CAT acts as a preventive antioxidant and plays an important role in protection against the deleterious effects of ROS. CAT reduces hydrogen peroxide and prevents production of hydroxyl radicals, thereby protecting the cellular components from oxidative destruction.[40] Recent studies have shown that significant decrease in the activity

of CAT over ethanol ingestion shows ineffective scavenging of H_2O_2.[41,42] Our results were found to be in parallel with the above observations in the ethanol group. GPx has a role in defending cells against oxidative stress and this in turn involves GSH as a cofactor. GPx catalyzes the oxidation of GSH to GSSG at the cost of H_2O_2. Decreased GPx activity was observed in the alcohol exposure group. This reduced activity may be involved in either free radical-dependent inactivation of enzyme or depletion of its cosubstrate (i.e., GSH) or NADPH on ethanol treatment.[43]

Our results indicate that ethanol ingestion significantly decreased hepatic GSH content in given time intervals. GSH plays significant role in the ROS scavenging and in the detoxification of chemical compounds in the liver.[44] GSH concentrations are higher in the liver compared to the lung and kidney; however, liver is extremely vulnerable to oxidative damage caused by ROS and GSH reduction. Moreover, acetaldehyde, the product of ethanol oxidation, elevates peroxidation reaction by binding to cysteine and/or to glutathione, a major cytosolic antioxidant in liver, and causes its exhaustion. Hepatic GSH depletion above 20% has been shown to attenuate the cell defense against ROS and has been known to cause hepatic injury.[45,46] Hence, reduction of hepatic GSH (28%–36%) observed in our study is indicative of ethanol-induced hepatic injury in mouse. Previous studies from Anuradha and Vijayalakshmi[47] have shown that the application of cysteine to alcohol-treated rats can reduce oxidative stress and cell injury by inhibiting lipid peroxidation and standardization of antioxidant levels. Furthermore, in our studies, the decrease in glutathione levels in the liver of 56-day alcohol-treated mice was associated with an increase in MDA levels in this organ. The ethanol-generated escalation of lipid peroxidation in hepatic tissues, which persisted in time, might be a repercussion of the increased production of free radicals as well as the inhibition of SOD and CAT activities during all periods. Moreover, the increased MDA level results from the increased oxidative stress tissues caused by ethanol and its oxidation. Oxidation of ethanol by alcohol dehydrogenase generates nicotinamide adenine dinucleotide phosphate (NADH) and increased production of ROS by NADH oxidases in different cell components after chronic ethanol treatment. Induction of the microsomal ethanol-oxidizing system and NADPH oxidase reaction can also simplify the free radical generation. Increased peroxidation has been reported by other investigators in the liver, kidney, lung,[48,49] and also in other tissues such as heart.[50]

Kidney is a significant organ actively engaged in preserving homeostasis of the body by reabsorbing significant substances and eliminating waste matters. It has been reported that regular intake of large volume of alcohol was connected with an increased risk of kidney damage in the general populations.[51] The kidney, which is the space for excretion of reactive metabolites, may also be affected by the ethanol-induced radical oxidant species. Chronic ethanol researches have shown enhances in ethanol oxidation as well as lipid peroxidation in the kidney.[52] However, the effect of acute ethanol on renal antioxidant defense is sparse. This examination established the ethanol influence on the levels of GSH, lipid peroxidation, and antioxidant enzyme activity in the kidney of mice. The noticed variations in renal antioxidants suggest that the kidney is influenced by ethanol-induced oxidative stress and that the kidney antioxidant defense system tries to dispose of augmented ROS flow to decline ethanol-induced oxidative injury. Proof of the ROS influx/imbalance is noticed in changes of enzymes activities between time intervals. The data indicate that acute ethanol ingestion importantly decreased kidney SOD, CAT, and GPx activity, showing the enzymes answer in the kidney to be time dependent. The fluctuation in kidney enzymes suggests that the kidney antioxidant defense system of the mice combats ethanol toxicity. MDA levels increased in the kidney. A significant MDA increase was observed in all time periods. Lipid peroxidation is a simple indicator that cell membrane damage has appeared in the kidney. Extended lipid peroxidation in the kidney after ethanol is compatible with other reviews that have shown augmented MDA levels.[53,54]

Alcohol is often consumed in combination with EDs because they reduce the depressant effects of alcohol. However, different research suggests that chronic use of these psychoactive substances in combination with alcohol can trigger an oxidative and inflammatory response.[20] Taurine administered alone has been reported to improve cellular antioxidant defense system, stabilize biomembranes, and reduce *in vivo* lipid peroxidation, thus preventing apoptosis and necrotic cell death.[55,56] Taurine supplementation has also been shown to attenuate steatosis and hepatotoxicity in several animal models.[57,58] The main organs involved in taurine metabolism are the gut, liver, and kidneys. The gut regulates taurine uptake from the diet by a specific taurine transporter.[59] The liver is involved in endogenous taurine biosynthesis from methionine or cysteine by their decarboxylation and subsequent oxidation of the sulfhydryl group and in the formation of bile acids containing taurine.[60] Whereas, the kidneys are the main sites of excretion of taurine.[61]

According to our data, coadministration of taurine with ethanol significantly changes both the enzymatic and nonenzymatic antioxidants to near-normal levels during alcohol exposure, which shows to be a strong antioxidant. Furthermore, the noted standardization of antioxidants is involved in the reduced levels of lipid peroxidation in the liver and kidney. These findings correlate with previous animal studies.[62,63] On the other hand, a study revealed that exposure to high dose of ED led to significant decreases in SOD, GPx, and CAT activities in blood samples and histopathological changes in hepatic and renal tissues of the ED-treated rats.[64] In our studies, we observe clear changes in the effect of taurine on the activity of antioxidative enzymes and glutathione concentrations during the course of the experiment. After 2 months of exposure to taurine and alcohol, a significant increase in SOD and GPx activities was observed in the liver, whereas in the kidneys only GSH concentration was significant increased. Certain studies have

shown that exposure to high levels of ED, which contained caffeine and taurine, induced a pro-oxidant environment in the cells, leading to increased protein oxidation.[65,66] It has been proved that caffeine increased blood urea nitrogen levels, resulting in the activation of xanthine oxidase which in turn stimulated the oxidation of xanthine to uric acid and generation of superoxide anion and H_2O_2. The interaction between H_2O_2 with O_2 produces free radicals.[67] However, there are no reports of similar effects of taurine. Surprisingly, according to Valle *et al*.,[68] the association of caffeine and taurine at concentrations similar to the highest dose of ED was different from the effects of the administration of the ED itself.

On the other hand, taurine supplementation prevents the decrease in the total thiol content. The free radicals may attack and transform sulfhydryl proteins into radical protein, which can simply act with disulfide bridges presenting free radicals. Taurine is efficient in maintaining -SH (sulfhydryl) groups, as well as in saving the total -SH supply from oxidation.[69] Unchanged -SH groups are essential for the constructive functions of many amino acids, particularly cysteine.[70] This supports glutathione synthesis and may help to clarify the decline in oxidative damage caused by alcohol administration. Therefore, some studies have described a near relationship between taurine administration and GSH synthesis/metabolism.[71,72] According to them, taurine advances the synthesis of GSH and rises the action of GPx, and this may be the mechanism of enzymatic antioxidant defense. The results of the conducted research shed new light on the effect of taurine (one of the EDs ingredients) and stimulate further exploration, which would allow to determine what dose of this substance taken with alcohol would have a positive effect on the body.

Conclusions

In summary, the exposure of male Swiss mice to alcohol revealed signs of toxicity that were evidenced by a reduction in antioxidant defense system. Moreover, the activities of SOD, CAT, GPx, and the concentration of MDA and GSH in the liver and kidney clearly indicate that taurine is able to inhibit the oxidative stress during the coexposure with ethanol, but its effect depends on the dose and time of exposure. On account of these findings, it is important to control the ingredients that are present in commercial products and the safety of these formulations. Further researches and regulations on marketed EDs and their combination with alcoholic beverages are needed.

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Conflicts of interest

There are no conflicts of interest.

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