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The effect of zinc on ethanol-induced oxidative stress in rat liver

[Rat karaciğerinde etanolle indüklenen oksidatif strese çinkonun etkileri]

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ABSTRACT

Aim Zinc is known as an essential trace element and has antioxidant functions. This study was planned to investigate the effects of zinc on oxidative stress induced by ethanol in the rat liver tissue.

Methods: Thirty-nine male rats were divided into four groups as control, ethanol (EtOH), zinc (Zn), ethanol plus zinc (EtOH+Zn). The control group (n=10) were injected intraperitoneally (i.p.) with 0.9% saline, EtOH group (n=10) with 2g kg/day ethanol, Zn group (n=10) received orally, $ZnSO_4.7H_2O$ at a dose of 7 mg kg/day and EtOH+Zn group (n=9) received zinc (orally) and ethanol (i.p.). On the 13th day, rats were euthanized. Liver tissues were removed and malondialdehyde (MDA), advanced oxidation protein products (AOPP), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR), and glutathione-S-transferase (GST) activities were measured. Tissue zinc levels were determined. Histological analyses of liver tissue specimens were also performed.

Results: In EtOH group MDA levels, GPx, SOD activities increased compared with control group. In accordance with histological findings, ethanol induced oxidative stress. But AOPP levels decreased in EtOH group due to supressive effect of ethanol on neutrophil activation and reactive oxygen species (ROS) production. In EtOH+Zn group, MDA, AOPP levels and GPx, SOD activities significantly changed. Besides, histological findings showed that zinc supplementation reduced oxidative stress. But in healthy rats MDA levels increased due to ROS mediated zinc damage.

Conclusion: Zinc supplementation was found to offer protection against ethanol-induced liver damage and oxidative stress in rats. However, zinc might increase oxidative stress in healthy rats.

Key Words*:* Ethanol, zinc; oxidative stress, antioxidant, liver

Conflict of interest: Authors did not declare any conflict of interest.

ÖZET

Amaç: Çinko antioksidan etkileri olan esansiyel bir eser elementtir. Bu çalışma, rat karaciğerinde etanolle indüklenen oksidatif strese çinkonun etkilerini incelemek amacıyla planlanmıştır.

Gereç ve Yöntemler: Otuz dokuz erkek rat 4 gruba ayrılmıştır. Kontrol, etanol (EtOH), çinko (Zn), etanol ve çinko (EtOH+Zn). Kontrol grubuna (n=10) intraperitonel (i.p.) % 0.9 tuz çözeltisi, EtOH grubuna (n=10) 2g/kg/gün etanol, Zn grubuna (n=10) oral olarak 7 mg/kg/gün $ZnSO_4.7H_2O$ ve ETOH+Zn grubuna (n=9) oral olarak çinko ve i.p. ethanol verilmiştir. Onüçüncü gün ratlar feda edilmiştir. Karaciğer dokuları çıkarılarak malondialdehit (MDA), ileri okside protein ürünleri (AOPP) düzeyleri, süperoksit dismutaz (SOD), glutatyon peroksidaz (GPx), glutatyon redüktaz (GR), ve glutatyon-S-transferaz (GST) aktiviteleri ve doku çinko düzeyleri ölçülmüştür.Karaciğer dokularının histolojik incelemeleri gerçekleştirilmiştir.

Bulgular: Etanol grubunda hepatik MDA düzeyleri, GPx, SOD aktiviteleri kontrol grubuna göre artmıştır ve histolojik bulguların da desteklediği gibi etanolün oksidatif stresi artırdığını göstermektedir.Ancak EtOH grubundaki AOPP düzeylerindeki düşüş, etanolün nötrofil aracılı ROS üretimini azalttığının göstergesidir.Etanol-çinko grubunda, MDA düzeylerinin EtOH grubuna göre azalırken, AOPP düzeylerinin kontrole göre düşmesi ve GPx, SOD aktivitelerinin ise kontrole göre artması oksidatif stresin azaldığını göstermektedir.Etanol-çinko grubundaki histolojik değişiklikler de bu bulguları desteklemektedir.Sağlıklı ratlarda çinkonun neden olduğu reaktif oksijen türleri (ROS) aracılı hasarın en önemli göstergesi ise MDA artışı olmuştur.

Sonuç: Bulgularımıza göre çinkonun etanole bağlı karaciğer hasarı üzerinde iyileştirici bir etki gösterdiği ve bu etkiyi karaciğerde oksidatif stresi azaltarak yaptığını söyleyebiliriz. Ancak, çinko sağlıklı ratlarda oksidatif stresi artırıcı yönde davranabilmektedir.

Anahtar Kelimeler: Etanol, çinko, oksidatif stres, antioksidan, karaciğer

Çıkar Çatışması: Yazarlar herhangi bir çıkar çatışması bildirmemişlerdir.

Introduction

Oxidative stress plays an important role in the pathogenesis of ethanol induced liver injury [1]. Increased reactive oxygen species (ROS) changes prooxidant-antioxidant balance and may lead to increase lipid peroxidation in gastrointestinal system, liver, heart and brain tissues. Liver is more vulnerable to ethanol induced damage than any other organ in the body, because ethanol metabolized mainly in liver [2,3].

Proteins regulate various metabolic processes in the cell. Damaged proteins in the ethanol treated rats may have altered the metabolism and the structure of subcellular organelles of the liver and contribute to fat accumulation. Protein oxidation is thought as a modification due to ethanol toxicity [4].

Zinc is known as an essential trace element necessary for protein metabolism. It is necessary for membrane integrity and also involved in the structure and function of over 300 metalloenzymes. It has important functions in skin and connecting tissue metabolism as well as in wound healing [5]. It exerts its antioxidant effects indirectly by maintaing membrane structures, involving in the structure of SOD, increasing the metallothionein concentrations and, competing with redox reactive metals, iron and cuprous for critical binding sites [6]. It is shown that hepatic and serum zinc levels of patients with alcoholic liver disease decreased depending on the degree of liver damage [3].

Data from the literature concerning alcohol related variations of antioxidative enzymes are conflicting and there is not much information available regarding the effects of zinc on lipid peroxidation, protein oxidation and antioxidative enzyme system in ethanol treated rats. Therefore, the primary purpose of the present study was: 1. to investigate the effects of zinc on ethanol induced liver injury by measuring antioxidant enzyme activities and oxidative stress markers and 2. to support the experimental oxidative stress with histological observations.

Materials and Methods

Chemicals

Zinc Sulfate ($ZnSO_4.7H_2O$) was obtained from Sigma (St Louis, MO, USA). All other reagents and chemicals were of analytical grade.

Animals

All experiments were conducted in accordance with the Committee of the Ethics on Animal Experiments of Gazi University (G.U.E.T-05.030, 49-5076, 15.04.2005). Thirty nine, six-month-old, male, Wistar rats (250-300 g) were used for the study. They were housed in a room maintained at 24° C with a 12-hour light-dark cycle. The animals were given standart rat chow and water ad libitum.

Treatments

After a quarantine period of 5 days, rats were randomly divided into four groups maintained for 13 days as follows:

Control group (n=10): Animals were fed standard was injected intraperitoneally (i.p.) with 0.9% saline.

Ethanol group (n=10): Ethanol was given intraperitoneally (i.p.) at a dose of 2g/kg/day.

Zinc group (n=10): Zinc sulphate ($ZnSO_4.7H_2O$) was administrated at a dose of 7 mg kg/ day orally. $ZnSO_4.7H_2O$ was dissolved in 0.5 ml distilled water.

Ethanol+Zinc group (n=9): Zinc sulphate was administrated orally at a dose of 7 mg kg/day 10 minutes after the ethanol was injected i.p. at a dose of 2g kg/day [7,8].

Sacrifice of Animals

On the 13th day, rats were euthanized with intravenous 40 mg/kg ketamine hydrochloride and 2.5 mg/kg xylazine injection. Liver tissues were quickly removed, washed with NaCl, immediately frozen in liquid nitrogen, and were kept at -80 °C until studied.

Determination of Superoxide Dismutase Activity

Superoxide dismutase (EC 1.15.1.1) activities of liver tissues were determined by the method of Sun *et al.* [9]. For SOD assay, liver tissues were homogenized in the ratio of 1/10 (w/v) distilled water and centrifuged at 5000 g for 30 min. The supernatant was carefully separated, the 3/5 (v/v) chloroform and ethanol were added. This mixture was centrifuged at 4°C and 5000 g for 2 hours. The supernatant was used for the determination of SOD. This assay involves xanthine oxidase used as superoxide generator. The results were expressed as unit per mg protein that inhibits the rate of nitroblue tetrazolium (NBT) reduction by 50%.

Determination of Malondialdehyde Levels

The malondialdehyde (MDA) levels were measured by using a high pressure liquid chromatography, (HPLC, Waters 486 USA) separation of MDA-TBA complex spectroflurometrically based on Agarwal *et al.* [10]. The mobile phase consisted of 40:60 ratio (v/v) of methanol to potassium monobasic phosphate at pH 6,8, pumped at a rate of 1.0 ml/min on a Hewlett-Packard Hypersil 5µ ODS 100x 4,6 mm placed in a column warmer set to 37°C. Fluorescence detector was set at excitation of 515 nm and emission of 553 nm. For MDA assay, liver tissues were homogenized in 1,15 % KCl and centrifuged at 14000 rpm. at 4°C. Supernatant was separated and treated with butylated- hydroxytoluene (BHT) and heat derivatized at 100°C for 1 hour with thiobarbituric acid at an acid pH. Samples were extracted with n-butanol and 20 µl of the extract was injected. Concentrations of MDA were calculated from a standart curve prepared

from 1,1,3,3 tetraethoxypropane (TEP) and expressed as nmol MDA/ g wet tissue.

Determination of Advanced Oxidation Protein Products Levels

Advanced oxidation protein products (AOPP) levels were measured spectrophotometrically according to the Witko-Sarsat *et al.* [11] and Çakatay *et al.* [12]. For AOPP assay, liver tissues were homogenized with 20 mM, pH 7,4 Tris-HCl buffer and centrifuged at 5000 g for 10 minutes. The supernatant was used for the determination of AOPP. The results were expressed as μmol/mg protein.

Determination of Zinc Levels

Liver tissues were weighed and kept at 110˚C for 48 hours and diluted 1/1 hydrochloric and nitric acid mixture (v/v) was added for digestion. After drying and a digestion procedure, zinc levels were measured by using Unicam 939 atomic absorption spectrometer. The results were expressed as μg/ g dry weight liver [13,14].

Determination of Glutathione Peroxidase Activity

Glutathione peroxidase (GPx; EC 1.11.1.9) activity was measured by a modification of the coupled assay procedure of Paglia and Valentine [15,16]. Liver tissues were homogenized in 20 mM, pH 7,4 Tris–HCl buffer and centrifuged at 5000 g for 10 minutes. The supernatant was used for the determination of GPx activity. The assay was performed by incubating 100 μl supernatant (diluted 1:5) in 1 ml reaction mixture (50 mM potassium phosphate buffer, pH 7,0, 3,6 mM sodium azide, 5 mM GSH, 0,3 mM NADPH, added in 10 μl glutathione reductase) for 5 minutes. The absorption was recorded at 340 nm during a 5 minute period, after which the reaction was started by addition of 25 μl H_2O_2. Enzymatic activity was calculated from the decrease in absorbtion of NADPH using the molar extinction coefficient for NADPH of $6.22x10^3$ $M^{-1}cm^{-1}$. The results were expressed as nmoles oxidized NADPH per minute per mg protein.

Determination of Glutathione Reductase Activity

Glutathione reductase (GR; EC 1.6.4.2) was assayed by the method of Riley *et al.* [17]. Liver tissues were homogenized in 20 mM, pH 7,4 Tris –HCl buffer and centrifuged at 5000 g for 10 minutes. The supernatant was used for the determination of GR activity. A volume of 10 μl supernatant was added to cuvettes containing 0,2 M KPO4 buffer, pH 7,0 and 50 μl NADPH in a volume of 0,4 ml and a reaction temperature of 30˚C. The absorbtion was recorded at 340 nm during a 5-minute period, after which the reaction was started by addition of 50 μl oxidized glutathione and followed for a further 5 minutes. The oxidation of NADPH was followed spectrophotometrically at 340 nm using the molar extinction coefficient for NADPH of $6.22x10^3$ $M^{-1}cm^{-1}$. The results were expressed as nmoles oxidized NADPH per minute per mg protein.

Determination of Glutathione-S-transferase Activity

Glutathione-S-transferase (GST; EC 2.5.1.18) activity was measured by following the absorbance increase due to formation of a tioether bond between glutathione and 1-chloro-2,4-dinitrobenzene (CDNB) at 340 nm, based on Habig *et al.* [18]. Liver tissues were homogenized in 0,1 M, pH 7,2 Tris –HCl buffer and centrifuged at 5000 g for 20 minutes at 4˚C. The supernatant was used for the determination of GST activity. The assay mixture (2 ml) contained 1 mM CDNB and 1 mM reduced glutathione in 0,1 M KPO_4 buffer, pH 6,5. After addition of 20 μl supernatant, the rate of increase of absorption was measured at 340 nm for 3 minutes at 25˚C. The molar extinction coefficient for CDNB was 9.6 $mM^{-1}cm^{-1}$. The enzyme activity was expressed in international units per milligram of protein.

Determination of Protein Concentration

The protein concentration of the supernatant was measured by the method of Lowry *et al.* [19].

Histological Evaluation

Liver tissues of all groups were fixed in phosphate-buffer containing 2.5% gluteraldehyde for 2-3 hours. Then they were postfixed in 1% osmium tetraoxide (OsO_4) and dehydrated in a series of graded alcohols (50, 60, 70, 80, 90, 96 and 100% ethanol). After passing through propylene oxide, the specimens were embedded in Araldyte CY 212, DDSA (2-dodecenyl succinic anhydride), BDMA (benzyldimethyl amine) and dibutylphitalate. Semithin sections were cut and stained with toluidin blue and examined with Olympus light microscope.

Statistical Analysis

Data were analyzed by SPSS 18.0 software for Windows version. Data were evaluated for normality of the distribution by the Shapiro-Wilk test and results were expressed as mean±S.D. or median (minimum-maximum). One way analysis of variance (ANOVA) was used for comparison between the groups that were normally distributed and Tukey's post hoc test was applied to locate the source of significant difference. For the groups that were not normally distributed, Kruskal Wallis Variance Analysis was used to determine the statistical significance of the differences. The significance of the median differences between groups was assessed by a Mann–Whitney U test with Bonferroni correction. Significant difference was accepted at $p<0.05$.

Results

Biochemical Results

The MDA levels were found to be significantly higher in the EtOH group than those in the control group ($p<0.05$). There was a significant decrease in the EtOH+Zn group

with respect to the ethanol group ($p<0.05$). In the Zn group MDA levels were higher with respect to the control and the EtOH+Zn group ($p<0.05$) (Table 1).

The AOPP levels decreased significantly in the EtOH group compared with the control group ($p<0.05$). There was a significant increase in the Zn group with respect to the EtOH group ($p<0.05$). In the EtOH+Zn group AOPP levels decreased significantly compared with the control group ($p<0.05$) (Table 2).

The effect of dietary zinc on the endojen zinc levels were investigated and the zinc levels were increased significantly only in the Zn group compared with the control group ($p<0.05$) (Table 1).

The GPx activity in the EtOH group were increased significantly with respect to the all groups ($p<0.05$). There was a significant increase in the EtOH+Zn group compared with the control group ($p<0.05$) (Table 2).

The glutathione reductase and glutathione-S-transferase activities were not changed significantly in all groups with respect to the control group. Glutathione reductase activity increased in the Zn group compared with the EtOH+Zn group ($p<0.05$) (Table 2).

The SOD activity in the EtOH group were increased with respect to the control group ($p<0.05$), and significantly decreased compared with the EtOH+Zn group ($p<0.05$). There was a significant increase in both EtOH+Zn and Zn groups compared with the control group ($p<0.05$) (Table 2).

Histological Results

In the ethanol group; dilatation in sinusoids, lipid granulles in hepatocyte cytoplasm, vacuolisation in sinusoidal endothelial cells, increase in fibrotic tissue between hepotocytes and apoptic appearence in some of the hepatocyte nucleuses and dilatation in biliary ducts were observed (Figure 2a-c). In contrast, in the ethanol-zinc group, the lipid granules were not observed. Both the dilatation in sinusoids and in biliary ducts, fibrotic tissue between hepatocytes, vacuolisation in sinusoidal endothelial cells were decreased (Figure3a-b). In the zinc group; although the general structure seemed to be normal, we observed minimal dilatation in sinusoids and vacuolization in endothelial cell cytoplasm (Figure 4).

It was observed that the most prominent changes were seen in the ethanol group following the EtOH+Zn group

Table1. Malondialdehyde and zinc levels of experimental groups.

	MDA (nmol/g wet tissue)	Zinc (mg/g dry weight)
Control (n=10)	14.33 (7.54-32.31)	40.56 (25.21-104.58)
Ethanol (n=10)	29.03 (22.62-45.43)*,**	55.84 (35.00-74.86)
Zinc (n=10)	33.12 (22.97-44.43)*,**	57.19 (48.10-132.54)*
Ethanol+Zinc (n=9)	21.70 (20.13-31.42)*	65.94 (34.50-101.72)

Results are expressed as median (min.-max.).The corrected p value (Mann-Whitney U test) was defined as 0.0083 (0.05/ 6 comparisons) after Bonferoni correction.; p values are shown as, $^{*}p < 0.05$ compared with control group, $^{**}p< 0.05$ compared with "ethanol+zinc" group.

Table2. Superoxide dismutase, glutathione peroxidase, glutathione reductase and glutathione-S-transferase activities and advanced oxidation protein products levels of experimental groups.

	SOD (U/mg protein)	GPx (nmoloxized NADPH/ min/mg/protein)	GR (mU/mg protein)	GST (U/mg protein)	AOPP (mmol/mg protein)
Control (n=10)	29.86±3.8	6.7±0.7	47.5±4.9	0.353±0.047	7.10±0.7
Ethanol (n=10)	39.00±4.3*	20.8±1.6*	47±4.3	0.330±0.037	5.80±0.5*
Zinc (n=10)	42.04±4.2*	7.0±1.3**	51±6.5	0.332±0.015	7.00±0.5**
Ethanol+Zinc (n=9)	47.01±4.3*,**	9.4±1.0*,**,***	42±6.3***	0.342±0.046	6.02±0.5*

Results are expressed as mean ± S.D.; p values are shown as; $^{*}p < 0.05$ compared with control group, $^{**}p < 0.05$ compared with ethanol,$^{***}p < 0.05$ compared with zinc group.

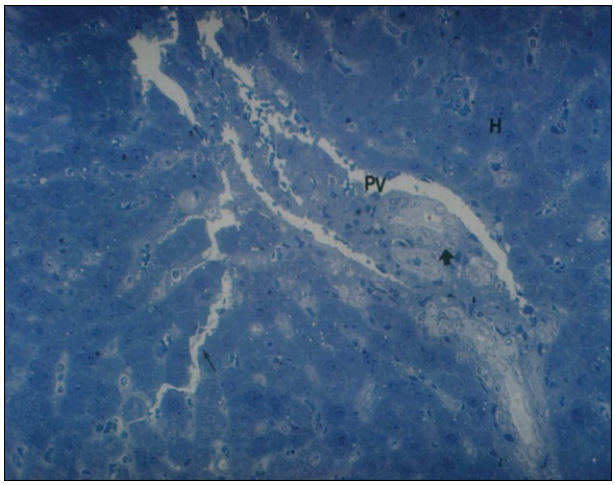

Figure 1 Normal rat liver histology. Hepatocytes (H), Portal Vein PV), biliary duct (thick arrow) and sinuosids (thin arrow), (Toluidine blue x 200).

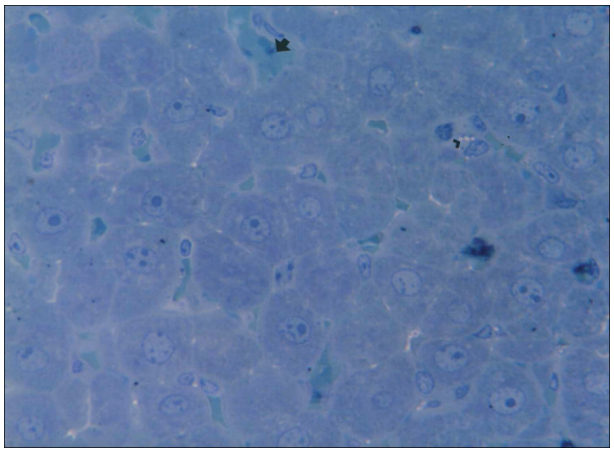

Figure 4 Effect of zinc administration on liver histology**.** Dilated sinusoids (↟), vacuolization in endothelial cytoplasm (>), **(**Toluidine blue x 400).

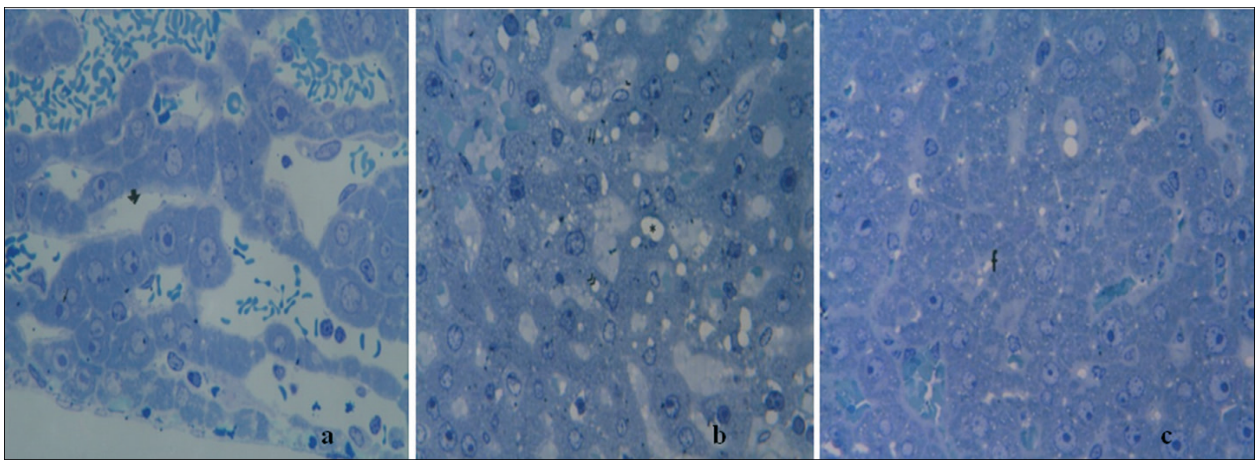

Figure 2 Effect of ethanol administration on liver histology. Dilated sinusoids (thick arrow), apoptic cell nucleus (thin arrow)**, (A)** Lipid granulles in hepatocyte cytoplasm (↟), dilatation in biliary ducts (*), vacuolization in endothelial cell cytoplasm (>), vacuolization in hepatocyte cell cytoplasm (>>)**, (B)** Fibrotic tissue between hepotocytes (f), **(C)**, (Toluidine blue x 400).

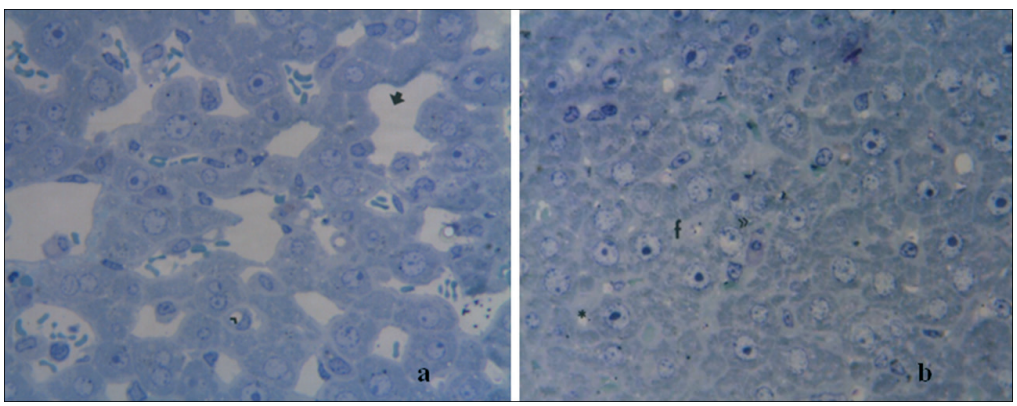

Figure 3 Effect of zinc on ethanol induced liver damage. Dilated sinusoids (↟), vacuolization in endothelial cell cytoplasm (>), **(A)** fibrosis (f), vacuolization in hepatocyte cytoplasm (↟↟) and dilatation in biliary ducts (*), **(B)** were decreased, (Toluidine blue x 400).

in the histopathological examinations. We also observed that changes in the zinc group were minimal and the structures in the control group were almost normal.

Discussion

It is well known that ethanol affects the oxidant-antioxidant balance in liver [3]. For this reason, the protective role of several substances like quercetin, taurine, melatonine ethylpyruvate on ethanol induced liver damage have been discussed by many researchers [1,20,21].

Studies with alcoholic patients and animal models showed that ethanol decreased the hepatic zinc levels and caused an elevation in lipid peroxidation products [3,22]. In some studies [23,24], the protective role of zinc from oxidative stress were emphasized, but we could not find any comprehensive literature about the effect of oral zinc supplementation on ethanol induced hepatic oxidative stress.

We found a significant increase in MDA level in the ethanol group similar to other studies [1,21]. There are several reasons for elevated lipid peroxidation. It is known that ethanol metabolized in liver by 3 ways: (1) alcohol dehydrogenase (ADH) in cytosol (2) CYP2E1 in endoplasmic reticulum (3) catalase in peroxisomes [25]. ADH is a zinc dependent metalloenzyme [3]. Metabolic way shifts from ADH to CYP2E1 with chronic ethanol intake and plays an important role for increase in lipid peroxidation [26]. Morgan *et al.* [26] showed that chronic ethanol supplementation increased the CYP2E1 activity and also the oxidative liver injury. Ethanol treatment might increase the redox active iron concentrations and caused lipid peroxidation. Iron produces highly reactive OH.- radical by fenton and Haber-Weiss reactions [27].

In some studies, the antioxidant role of the zinc was emphasized [17,28]. In our study, MDA decrease in the EtOH+Zn group compared with the EtOH group is probably due to the protective effect of zinc by binding the negatively charged phospholipids instead of iron. Recent studies showed that metallothionein might be effective for attenuating the oxidative hepatotoxicity [29]. Although the antioxidative mechanism of metallothionein was not obvious, it may increase the tissue concentration of zinc under oxidative stress.

Histological findings of our study confirmed that ethanol was hepatotoxic and zinc supplementation ameliorated the changes in our experimental conditions. We thought that histological observations of our study supported the decreased levels of lipid peroxidation in the EtOH+Zn group.

We found that the highest elevation of MDA levels were in the Zn group. Bishop G M et al. [30] reported zinc toxicity in astrocyte cultures associated with the generation of intracellular ROS. In a study of Cao G [28] zinc caused increased hepatic MDA levels with a dose of 500 ppm in mice. Similarly, lipid peroxidation increase in the healthy zinc group were observed with 7 mg/kg dose of zinc in our experimental conditions. We thought that ROS increase in the zinc group might cause significant MDA elevation. Our results showed that oral zinc supplementation caused an increase in the oxidative stress in the healthy group. Minimal degenerative changes that can be observed in the histopathological examinations in this group seemed to support our findings demonstrating the reflection of the oxidative stress on the tissue morphology.

In our experimental model, increased hepatic zinc levels in the healthy Zn group showed the uptake of the oral zinc by liver tissue. Previous studies showed that chronic liver disease caused zinc deficiency and therefore zinc supplementation may be useful for these patients [3]. But, zinc additives have been widely used by healthy people in different doses except for therapeutic causes such as cancer, infection, growth retardation, and skin lesions. Studies on rats have shown that excessive dietary zinc in these animals includes deficiencies of copper and iron producing poor growth and anemia. These findings indicate that excessive intake of zinc supplements have also potential risk to humans [6]. Reports of the studies examining the zinc effects with different doses on healthy rats are limited and more studies are needed [6,31].

Reseachers observed that zinc deficiency was not only related with the ethanol intake directly but also dependent on the level of the liver damage [3]. The spectrum of alcoholic liver disease includes fatty liver, alcoholic hepatitis, fibrosis and cirrhosis [4]. Based on the histological findings of our experimental model, early phase of the liver damage might cause the insignificant results for zinc levels in the EtOH group.

Proteins, especially thiol containing proteins are major targets for free radicals [4]. Advanced oxidation protein products (AOPP) was defined as protein products containing crosslinked dityrosine bonds [11]. It is well-known that several highly rective oxygen radicals and metabolites are generated during the respiratory burst of activated neutrophils, myeloperoxidase (MPO) is released into the extracellular fluids together with superoxide anion radicals and hydrogen peroxide (H_2O_2). Whereas H_2O_2, superoxide ($O_2^{\cdot-}$) and hydroxyl radical are also released from sources other than neutrophils. Hypochlorous acid (HOCl) is a neutrophyl specific nonradical oxidant and is synthesized by an MPO-catalyzed reaction from H_2O_2 and Cl^-. Hypochlorous acid rapidly attacks biological molecules and participates in the generation of chloramines. Each of these neutrophil-derived oxidants, notably HOCl with its greater toxicity and lifespan, has been shown to cause oxidative modification of functional groups of proteins and aminoacids, especially of free thiol groups, and thus can cause damage through oxidative stress [32]. Advanced oxidation protein products are formed with activation of

proteins with chloronized oxidants and AOPP are tightly linked to the degree of monocyte activation, which is a key component of the innate immune system [33].

Scientific data related with the oxidative stress at hepatocellular proteins due to ethanol toxicity assigned as AOPP levels are limited. Zwala–Jagiello *et al.* [33] reported that elevated AOPP levels in alcohol related cirrhosis were related to the severity of the disease. Several studies showed that ethanol intoxication might cause suppressed innate immunity and reduction of the hepatic gene expressions of inflammatory cytokines, such as tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) as well as the hepatic expression of intercellular adhesion molecule 1 (ICAM-1) and hepatic polymorphonuclear cells (PMNL) infiltration [33,34]. In our study, the decrease of AOPP levels in the ethanol group might be interpreted as diminished neutrophil activation and reduced oxidative stress due to ethanol exposure with our experimental model. It is known that activated polimorphonuclear neutrophils (PMN) generate ROS via NADPH oxidase complex. The NADPH oxidases are group of enzymes, which catalyze the production of O_2^- from oxygen by using NADPH as the electron donor. When activated, NADPH oxidase transfers electrons from NADPH to O_2 to produce O_2^-, which can react with nitric oxide forming peroxinitrite or it can be spontaneously or enzymatically converted to H_2O_2. Zinc is an inhibitor of this enzyme [24]. Besides, zinc has been shown to inhibit nuclear factor kappa B (NF-κB) acivation in human hepatocellular carcinoma derived cell line [35]. In our study, the decrease in AOPP levels in the EtOH+Zn group might be related with reduced ROS due to both inhibitory effect of zinc on NADPH oxidase system and the suppresive effect of ethanol on neutrophil activation.

Studies showed that antioxidant enzyme activities varied due to ethanol induced hepatotoxicity [36,37]. The mode, dose and duration of the ethanol intake in different experimental models might cause the results to be different.

Aykaç G. *et al.* [37] and Pathak A *et al.* [36] reported a significant increase for hepatic GPx activities after 2 weeks of oral ethanol intake with different doses in rats. Similarly, we observed a significant increase for GPx activity in the EtOH group on the 13th day. We found that GPx activity decreased in the EtOH+Zn group compared with the EtOH group. Pathak A *et al.* [36] also reported a decrease in rat hepatic GPx activities at the fourth weeks of their study.

In accordance with the study of Pathak A *et al.* [36], in our study both the SOD and GPx activities increased significantly with chronic ethanol intake. Superoxide dismutase and GPx increase were in coordination with the lipid peroxidation increase. Since these enzymes were known as important defence lines against ethanol induced oxidative stress.

Glutathione-S-transferase (GST) is major detoxifying system in hepatocytes [37]. Aykaç G. *et al.* [37] observed a significant increase for hepatic GST activity with ethanol consumption of rats. Rouach H *et al.* [38] reported unaffected GST activities after 4 weeks of ethanol consumption. Glutathione reductase catalyzes the reduction of oxidized glutathione to reduced form in the presence of NADPH as a reducing power. Macdonald D *et al.* [39] showed that GR activities of rat hepatocytes increased after 1-7 weeks of 5 mg/kg ethanol supplementation. Zhou Z *et al.* [29] showed that hepatic GR activity did not change after 12 weeks of ethanol treatment to mice. But, simultaneous supplementation of zinc and ethanol caused a significant increase for hepatic GR activities. In our experimental conditions, both GR and GST activities were not changed significantly in all groups with respect to the control group. We thought that, after 13 days of ethanol and zinc treatment, these enzymes were in an adaptation period against oxidative stress induced by ethanol.

Among all groups, we observed the most increase for SOD activity in the EtOH+Zn group. In a study of Genc S *et al.* [21], in which the melatonin effects on ethanol induced liver damage were investigated, similar results were observed for SOD activity when the antioxidant and ethanol applied together. By virtue of that study we thought that, zinc, the constitunal part of SOD might increase the antioxidative effect of this enzyme when applied with ethanol.

The biochemical and histological results of our study showed that ethanol caused oxidative stress in liver of rats. According to our results, zinc was an antioxidant ameliorating the oxidant effects of ethanol in the liver. However, zinc might increase the oxidative stress in healthy rats. For this reason, further animal and human studies were needed to clarify the suitable antioxidant dose of zinc.

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