

Effects of Zinc on Intestinal Injury and Some Serum Parameters in Ethanol-Administered Rats

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This study was designed to determine the morphological and biochemical effects of zinc sulfate and the role of metallothionein in ethanol-induced intestinal injury. Rats received zinc sulfate (100 mg/kg/d) for 3 consecutive d, 2 h prior to the administration of ethanol by gavage. Ethanol administration caused intestinal injury as determined by increased serum lactate dehydrogenase activity, urea, creatinine, uric acid, and sialic acid levels, intestinal lipid peroxidation level, decreased serum catalase activity, intestinal glutathione level, and metallothionein expression. Zinc sulfate pretreatment of the ethanol group caused a decrease in histological damage, serum lactate dehydrogenase activity, urea, creatinine, uric acid, sialic acid levels, and intestinal lipid peroxidation level, but increases in serum catalase activity, intestinal glutathione level, and metallothionein expression. The present study indicates that zinc sulfate has a protective effect against ethanol-induced intestinal injury. In addition, the protective effect of zinc on ethanol-induced intestinal injury might be mediated by metallothionein, as well as having antioxidative potential.

Key words: ethanol; intestinal injury; metallothionein; serum parameters; zinc sulfate

Ethanol is a direct systemic toxin that produces injury to all tissues, depending on the dose and duration of exposure.¹ The stomach and upper gastrointestinal tract is a major site of ethanol metabolism.² Therefore, acute ethanol exposure causes significant damage in the intestinal mucosa in experimental animals and humans. Ethanol impairs absorption of vitamins and nutrients, increases mucosal permeability to macromolecules, and alters motility.^{3,4} Alcoholics and patients with inflammatory bowel disease are commonly known to be zinc deficient, which might be attributed to chronic intestinal injury.^{5,6} The pathogenesis of ethanol-induced intestinal injury has been associated with free radical production and oxidative stress.⁷

Zinc is an essential micronutrient that is involved in vital functions. It has been found to be essential to the structure and function of various proteins, hormones,

and nucleotides, and in over 300 enzyme reactions. It plays a critical role in many physiological functions, including DNA, RNA, and protein synthesis; cell growth, proliferation, and regeneration; and stabilization of biomembranes.⁸ A major biochemical function of zinc is to retard oxidative processes, and it serves as a potential antioxidant.⁹ It has been documented that zinc ions are indispensable in tissue repair¹⁰ and also possess gastrohepatic protective effects.¹¹ The homeostatic regulation of zinc metabolism in the cell is maintained by metallothionein (MT).¹² Metallothionein is a low molecular weight metal binding protein characterized by high cysteine content and high affinity for bivalent heavy metal ions, such as zinc, cadmium, and copper.^{8,13} This metalloprotein is involved in many physiological processes, such as trace metal homeostasis and detoxification, cell proliferation and differentiation, apoptosis, and protection against oxidative damage.¹⁴

The aim of this study was to determine whether zinc has a protective effect on intestinal injury produced by ethanol. In addition, we also investigated the role of intrinsic metallothionein in ethanol-induced intestinal injury, immunohistochemically.

Materials and Methods

Experimental design and treatment of animals. In this study, 2.5 to 3-month old male Wistar albino rats ($n = 41$) from DETAE (Istanbul University Centre for Experimental Medical Research and Applications) were used. The experiments were reviewed and approved by the Animal Care and Use Committee of Istanbul University. The animals were fed Purina Laboratory Rodent Diet 5001 (including 79 ppm zinc) and tap water *ad libitum*, but were fasted for 18 h prior to the experiments. The animals were randomly divided into four groups: group I, intact control rats ($n = 8$), group II, control animals given only zinc sulfate (100 mg/kg/d, for 3 consecutive d) ($n = 8$), group III, animals receiving 1 ml absolute ethanol ($n = 14$), group IV, animals receiving zinc sulfate and absolute ethanol at the same dose and time ($n = 11$). Zinc sulfate and absolute ethanol were given to the rats by the gavage technique. The animals were sacrificed by ether anesthesia 2 h after ethanol administration or 4 h after the last zinc sulfate treatment.

Histological assays. Samples from the duodenum were taken from the animals for histological studies. The tissues were fixed in Bouin's

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Abbreviations: AEC, 3-amino-9-ethylcarbazole; ANOVA, analysis of variance; CAT, catalase; DETAE, Istanbul University Centre for Experimental Medical Research and Applications; GSH, glutathione; LDH, lactate dehydrogenase; LPO, lipid peroxidation; LDL, low density lipoprotein; MDA, malondialdehyde; MT, metallothionein; NS, no significance; PAS, periodic acid Schiff; Zn, zinc

solution and embedded in paraffin after completion of the routine follow-up. Serial sections 5 μ m in thickness were stained with Masson's triple dyes and periodic acid Schiff (PAS), and examined under an Olympus CX41 light microscope.

Immunohistochemical assay. Deparaffinized sections were permeabilized with 0.3% Triton X-100 for 10 min, and then were heated in 10 mM citrate buffer (pH 6.0) for 10 min in a microwave oven. After blocking of endogenous peroxidase activity with 3% hydrogen peroxide, the Ultra Vision Detection System (Lab Vision) for the streptavidin-biotin-peroxidase technique was employed. Sections were incubated with metallothionein antibody (mouse anti-MT E9; Zymed Laboratories) at room temperature for 60 min. Highly specific monoclonal antibody was diluted with PBS containing 0.3% Triton-X 100 at 1:50. The enzyme activity was developed using 3-amino-9-ethylcarbazole (AEC), and the sections were counterstained with hematoxylin.

Quantitative analysis of MT-positive crypt cells was made using a micrometer $6.25 \times 10^{-2} \text{ mm}^2$ in area mounted within the ocular of Olympus CX41 light microscope. In a zoom of X400, MT-positive crypt cells were counted in every crypt for 10 randomly selected regions, and the numbers of MT-positive cells per 100 crypts in total were calculated.

Biochemical assays. Blood samples were withdrawn by syringe from hearts of the animals. Biochemical investigation was made in serum and tissue. Catalase (CAT) activity was assayed by the method of Aebi.¹⁵⁾ Lactate dehydrogenase (LDH) activity was determined by the method of Caboud and Wroblewski.¹⁶⁾ Serum urea and creatinine levels were determined by the acetylmonooxime method¹⁷⁾ and by the Jaffe reaction,¹⁸⁾ respectively. Uric acid levels were estimated by the method of Caraway.¹⁹⁾ Serum sialic acid levels were determined by Lorentz's method, with slight modifications.²⁰⁾ Intestinal tissues were homogenized in cold 0.9% saline by means of a glass homogenizer to make up a 10% (w/v) homogenate. The homogenates were centrifuged, and the clear supernatants were used in glutathione (GSH), lipid peroxidation (LPO), and protein analysis. GSH levels were determined by the method of Beutler using Ellman's reagent.²¹⁾ The malondialdehyde (MDA) content as an index of lipid peroxidation in intestinal tissue homogenates was determined by the method of Ledwozyw.²²⁾ The protein content in the supernatants was estimated by the method of Lowry using bovine serum albumin as standard.²³⁾

Statistical analysis. Microscopic and biochemical data were evaluated statistically with SPSS 15 software. All data were expressed as mean $\pm$ standard deviation (SD). Statistical analysis was performed by two-way analysis of variance (ANOVA) taking interactions between effects (ethanol and zinc administrations). Using a *post hoc* multiple comparison test, the Duncan's and Scheffe's multiple range tests were used to find the significance among means. Differences were considered statistically significant at $P < 0.05$.

Results

Histological results

A normal histological appearance was observed in intact controls and the other controls given zinc sulfate. Discontinuity and ruptures in the epithelium of the ends of the villi, expansion, compression, and loss of the villi, and edema inside of the villi were observed in the duodenal mucosa of the animals given ethanol. In addition, hyperemia, a few necrotic areas, inflammatory cell infiltration, increases in the numbers of eosinophilic cells in the epithelium, picnotic nuclei, and vacuolization and hyperplasia in the epithelial cells were detected in this group. PAS positive reaction in the goblet cells, in the brush border of villi, and between in the villi of the duodenal mucosa of the animals receiving ethanol decreased with respect to the control animals. However, the PAS positive reaction in the duodenal mucosa of the

animals given ethanol and zinc sulfate was the same as the control animals. Zinc sulfate pretreatment decreased these ethanol-induced degenerative morphological changes (Figs. 1, 2).

Immunohistochemical results

The quantitative assessment of MT immunoreactivity in the duodenal mucosa is presented in Fig. 3. MT immunoreactivity in the cytoplasm of the surface epithelial cells and goblet cells of the villi was determined only in the groups given zinc sulfate. No MT staining was detected in the cytoplasm or nuclei of the surface epithelial cells or the goblet cells in the intact rats or in the animals receiving only ethanol. MT was predominantly located in the cytoplasm and/or nucleus of the Paneth cells at the base of the crypts of Lieberkühn in all the groups (Fig. 4). According to two-way ANOVA results, there was a major effect of zinc on MT immunoreactivity in the duodenal mucosa. Regardless of ethanol administration, the numbers of MT immunoreactive crypt cells in zinc groups increased significantly with respect to those who did not receive zinc ($P < 0.001$). However, no major effect of ethanol on MT immunoreactivity was observed. The effect of interaction among zinc and ethanol on MT expression in the duodenal mucosa was also found to be insignificant (Table 1). In the control group given zinc sulfate and the experimental group given zinc sulfate and ethanol, MT expression in the crypt cells was significantly greater than in the intact control group ($^bP < 0.01$). The number of immunoreactive crypt cells decreased statistically significantly in the duodenum of all animals of the group given only ethanol as compared with the intact control group ($^cP < 0.05$). Zinc sulfate pretreatment of the ethanol group markedly stimulated MT expression in the crypt cells of the duodenal mucosa ($^aP < 0.001$).

Biochemical results

Serum LDH and CAT activities are presented in Table 2. The results of two-way comparisons revealed that there was a major effect of ethanol on serum LDH activities. Regardless of zinc sulfate administration, the activities of LDH in the groups administered ethanol showed an increase as compared with the control group, which did not ($P < 0.05$). There was also a major effect of zinc on the activities of LDH in the serum. Regardless of ethanol administration, the serum LDH activities in the animals receiving zinc sulfate were significantly lower than those not receiving it ($P < 0.001$). The effect of interaction among zinc and ethanol was found to be significant ($P < 0.001$) (Table 1). The activities of LDH in the serum in the group administered ethanol showed a significant increase as compared with the control group ($^aP < 0.001$), and a significant decrease was noted in the group administered ethanol + zinc sulfate as compared to the group administered ethanol ($^cP < 0.0001$). According to the two-way ANOVA results, there was a major effect of ethanol on serum CAT activities. In other words, regardless of zinc sulfate administration, the CAT activities of the ethanol group were significantly lower than those of the no-ethanol group ($P < 0.01$), but no major effect of zinc on CAT activities was observed. The effect of the zinc $\times$ ethanol interaction on serum CAT activities was also found to be insignificant

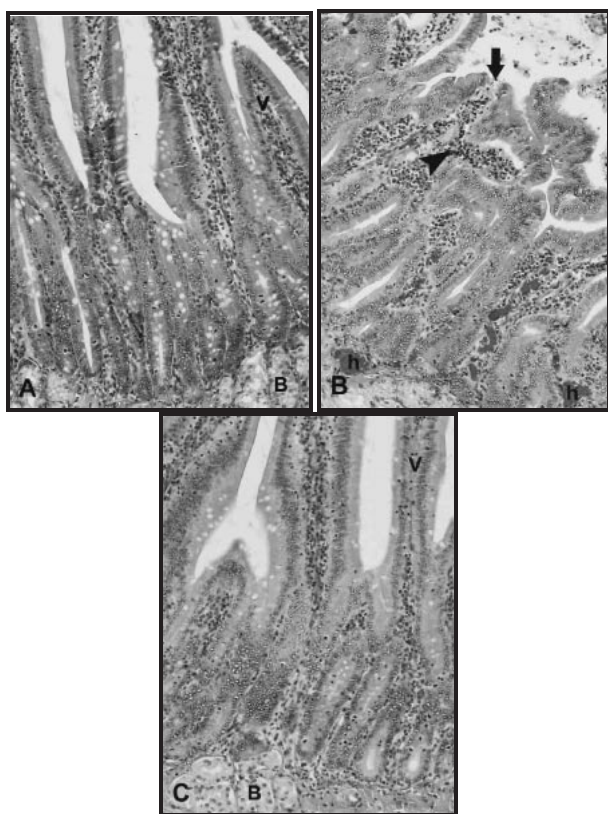


Fig. 1. Histological Appearance of the Duodenal Mucosa in All Groups.

A, Normal histological appearance of duodenal mucosa of control rats. Villi (V) and Brunner's glands (B). B, The histological appearance of duodenal mucosa of rats given ethanol. Discontinuity and ruptures in the epithelium of villi (➔), inflammatory cell infiltration (➤), hyperemia (h). C, Zinc sulfate pretreatment significantly decreased ethanol-induced degenerative changes in the duodenal mucosa. Villi (V) and Brunner's glands (B). Masson's triple dyes. Original magnification $\times 135$.

(Table 1). Serum CAT activity in the ethanol group was significantly lower than the control group ($^bP < 0.05$). In ethanol + zinc sulfate group, serum CAT activities were higher than in the ethanol group. Serum CAT activity in the ethanol + zinc sulfate group was significantly lower than in the control + zinc sulfate group ($^dP < 0.0001$).

Table 3 shows the mean serum urea, creatinine, uric, and sialic acid levels of all the groups. The results indicate that there was a major effect of ethanol on serum urea levels. In other words, regardless of zinc administration, those that received ethanol scored significantly higher than those that did not ($P < 0.001$). The effect of zinc $\times$ ethanol interaction also proved to be significant ($P < 0.001$). However, no main effect of zinc on urea levels was observed (Table 1). Serum urea levels were significantly higher in the ethanol group than in the controls ($^aP < 0.0001$).

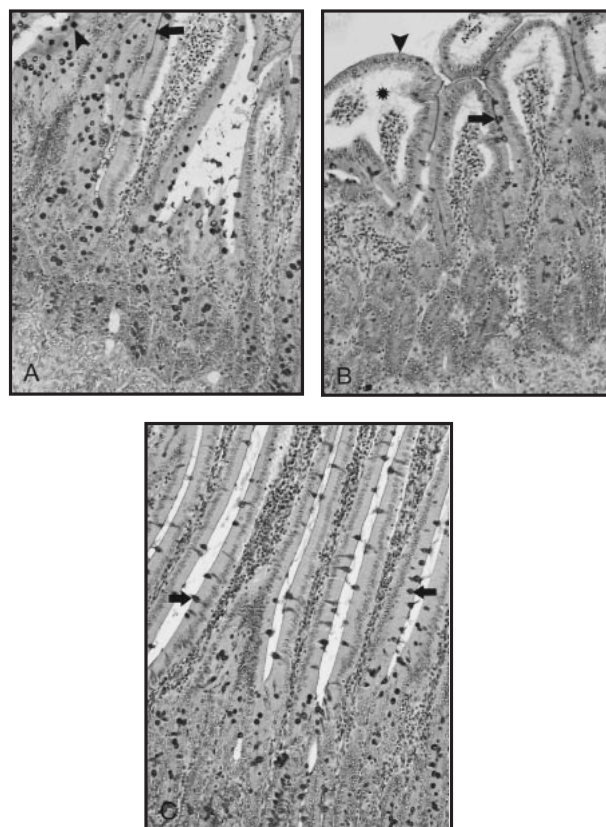


Fig. 2. PAS Positive Reaction in the Duodenal Mucosa in All Groups.

A, PAS positive reaction in goblet cells (➤), and in the brush border of villi (➔) of the duodenal mucosa of control rats. B, A decrease in the PAS positive reaction (➔), expansion and compression of the villi (➤), edema inside the villi (*) in the duodenal mucosa of the rats given ethanol. C, PAS positive reaction (➔) in the duodenal mucosa of the animals administered ethanol and zinc sulfate was the same as the control animals. Periodic acid Schiff. Original magnification $\times 135$.

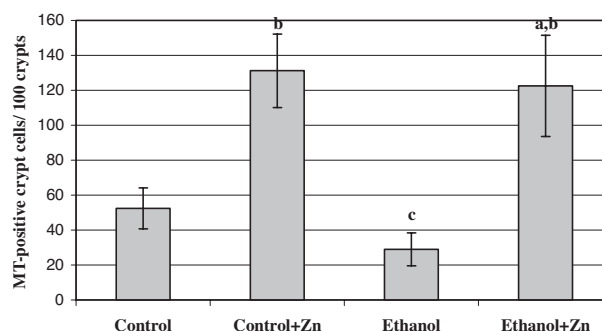


Fig. 3. Quantitative Assessment of MT Immunoreactivity in the Duodenal Crypts.

The data are given as mean $\pm$ SD per group: control, 52.48 $\pm$ 11.72, control + Zn, 131.21 $\pm$ 20.99, ethanol, 28.93 $\pm$ 9.35, ethanol + Zn, 122.46 $\pm$ 28.96. $^aP < 0.001$ versus ethanol group, $^bP < 0.01$ versus control group, $^cP < 0.05$ versus control group.

Table 1. Significant Results of All Parameters by Two-Way ANOVA

Source	MT	LDH	CAT	Urea	Creatinine	Uric acid	Sialic acid	LPO	GSH
Ethanol	NS	$P < 0.05$	$P < 0.01$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.01$	$P < 0.001$	$P < 0.001$
Zinc	$P < 0.001$	$P < 0.001$	NS	NS	NS	$P < 0.001$	NS	$P < 0.001$	$P < 0.001$
Ethanol $\times$ Zinc	NS	$P < 0.001$	NS	$P < 0.001$	NS	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$

NS, no significance

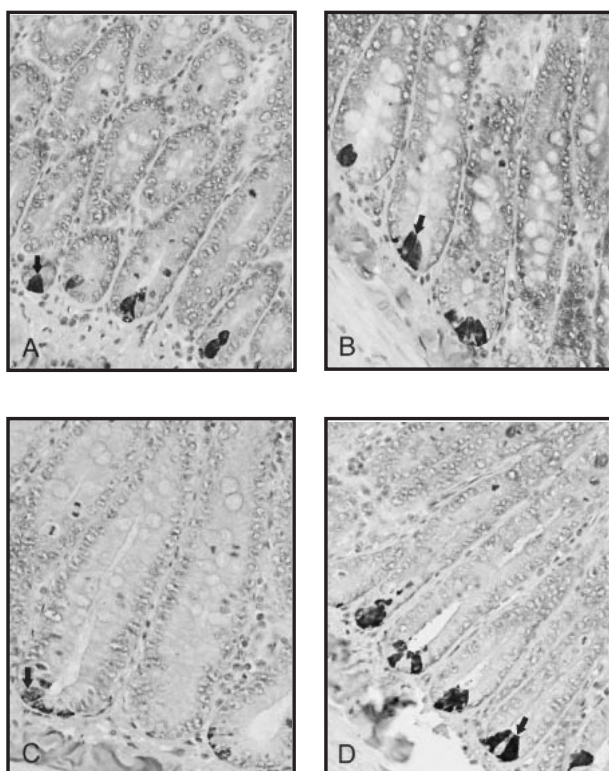


Fig. 4. MT Immunoreactivity in the Duodenal Mucosa of in All Groups.

A, Immunohistochemical staining for metallothionein in Paneth cells in the duodenal mucosa of intact rats (◆). B, Increased MT expression in control animals given zinc sulfate (◆). C, The numbers of MT immunoreactive crypt cells decreased in ethanol-treated rats (◆). D, Increased numbers of MT immunoreactive crypt cells in the duodenal mucosa of animals that received zinc sulfate and ethanol with respect to the rats given only ethanol (◆). Original magnification $\times 270$.

Administration of zinc sulfate caused a decrease in serum urea levels in the ethanol group. According to the statistical analysis, there was a major effect of ethanol on serum creatinine levels ($P < 0.001$). In other words, regardless of zinc sulfate administration, the creatinine levels in the ethanol group significantly increased as compared with the no-ethanol group ($P < 0.001$), but no major effect of zinc on creatinine levels was observed. There was also no statistically significant interaction between zinc and ethanol (Table 1). Serum creatinine levels were significantly higher in the ethanol group than in the controls ($^bP < 0.0001$). In the ethanol group treated with zinc sulfate, the serum creatinine levels significantly decreased when compared with the ethanol group ($^cP < 0.0001$). In the ethanol group treated with zinc sulfate, the serum creatinine levels decreased significantly as compared with the control + zinc sulfate group ($^dP < 0.0001$). Two-way ANOVA revealed that there was a major effect of ethanol and zinc sulfate on serum uric acid levels. Regardless of zinc administration, those that received ethanol scored significantly higher than those that did not ($P < 0.001$). However, regardless of ethanol administration, the uric acid levels of the zinc sulfate groups were lower than those of the no-zinc sulfate groups ($P < 0.001$). The effect of interaction among zinc and ethanol was also found to be significant ($P < 0.001$) (Table 1). Ethanol administration markedly increased

Table 2. Mean Levels of Serum Parameters

Group	n	LDH activity (U/ml)*	CAT activity (U/ml)*
Control	8	4416.66 $\pm$ 144.33	3249.42 $\pm$ 860.39
Control + Zinc sulfate	8	4475.00 $\pm$ 342.58	2702.00 $\pm$ 500.15
Ethanol	14	5785.71 $\pm$ 419.04 ^a	2371.42 $\pm$ 431.38 ^b
Ethanol + Zinc sulfate	11	4000.00 $\pm$ 500.00 ^{c,d}	2464.00 $\pm$ 188.07

*Mean $\pm$ SD

n, number of animals

^a $P < 0.001$ versus control group

^b $P < 0.05$ versus control group

^c $P < 0.0001$ versus ethanol group

^d $P < 0.0001$ versus control + zinc sulfate group

serum uric acid levels as compared to the control animals ($^cP < 0.0001$), but administration of zinc sulfate was found to decrease serum uric acid levels in the ethanol group ($^cP < 0.0001$). Statistical analysis revealed that there was a major effect of ethanol on serum sialic acid levels. Regardless of zinc sulfate administration, the sialic acid levels significantly increased after ethanol administration to the rats ($P < 0.01$). The effect of interaction between zinc and ethanol also found to be significant ($P < 0.001$). On the other hand, no major effect of zinc on sialic acid levels was observed (Table 1). Serum sialic acid levels were significantly higher in the ethanol group than in the controls ($^dP < 0.0001$). Oral administration of zinc sulfate significantly decreased the serum sialic acid levels in the ethanol group ($^fP < 0.001$).

The mean intestinal GSH and LPO levels of the four groups are shown in Table 4. Statistical analysis revealed that there was a major effect of ethanol and zinc on intestinal LPO levels. Regardless of zinc sulfate administration, the intestinal LPO levels in the groups given ethanol were found to increase as compared with the groups that did not receive ethanol ($P < 0.001$). Regardless of ethanol administration, the LPO levels in the animals receiving zinc were significantly lower than those that did not receive zinc ($P < 0.001$). The effect of interaction between zinc and ethanol also found to be significant ($P < 0.001$) (Table 1). Ethanol administration markedly stimulated LPO levels in intestinal tissues, and the MDA content was elevated by about 31.48% as compared to the control animals ($^aP < 0.0001$). Treatment with zinc sulfate decreased the LPO levels in rat intestinal tissue as compared to the animals given alcohol alone ($^dP < 0.0001$). The results of two-way comparisons revealed that both ethanol and zinc had major effects on intestinal GSH levels. Regardless of zinc administration, those that received ethanol scored significantly lower than those who did not ($P < 0.001$). However, regardless of ethanol administration, the GSH levels of zinc sulfate groups were markedly high as compared to animals who did not receive any zinc ($P < 0.001$). The effect of ethanol $\times$ zinc interaction on intestinal GSH levels was also found to be significant ($P < 0.001$) (Table 1). GSH levels were significantly decreased by ethanol administration in the intestine ($^bP < 0.001$), but with the administration of zinc sulfate, a considerable increase was observed in the GSH levels of the ethanol group ($^dP < 0.0001$). In the

Table 3. Mean Levels of Serum Urea, Creatinine, Uric Acid, and Sialic Acid for All Groups

Group	n	Urea* (mg/dl)	Creatinine* (mg/dl)	Uric acid* (mg/dl)	Sialic acid* (mmol/l)
Control	8	34.90 ± 0.41	0.27 ± 0.04	2.45 ± 0.01	2.00 ± 0.08
Control + Zinc sulfate	8	37.51 ± 1.28	0.27 ± 0.03	2.52 ± 0.02	2.15 ± 0.11
Ethanol	14	46.55 ± 3.55 ^a	0.64 ± 0.05 ^b	3.11 ± 0.01 ^c	2.31 ± 0.16 ^d
Ethanol + Zinc sulfate	11	43.30 ± 4.55 ^c	0.57 ± 0.04 ^{c,g}	2.55 ± 0.02 ^e	2.11 ± 0.07 ^f

*Mean ± SD n, number of animals ^{a,b,c,d}*P* < 0.0001 versus control group^e*P* < 0.0001 versus ethanol group^f*P* < 0.001 versus ethanol group^g*P* < 0.0001 versus control + zinc sulfate group**Table 4.** Mean Levels of Intestinal Glutathione and Lipid Peroxidation for All Groups

Group	n	LPO* (nmol MDA/mg protein)	GSH* (nmol GSH/mg protein)
Control	8	0.54 ± 0.04	17.25 ± 0.72
Control + Zinc sulfate	8	0.56 ± 0.06	20.91 ± 0.77
Ethanol	14	0.71 ± 0.05 ^a	7.95 ± 0.46 ^b
Ethanol + Zinc sulfate	11	0.56 ± 0.04 ^d	22.41 ± 0.44 ^{d,e}

*Mean ± SD

n, number of animals

^a*P* < 0.0001 versus control group^b*P* < 0.001 versus control group^d*P* < 0.0001 versus ethanol group^e*P* < 0.0001 versus control + zinc sulfate group

ethanol group treated with zinc sulfate, intestinal GSH levels increased significantly as compared with the control + zinc sulfate group (^e*P* < 0.0001).

Discussion

During alcohol ingestion, the upper gastrointestinal tract is exposed to high alcohol concentrations. Acute alcohol administration produces morphological and biochemical changes in the proximal part of the small intestine.^{3,24} Exposure of the intestinal mucosa to ethanol might result in structural damage, increased mucosal permeability, altered gut motility, impaired absorption of vitamins and nutrients, and promotion of bacterial overgrowth.⁴ In the present study, acute ethanol exposure caused serious degenerative morphological changes in the duodenal tissue. However, zinc sulfate pretreatment in ethanol group significantly reduced the degree of damage in the architecture of duodenal mucosa and increased the content of mucosal glycoprotein. The morphological findings of this study suggest that zinc sulfate has a protective effect on ethanol-induced duodenal injury in rats.

Metallothionein, an intracellular zinc-binding protein, is an important component in the antioxidant defense system in cells. Many studies have shown that metallothionein has antioxidant effects under various conditions of oxidative stress, including radiation exposure,²⁵ the toxicity of anticancer agents such as cisplatin, adriamycin, and doxorubicin,²⁶ and various chemicals, such as carbon tetrachloride, paraquat, and t-butyl hydroperoxide,²⁷ but the mechanism by which MT functions as a potent antioxidant is unclear. It has been suggested that MT serves as an endogenous zinc reservoir and that it releases zinc as a consequence of

stress-induced changes in the cellular redox state. Thus zinc might mediate the protective action of MT. However, MT is required to maintain high levels of zinc in the tissues.¹² The present study examined the immunohistochemical localization of MT in the duodenum in order to determine whether the protective effect of zinc on ethanol-induced duodenal injury is mediated by MT or is independent of it. Few immunohistochemical studies have examined the regional distribution of MT in the gastrointestinal tract in response to changes in dietary zinc status.^{28,29} No other study has immunohistochemically investigated the distribution of MT in the duodenal mucosa after zinc administration in ethanol-induced duodenal injury. We observed that MT immunoreactivity in the duodenal mucosa increased in the control group given zinc sulfate as compared to the intact control group. It was noted that the number of MT immunoreactive cells decreased in the duodenum of all animals of the group given only ethanol, but zinc sulfate administration to the ethanol group increased MT immunoreactivity remarkably when as compared to the group that received ethanol only. These results accord with our earlier findings on the liver³⁰ and gastric musosal tissue.³¹ We found that MT is located predominantly in the Paneth cells at the base of the crypts of Lieberkühn in all the groups. Paneth cells play important roles in the elimination and storage of heavy metals and regulate the microbial flora of the intestine.^{29,32} Paneth cells are known to express antimicrobial peptides, including alpha-defensins, also termed cryptdins, which are zinc-binding proteins.³³ Selective localization of MT in Paneth cells and zinc sulfate-induced MT expression in Paneth cells perhaps support the hypothesis that MT contributes to gut immunity as a storage protein for zinc. In this study, the presence of MT was demonstrated in surface epithelial cells and goblet cells only in the groups given zinc sulfate. Zinc is absorbed in the gut through the apical surfaces of enterocytes. Previous studies with radioactive isotopes have established that synthesis of MTs is induced within the enterocyte by parenteral or oral administration of zinc.^{34,35} Bauman *et al.*³⁶ reported that goblet cells in the duodenum of chicks concentrate zinc and excrete it into the lumen. These findings provide strong evidence for a regulatory role of metallothionein in zinc absorption and homeostasis. In view of these findings, we assume that the exogenous zinc has a MT-mediated protective role in ethanol-induced intestinal injury in rats.

Free radicals are directly implicated in the pathogenesis of acute and chronic duodenal ulceration induced by ethanol.³⁷ Production of reactive oxygen species is a physiological process, but increase of it and dysbalance between the production of radicals and the

antioxidant defense system can lead to oxidative stress. Acute or chronic ethanol administration increases lipid peroxidation of the cell membrane, and oxidative stress in several tissues.⁷⁾ Lipid peroxidation mediated by oxygen free radicals causes destruction and damage to cell membranes, and destroys the integrity of the duodenal mucosa.^{3,37)} In our study, the production of malondialdehyde as an index of lipid peroxidation was determined in intestinal tissues. Ethanol administration markedly elevated LPO levels in small intestine tissues with respect to the control animals. Zinc sulfate administration to the ethanol group reduced formation of MDA in the small intestine as compared to the animals given ethanol only; it even fell to the level of the control rats.

Endogenous antioxidant defense systems have a critical role in protecting against the development of acute intestinal injury. Glutathione is a major non-protein thiol in living organisms that plays an important role in protection against free radicals, peroxides, and other toxic compounds.^{38,39)} In the present study, ethanol significantly decreased the intestinal GSH concentration as compared to the control group. This remarkable reduction in intestinal GSH levels can be the result of oxidation of GSH due to the generation of toxic oxygen metabolites induced by ethanol or the binding of glutathione to acetaldehyde generated through the oxidation of ethanol by alcohol dehydrogenase activity,⁴⁰⁾ or to increased utilization of GSH by antioxidant enzymes such as glutathione peroxidase.³⁸⁾ Our results are in agreement with observations of ethanol-induced injury by other researchers.^{38,41)} The results indicate that ethanol-induced intestinal damage is associated with a significant reduction in nonprotein sulfhydryl concentrations in rats. The significant rise in GSH levels observed in the present study after zinc administration to animals in the ethanol group provides strong evidence for the antioxidative potential of zinc.

Catalase, which is included in the ethanol-metabolizing system, plays an important role in alcohol oxidation.^{7,42)} It acts as a preventative antioxidant and protects aerobic organisms from the deleterious effects of hydroxyl radicals.³⁹⁾ It is known that ethanol ingestion has effects on the plasma antioxidant system of rats.⁴³⁾ In this study, administration of acute ethanol led to a significant decrease in serum CAT activity. The decrease in the catalase activity might have been due to depletion or inhibition of the enzyme as a result of increased production of free radicals,⁴⁴⁾ but serum CAT activity in the ethanol + zinc sulfate group increased insignificantly as compared to the ethanol group.

Lactate dehydrogenase is one of the metabolic requirements of tissue and is involved in energy production. It can be used as an indicator for cellular damage in clinical practice and at the cytotoxicity of toxic agents.⁴⁵⁾ LDH has been reported to increase in alcoholic liver injury and alcoholic myopathy.⁴⁶⁾ When these tissues or organs are damaged, LDH is released into the blood from the cells. Leakage of this enzyme increases due to changes in the permeability of the plasma membrane as a consequence of peroxidation of the membrane by oxygen- and ethanol-derived free radicals.⁴⁷⁾ In our study, serum LDH activity increased in the ethanol group. Administration of zinc sulfate to

the ethanol group caused decreases in serum LDH activity. This effect might be related to the membrane stabilizing effect and radical scavenging potency of zinc sulfate.^{8,9)}

Serum levels of urea and creatinine have been used as indicators of renal protection.⁴⁸⁾ Elevated blood urea is known to be correlated with increased protein catabolism in mammals, and/or the conversion of ammonia to urea as a result of increased synthesis of the arginase enzyme involved in urea production. In agreement with this, other study results of ours indicate that serum total protein concentrations decreased in animals treated with ethanol.¹⁾ Administration of absolute ethanol to rats leads to marked elevations of serum urea and creatinine levels. The increased blood urea and creatinine are in agreement with the results obtained by other researchers.⁴⁹⁾ This elevation of urea and creatinine levels reflects the degree of renal injury. In our study, serum urea and creatinine levels were increased by the application of ethanol, but treatment with zinc sulfate reduced the serum urea and creatinine levels in the ethanol groups, and this indicates that zinc sulfate prevents the damage caused by ethanol. It can be concluded from these results that zinc sulfate possess a remarkable protective effect on absolute ethanol-induced renal injury.

In our study, the rats in the ethanol group showed significant rises in serum uric acid concentrations. Hyperuricemia has been reported to be an independent predictor of cardiovascular risk.⁵⁰⁾ In another report, it was stated that hyperuricemia is a renal prognostic factor.⁵¹⁾ One of the mechanisms by which hyperuricemia might lead to renal prognosis is the linkage between hyperuricemia and the inflammatory response. Another mechanism is the relationship between hyperuricemia and atherosclerosis. Increased oxidant stress and oxidation of low density lipoprotein (LDL) in the arterial wall due to peroxynitrite play important roles in the development of atherosclerosis. In this sense, the finding of elevated serum uric acid concentrations might reflect the body's response to increased production of endogenous oxygen species, because uric acid is a potent scavenger of peroxynitrite.⁵²⁾ Suarna *et al.*⁵³⁾ reported a more direct role of uric acid in the progression of atherosclerotic process: urate crystals trigger an inflammatory response in the vascular plaque. In our study, serum uric acid levels were significantly higher in the ethanol group than in control group. Administration of zinc sulfate significantly decrease uric acid in the serum of the ethanol group. A decrease in serum uric acid levels indicates a beneficial effect in renal and cardiovascular injury.

Sialic acid is a terminal component of the non-reducing end of the carbohydrate chains of glycoproteins and glycolipids.⁵⁴⁾ Serum sialic acid has been suggested as a new marker of excessive alcohol consumption.⁵⁵⁾ It has been reported that ethanol administration to rats enhanced the sialic acid content of microvillus membranes in the intestine.⁵⁶⁾ Romppanen *et al.*⁵⁵⁾ found that serum sialic acid concentrations significantly increased in alcoholics. In the present study, the serum sialic acid levels were significantly higher in the ethanol group than in the control rats. However, zinc sulfate pretreatment in the ethanol group caused a significant decrease in the sialic acid concentration.

In conclusion, microscopic and biochemical evaluations indicate that zinc sulfate prevents ethanol-induced intestinal injury. We suggest that zinc used against intestinal injury has a protective effect both by stimulation of MT synthesis and through GSH and CAT, as well as having antioxidative potential.

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