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Protective Effects of Ascorbic Acid, DL-α-Tocopherol Acetate, and Sodium Selenate on Ethanol-Induced Liver Damage of Rats

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ABSTRACT

In this study, the effect of a combination of vitamin C (ascorbic acid), vitamin E (DL-α-tocopherol acetate), and selenium (sodium selenate) on ethanol-induced liver damage in rats was investigated, morphologically and biochemically. The ethanol-induced injury was produced by the administration of 1 mL of absolute ethanol to each rat. Animals received vitamin C (250 mg/kg), vitamin E (250 mg/kg), and selenium (0.5 mg/kg) (ViCESe) for 3 d 1 h prior to the administration of absolute ethanol. In the liver of the animals given ethanol, the degenerative changes such as extreme hyperemia, vacuolization in cells of portal areas, a dilation in sinusoids, mononuclear cell infiltration, a swelling in cisternae of granular endoplasmic reticulum and in mitochondrial cristae, an increase in smooth endoplasmic reticulum, many lipid vacuoles were observed both light and electron microscopically. A similar structure was usually distinguished when compared with control animals, in rats given ethanol + ViCESe. In this group, the findings indicating cellular damage were either not observed at all or were decreased. In the group administered ethanol, a reduction of the blood glutathione (GSH) level and increases in serum values of alanine aminotranserase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), and γ-glutamyl

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transferase (GGT) activities were observed, whereas in the control group, the reverse was found to occur. On the other hand, in the group in which ethanol + ViCESe was administered, it was observed that the blood GSH value and serum ALP and ALT activities increased and serum AST, LDH, and GGT activities decreased. As a result, the present study indicates that ViCESe because of their antioxidant activity against ethanol damage have a protective effect on the liver.

Index Entries: Alcohol; liver damage; ascorbic acid; DL-α-tocopherol acetate; sodium selenate; selenium; rat.

INTRODUCTION

Ethanol is a direct systemic toxin that produces injury to all tissues, depending on the dose and duration of exposure. The degree of injury varies among organ systems. The liver is predominantly responsible for ethanol metabolism *(1).* Ethanol is extensively metabolized into cytotoxic acetaldehyde by alcohol dehydrogenase enzyme in the liver, and acetaldehyde is oxidized to acetate by aldehyde oxidase or xanthine oxidase, giving rise to reactive oxygen species (ROS) *(2).* The role of free radicals in the development of alcoholic liver damage has been suspected since the early 1960s *(3).* Although there are various hypotheses, little is known about the pathophysiology and underlying cellular and molecular mechanisms of ethanol-induced liver damage *(4).* Alcohol-induced oxidative stress is linked to the metabolism of ethanol *(3).* Oxidative stress in the cells or tissues refers to enhanced generation of ROS and/or a depletion in the antioxidant defense system causing an imbalance between pro-oxidants and antioxidants *(5).* The development of new and effective strategies to diminish the production of oxidants and/or enhance intracellular and extracellular antioxidant defenses in the liver offers great promise for the prevention and treatment of liver disease *(4).* Among the mechanisms implicated in alcohol-dependent liver disease, free-radical generation and lipid peroxidation appear to play important roles *(6).* It has been reported that there is an increase in the formation of free radicals following ethanol administration *(7).*

Antioxidants are essential in preventing the cellular damage caused free radicals and free-radical-modified lipid peroxidation. In normal metabolism, there is a balance between the generation of free radicals and antioxidant defense mechanism. Excessive ethanol use commonly leads to vitamin deficiency *(1).* Recent studies have shown that both vitamins C and E are reduced in alcoholics *(8).* A number of investigations have revealed that vitamin C, vitamin E, and selenium levels decreased by exposure to ethanol *(9).* It is suggested that decreased hepatic α-tocopherol and increased lipid peroxidation after ethanol feeding may be of relevance for the issue of vitamin E requirement *(10).* The antioxidant pretreatment data clearly indicated that both vitamins C and E can prevent the generation of 1-hydroxyethyl radicals following acute ethanol treatment. These antioxidants probably exert their effects through their ability to scavenge reactive oxidants *(9).*

Vitamins C and E or vitamin E and selenium exert synergistic effects in the prevention of biological membranes from oxidants *(9).* Vitamin C is a water-soluble hydrophilic antioxidant that protects cells from oxidative stress by scavenging free radicals *(11).* Vitamin E is a family of lipid-soluble vitamins and acts as an antioxidant in cells, interrupting the propagation of lipid peroxidation in the plasma membrane and thus preserving membrane integrity *(12).* Selenium is an essential trace element and it may play several roles in the human body and is an essential component of glutathione peroxidase *(3).* It is known that the antioxidant effect of selenium is associated with its presence in the glutathione peroxidases, which are known to protect DNA and other cellular components *(13)* and that α-tocopherol and selenium are decreased in human blood plasma by excessive alcohol intake *(14).* In this study, we examined whether vitamin C, vitamin E, and selenium (ViCESe) have a protective effect against alcohol-induced liver injury in rats.

MATERIALS AND METHODS

Animals

Forty, 4- to 5-mo-old, female Spraque–Dawley rats weighing 200–250 g (Istanbul University Centre for Experimental Medical Research and application [DETAM]) were used in this study. The animals were fed with pellet chow and tap water *ad libitum* before the experiments and fasted for 24 h prior to the experiments. All rats were clinically healthy.

Experimental Design and Treatment of Animals

The animals were randomly divided into four groups: group I, intact animals (control); group II, control animals receiving vitamin C (250 mg/kg/d for 3 d), vitamin E (250 mg/kg/d for 3 d), and sodium selenate (0.5 mg/kg/d for 3 d); group III, animals receiving 1 mL absolute ethanol; group IV, animals receiving ViCESe for 3 d 1 h prior to the administration of absolute ethanol (in the same dose and time). The antioxidants and absolute ethanol were given to rats by gavage.

Animal Model for Liver Damage

The damage was induced by oral administration at a constant volume by 1 mL absolute ethanol per rat. The animals were sacrificed by ether 1 h after treatment with absolute ethanol.

Histological Examination

The liver samples that were obtained were fixed in Bouin fixative and embedded in paraffin after having completed the routine follow-up. Sections of 5 μm thickness stained with hematoxylin–eosin and Masson tridye were examined under a Carl Zeiss Ultraphot II light microscope.

Ultrastructural Examination

The liver tissue samples of 1 mm thickness were embedded in Epon 812 after having been prefixed in 2% glutaraldehyde prepared with Sörensen's phosphate buffer (pH 7.2), and postfixation in osmium tetroxide of 1% was prepared with the same buffer. Ultrathin sections that were obtained were examined under a Carl Zeiss EM 9 S-2 electron microscope.

Biochemical Assays

The blood samples were withdrawn by syringe from the heart. Biochemical investigation was made in serum and blood. Biochemical investigations of alanine amino transferase (AST), aspartate amino transferase (ALT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), and γ-glutamyl transferase (GGT) in serum were measured by means of an autoanalyzer (Targa 3000 Biotechnica, Rome, Italy). Blood glutathione (GSH) levels were measured according to the Beutler, Duron, and Kelly method using Ellman's reagent *(15).* GSH is reacted with 5,5-dithiobis-2-nitrobenzoic acid (DTNB), resulting in the formation of a product with a maximal absorbance at 412 nm. Results were expressed as milligram per deciliter.

The results were evaluated using an unpaired *t*-test and analysis of variance (ANOVA) using the NCSS statistical computer package (Kaysville, Utah, USA).

RESULTS

Light Microscopic Results

In the liver of the animals given ethanol, extreme hyperemia, vacuolization in cells of portal areas, a dilation in sinusoids, and mononuclear cell infiltration were observed. A similar structure was distinguished, in comparison to control group, in rats given ethanol + ViCESe. In this group, the findings indicating cellular damage were either not observed or were decreased (see Figs. 1–4).

Ultrastructural Results

A swelling in the cisternae of granular endoplasmic reticulum and mitochondrial cristae, a dilation in perinuclear area, an increase in smooth endoplasmic reticulum, and many lipid vacuoles were noted in the hepatocytes of the animals given ethanol. There was no detectable difference except less lipid vacuoles when compared with control animals in hepatocytes of rats given ethanol + ViCESe. In this group, the findings indicating cellular damage were either not observed at all or were decreased (*see* Figs. 5–7).

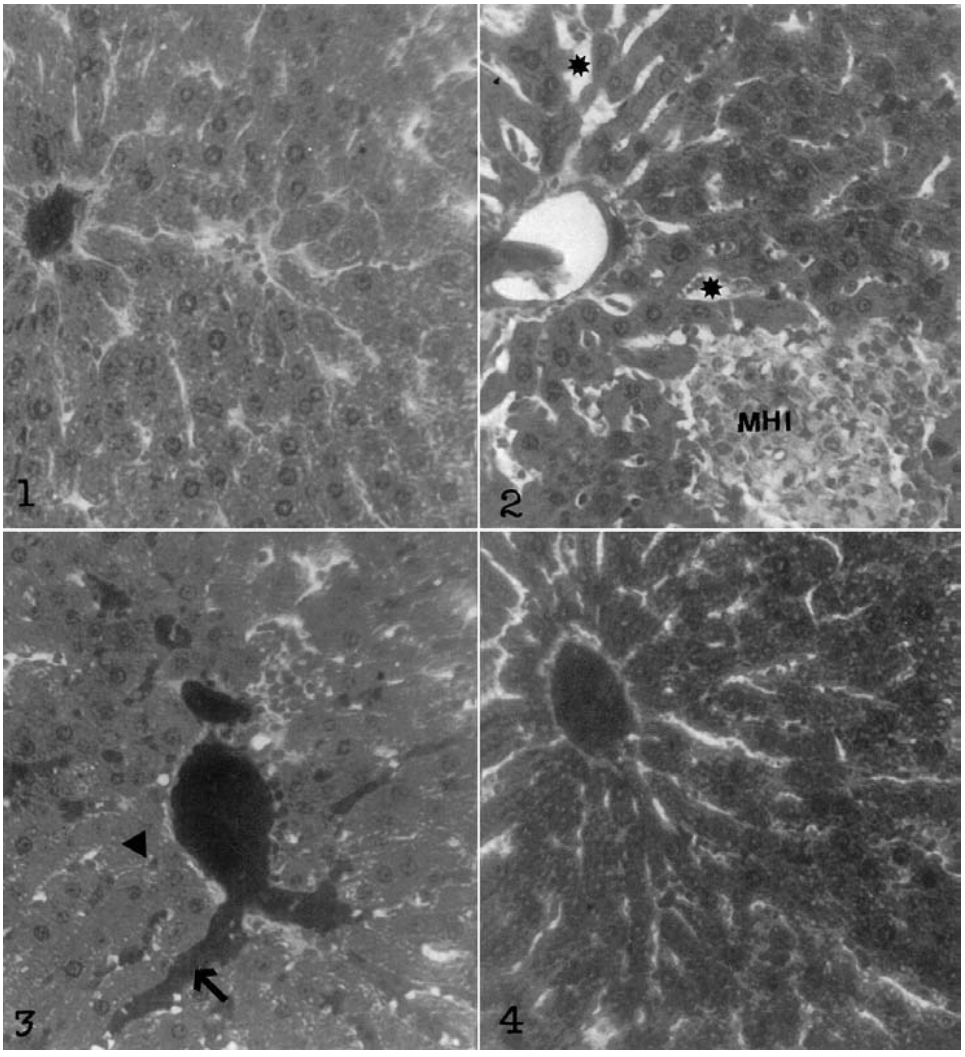

Fig. 1. Normal histological appearance of the liver tissue of a control rat. Masson. Magnification = 400×.

Fig. 2. Histological appearance of the liver tissue of the animal given ethanol. Dilation in sinusoids (*), and mononuclear cell infiltration (MHI). Masson. Magnification = 400×.

Fig. 3. Histological appearance of the liver tissue of the animal given ethanol. Extreme hyperemia (→), vacuolization (▲). Masson. Magnification = 400×.

Fig. 4. Histological appearance of the liver of the animal given ethanol+ViCESe. An ordinary appearance of the liver tissue as controls. Masson. Magnification = 400×.

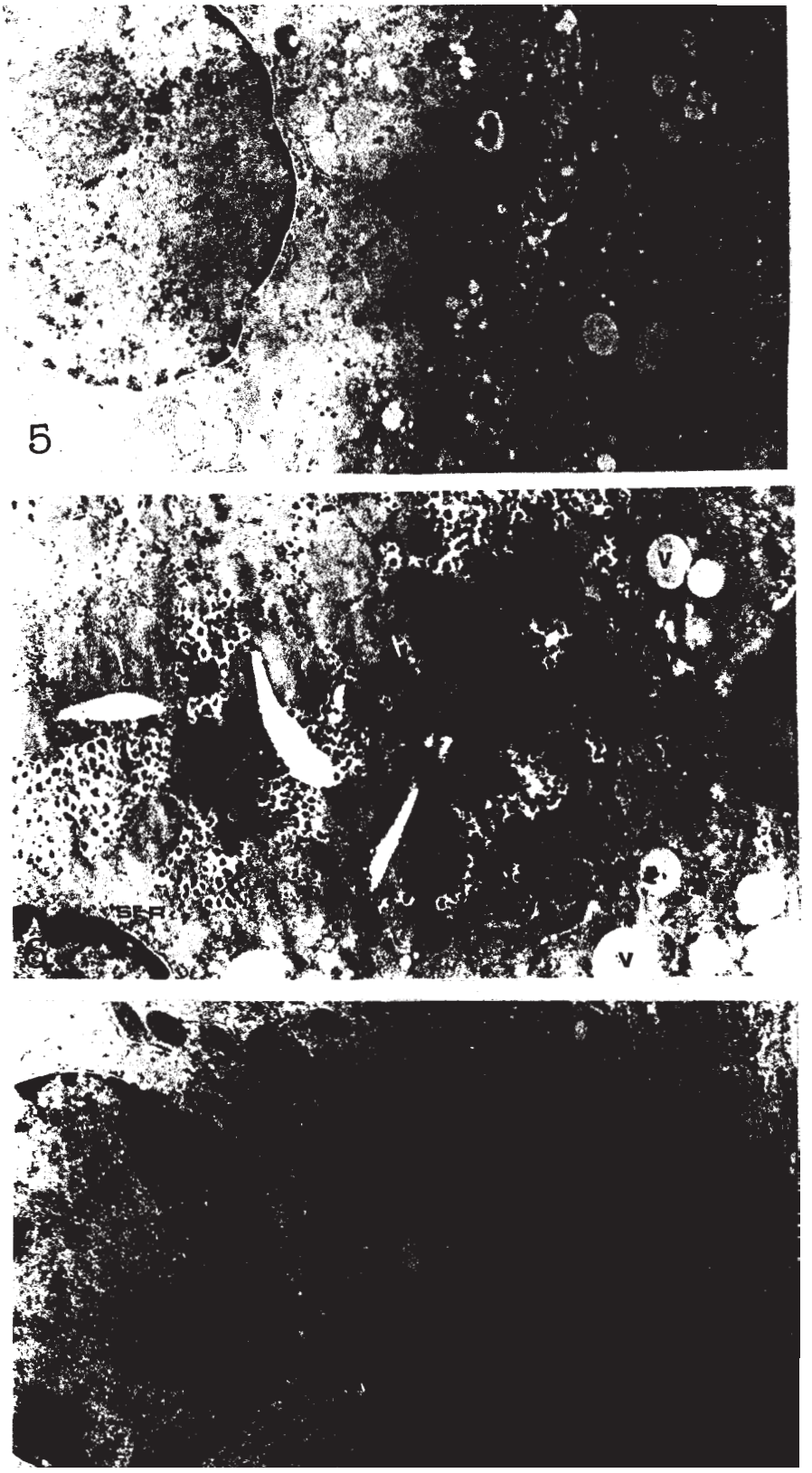
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Fig. 5. An electron micrograph of the hepatocyte of a control animal. Magnification = 10,800×.

Fig. 6. An electron micrograph of the hepatocyte of the animal given ethanol. An increase of smooth endoplasmic reticulum (SER) and many lipid vacuoles (V). Magnification = 10,800×.

Fig. 7. An electron micrograph of the hepatocyte of the animal given ethanol+ViCESe. An ordinary appearance of hepatocytes as controls. Magnification = 10,800×.

Biochemical Results

Serum AST, ALT, ALP, LDH, and GGT activities are presented in Table 1. From the obtained results, values of AST in the serum in the group administered ethanol have shown a significant increase when compared with the control group (Pt-test = 0.0001); also, a significant decrease was noted in the group administered ethanol + ViCESe compared to the group administered ethanol (Pt-test =0.0001). According to Table 1, a significant difference in the AST activities of the four groups was observed (p_{ANOVA} =0.0001). When the ALT activities of rats administered ethanol were compared with the values of the control group, a statistically significant increase was determined (Pt-test= 0.0001). After the administration of ethanol + ViCESe, an increase in ALT values in comparison to the group that was administered only ethanol was observed (Pt-test=0.0001). A significant increase of serum LDH activities in the group administered ethanol was determined in comparison (Pt-test = 0.0001) to the control group, and when LDH activities of the group administered ethanol + ViCESe were compared with the group administered only ethanol, a statistically significant decrease (Pt-test =0.0001) was determined. The activities of ALP in the serum in the group administered ethanol showed a statistically insignificant increase when compared with the control group (Pt-test = 0.001); also, a significant increase in the group administered ethanol + ViCESe when compared to the administered ethanol (Pt-test = 0.0001) was noted. According to Table 1 values, a significant difference in the serum LDH and ALP values of the four groups were observed (p_{ANOVA} = 0.0001). In this study, a statistically significant increase was observed in serum GGT values of the group administered ethanol, in comparison with the control group (Pt-test = 0.0001). On the other hand, in the group to which ethanol + ViCESe was administered, the serum GGT activities decreased. A significant difference in the serum GGT activities of the four groups was observed (p_{ANOVA} = 0.0001).

Blood GSH levels are presented in Table 2. From the obtained results, values of glutathione in blood in the group administered ethanol have shown a significant decrease when compared with the control group (Pt-test = 0.004). Also, a significant increase in the group administered ethanol + ViCESe when compared to the group administered ethanol (Pt-test = 0.0001) was observed. According to Table 2, a significant difference in the blood GSH levels of the four groups was observed (p_{ANOVA} = 0.0001).

Table 1
Mean (Mean ± SD) Levels of all Serum Parameters[e]

Groups	n	AST (U/L)	ALT (U/L)	LDH (U/L)	ALP (U/L)	GAMA –GT (U/L)
Control	10	188.43 ±7.59	49.44 ± 1.98	439.45 ± 8.57	51.88 ± 4.97	4.73 ± 1.55
Control + ViCESe	10	160.83 ± 7.49[a]	72.85 ± 2.39[a]	782.33 ± 29.50[a]	137.75 ±5.44[a]	8.01 ± 0.82[a]
Ethanol	10	239.90 ± 5.69[c]	61.62 ± 3.15[c]	1158.74 ± 93.06[c]	64.84 ± 5.46[b]	14.99 ± 2.06[c]
Ethanol + ViCESe	10	161.64 ± 9.59[d]	77.13 ± 2.10[d]	818.07 ± 22.39[d]	120.22 ± 6.43[d]	7.76 ± 1.41[d]
P_{ANOVA}		0.0001	0.0001	0.0001	0.0001	0.0001

[ac] P_t-test = 0.0001 vs control groups.
[b] P_t-test = 0.001 vs control groups.
[d] P_t-test = 0.0001 vs ethanol groups.
[e] *n* = Number of animals.

Table 2
Mean (Mean ± SD) Levels of Blood Glutathione for all Groups

Groups	Number of Animals	Glutathione (mg/dl)
Control	10	35.92 ± 4.45
Control+ ViCESe	10	49.91 ± 9.15[a]
Ethanol	10	25.89 ± 7.32[b]
Ethanol+ ViCESe	10	58.43 ± 10.18[c]
P_{ANOVA}		0.0001

[a] P_t-test = 0.002 vs control groups.
[b] P_t-test = 0.004 vs control groups.
[c] P_t-test = 0.0001 vs ethanol groups.

DISCUSSION

Hepatocyte damage is an important event in the course of alcoholic liver injury *(3)*. Chronic ethanol administration induces oxidative stress and increases lipid peroxidation of the cell membrane. This leads to increased membrane fluidity, disturbances of calcium homeostasis, and, finally, cell death *(14)*. Oxygen-free-radical production in alcoholic liver is believed to be a major mechanism of tissue injury. This hypothesis has found experimental support by the use of an intragastric tube feeding model of chronic ethanol administration. Intragastric infusion of ethanol results in the oxidation of lipids and proteins, and the decrease in the antioxidant defense may contribute to the extent of liver oxidative damage *(16)*. Ethanol-induced hepatic injury was indicated when it was found that acute ethanol intoxication resulted in increased lipid peroxide formation and in a reduction in GSH levels in the livers of rats. Reduction in the GSH content of the liver is a common feature of hepatotoxicity *(17)*.

Vitamin E is an important antioxidant in biological systems and is readily absorbed by the intestine. α-Tocopherol is present in the lipid bilayers of biological membranes, where it may play a structural role *(18)*. This vitamin very efficiently scavenges lipid peroxyl radicals and thereby prevents the lipid peroxidation process in an unhibited chain reaction. Tocopherol defi-

ciency is characterized by a number of chronic health problems; there is damage to cell membranes as a result of increased lipid peroxidation. Ascorbate has been shown to efficiently scavenge superoxide, hydrogen peroxide, hypochloride, hydroxyl radicals, and peroxyl radicals and to restore the antioxidant properties of fat-soluble α-tocopherol *(19).* Selenium is a naturally occurring antioxidant and appears to preserve tissue elasticity by delaying oxidation of polyunsaturated fatty acids. Selenium is an essential component of glutathione peroxidase, whose main role is to decompose safely mainly hydrogen peroxide and organic peroxides with the help of reduced GSH *(3).* When combined, ViCESe play a complementary role in the prevention of oxidative damage in cellular and subcellular membranes *(20).*

It is reported that the administration of α-tocopherol acetate to rats reduced the hepatic triglyceride accumulation caused by ethanol *(21).* The many lipid vacuoles observed in the group given ethanol in the present study may be explained by hepatic triglyceride accumulation. In addition, the increase in smooth endoplasmic reticulum indicates the accumulation of lipid vacuoles, including triglycerides, in this group. Antioxidants, mainly α-tocopherol, protect low-density lipoprotein (LDL) particles against oxidation. This leads to their consumption, if they are not effectively regenerated by antioxidants in the hydrophilic compartment, especially by ascorbic acid. Regular intake of vitamin E and other antioxidants can thus retard the oxidative modification of LDL *(22).* Owing to its role in the regeneration of α-tocopherol, ascorbate supplementation may be useful. A supplementation in antioxidant micronutrient such as selenium may present another effective means to reduce ethanol toxicity *(23).* Pharmacological antioxidants could have beneficial effects in reducing the incidence of ethanol-induced changes in cellular lipids, proteins, and nucleic acids. The antioxidants considered could act by reducing free-radical production, trapping free radicals themselves, interrupting the peroxidation process, or reinforcing the natural antioxidant defense *(14).* Oxygen-derived free radicals mediate tissue damage. Among these radicals are the superoxide anion and hydroxyl radical. These radicals promote lipid peroxidation and membrane damage by crosslinking to macromolecules *(24).* Therefore, the radicals are detrimental to the integrity of biological tissues and may be responsible for their injury *(25).* In this study, the results might also indicate that free radicals might be involved in the pathogenesis of the histological damage caused by ethanol in the liver.

Ethanol administration affects the generation of mitochondrial free radicals *(4).* Short-term ethanol administration has been shown to increase superoxide generation in liver mitochondria and in perfused rat liver *(26).* The enhanced superoxide generation increases lipid peroxide and induces mitochondrial dysfunction in rats that have been subjected to acute ethanol exposure *(27).* Depletion of GSH may markedly increase the susceptibility to further mitochondrial dysfunction from oxidant stress produced by ethanol or simply from physiological consequences of aerobic respiration and mitochondrial GSH as histological liver disease progresses

(28). The opening of the pore is thought to induce the influx of water and solutes, followed by mitochondrial swelling and the rupture of its outer membrane, resulting in the release of cytochrome-*c (4).* The changes in the electron transport chain and in the ATPase complex lead to slower respiration in the presence of ADP in the ethanol-fed animals, leading to a lower rate of ATP synthesis *(29).* The swelling in mitochondrial cristae observed in our study may indicate a decrease in oxidative phosphorylation and a sign of histological liver damage.

Glutathione is synthesized in the cytoplasm of the liver cells and then distributed through the circulatory/transport system into different organs and subcellular compartments. Glutathione plays a major role as a reductant in oxidation–reduction process and also serves in detoxification *(30).* In healthy subjects, GSH is very important for its powerful reducing capability of free radicals.

The ethanol or its metabolites can alter the balance in the liver toward auto-oxidation, either acting as pro-oxidants, or reducing the antioxidant levels, or both *(8).* The pathogenesis of alcohol-induced liver disease involves the adverse effect of ethanol metabolites and oxidative tissue injury *(31).*

The liver is the most important organ in the metabolism of drugs and other substances *(32).* Morphological alterations that occur in the liver affect many metabolic processes in the organism. Chronic and acute administration of ethanol to rats could lead to a variety of pathological changes and ultrastructural abnormalities in the liver *(33).* Liver cell destruction shows its effects by an impairment in liver cell membrane permeability and a resultant release of some enzymes into blood plasma and increase in their activity. The increase in AST, ALT, LDH, ALP, and GGT activities in serum is an indicator of liver destruction.

In our study, it was observed that as a result of ethanol, enzymes such as AST, ALT, LDH, ALP, and GGT were released into the blood. Their increase in the serum activities of these enzymes was directly proportional to the degree of cellular damage. In our study, these values (AST, LDH, GGT) decreased by administration of ViCESe. The decrease in those increasing enzyme levels shows that ViCESe prevented damage in the liver.

Ethanol also enhances oxidative stress by reducing the level of antioxidants. A putative role for ethanol-induced oxidative stress in the process of mitochondrial dysfunction and cell death is suggested *(5).* Its increase and imbalance between the production of radicals and antioxidants could lead to oxidative stress with the affection of various biological functions and structural changes *(34).* Hepatic tissue damage significantly increased in comparing ethanol administered rats and control rats. The degenerative changes such as extreme hyperemia, vacuolization, mononuclear cell infiltration, the swelling in cellular organelles, many lipid vacuoles are very important changes. These changes show a decrease in metabolical functioning of the cells. Histological and ultrastructural damage caused by ethanol were also diminished in the liver of the animals given ethanol + ViCESe in our study and a similar structure was almost distinguished in

comparison to the control group. As a result of all the obtained morphological and biochemical findings, it has been concluded that ViCESe have a protective effect against ethanol hepatotoxicity.

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REFERENCES

1 G. Kanbak, M. Inal, and C. Baycu, Ethanol-induced hepatotoxicity and protective effect of betaine, *Cell Biochem. Funct.* **19,** 281–285 (2001).
2 I. Fridovich, Oxygen radicals from acetaldehyde, *Free Radical Biol. Med.* **7,** 557–558 (1989).
3 T. Zima, L. Fialova, O. Mestek, et al., Oxidative stress, metabolism of ethanol and alcohol-related diseases, *J. Biomed. Sci.* **8,** 59–70 (2001).
4 M. Adachi and H. Ishii, Role of mitochondria in alcoholic liver injury, *Free Radical Biol. Med.* **32,** 487–491 (2002).
5 E. C. Schlorff, K. Husain, and S. M. Somani, Dose- and time-dependent effects of ethanol on plasma antioxidant system in rat, *Alcohol* **17,** 97–105 (1999).
6 D. J. Tuma, Role of malondialdehyde–acetaldehyde adducts in liver injury, *Free Radical Biol. Med.* **32,** 303–308 (2002).
7 R. Polavarapu, D. R. Spitz, J. E. Sim, et al., Increased lipid peroxidation and impaired antioxidant enzyme function is associated with pathological liver injury in experimental alcoholic liver disease in rats fed diets high in corn oil, and fish oil, *Hepatology* **27,** 1317–1323 (1998).
8 M. V. Suresh, C. V. S. Kumar, J. J. Lal, et al., Impact of massive ascorbic acid supplementation on alcohol induced oxidative stress in guinea pigs, *Toxicol. Lett.* **104,** 221–229 (1999).
9 P. Navasumrit, T. H. Ward, N. C. F. Dodd, et al., Ethanol-induced free radicals and hepatic DNA strand breaks are prevented in vivo by antioxidants: effects of acute and chronic ethanol exposure, *Carcinogenesis* **21,** 93–99 (2000).
10 T. Kawase, S. Kato, and C. S. Lieber, Lipid peroxidation and antioxidant defense systems in rat liver after chronic ethanol feeding, *Hepatology* **10,** 815–821 (1989).
11 D. A. Stoyanovsky, D. Wu, and A. I. Cedrebaum, Intraction of hydroxyethyl radical with glutathione, ascorbic acid and α-tocopherol, *Free Radical Biol. Med.* **24,** 132–138 (1998).
12 S. Warren, S. Patel, and C. M. Kapron, The effect of vitamin E exposure on cadmium toxicity in mouse embryo in vitro, *Toxicology* **142,** 119–126 (2000).
13 G. N. Schrauzer, Anticarcinogenic effect of selenium, *Cell Mol. Life Sci.* **57,** 1864–1873 (2000).
14 R. Nordmann, Alcohol and antioxidant systems, *Alcohol Alcohol.* **29,** 513–522 (1994).
15 E. Beutler, O. Duron, and B. M. Kelly, Improved method for the determination of blood glutathione, *J. Lab. Clin. Med.* **61,** 882–888 (1963).
16 H. Rouach, V. Fataccioli, M. Gentil, et al., Effect of chronic ethanol feeding on lipid peroxidation and protein oxidation in relation to liver pathology, *Hepatology* **25,** 351–355 (1997).
17 C. M. Macdonald, J. Dow, and M. R. Moore, A possible protective role for sulphydryl compounds in acute alcoholic liver injury, *Biochem. Pharmacol.* **26,** 1529–1531 (1977).

18 G. M. Kostner, D. Otti, M. Jauhiainen, et al., Human plasma phospholipids transfer protein accelerates exchange/transfer of α-tocopherol between lipoproteins and cells, *Biochem. J.* **305,** 659–667 (1995).
19 E. Skrzdlewska and R. Farbiszewski, Lipid peroxidation and antioxidant status in the liver erythrocytes, and serum of rats after methanol intoxication, *J. Toxicol. Environ. Health* **53,** 637–649 (1999).
20. M. Nazıroğlu, N. Dilsiz, and M. Cay, Protective role of intraperitoneally administered vitamin C and E selenium on the levels of lipid peroxidation in the lens of rats made diabetic with streptozotocin, *Biol. Trace Element Res.* **70,** 223–232 (1999).
21. N. R. Di Luzio and A. D. Hartman, Role of lipid peroxidation in the pathogenesis of the ethanol-induced fatty liver, *Fed. Proc.* **26,** 1436–1442 (1967).
22. H. Esterbauer and P. Ramos, Chemistry and pathophysiology of oxidation of LDL, *Rev. Physiol. Biochem. Pharmacol.* **127,** 31–64 (1996).
23. N. I. Krinsky, Antioxidant functions of carotenoids, *Free Radical Biol. Med.* **7,** 617–635 (1989).
24. R. F. Del Maestro, H. H. Thaw, J. Bjork, et al., Free radicals as mediators of tissue injury, *Acta Physiol. Scand.* **492(suppl.),** 43–57 (1980).
25. A. W. S. S. Salim, Role of oxygen-derived free radicals in mechanism of acute and chronic duodenal ulceration in the rat, *Dig. Dis. Sci.* **35,** 73–79 (1990).
26. A. P. Bautista and J. J. Spitzer, Acute ethanol intoxication stimulates superoxide anion production by in situ perfused rat liver, *Hepatology* **15,** 892–898 (1992).
27. A. Masini, D. Ceccarelli, D. Gallesi, et al., Lipid hydroperoxide induced mitochondrial dysfunction following acute ethanol intoxication in rats: the critical role for mitochondrial reduced glutathione, *Biochem. Pharmacol.* **47,** 217–224 (1994).
28. T. Hirano, N. Kaplowitz, H. Tsukamoto, et al., Hepatic mitochondrial glutathione depletion and progression of experimental alcoholic liver disease in rats, *Hepatology* **16,** 1423–1427 (1992).
29. C. C. Cunningham, W. E. Coleman, and P. I. Spach, The effects of chronic ethanol consumption on hepatic mitochondrial energy metabolism, *Alcoholism* **25,** 127–136 (1990).
30. C. V. Smith, D. P. Jones, T. M. Guenther, et al., Compartmentation of glutathione: Implications for the study of toxicity and disease, *Toxicol. Appl. Pharmacol.* **140,** 1–12 (1996).
31. O. Niemela, S. Parkkila, S. Yla-Herttuala, et al., Sequential acetaldehyde production, lipid peroxidation and fibrogenesis in micropig model of alcohol-induced liver disease, *Hepatology* **24,** 1208–1214 (1995).
32. W. W. Souba and D. W. Wilmore, Postoperative alteration of arteriovenous exchange of amino acids across the gastrointestinal tract, *Surgery* **94,** 342–350 (1983).
33. C. Coundary, M. J. Richard, H. Faure, et al., Blood and liver lipid peroxide status after chronic ethanol administration in rats, *Clin. Chim. Acta* **219,** 35–45 (1993).
34. B. Halliwell, Drug antioxidant effect: a basis for drug selection? *Drugs* **42,** 569–605 (1991).