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Role of oxidants-antioxidants and radical scavengers in alcohol-induced liver injury in rats

Sıçanlarda alkolle oluşturulmuş karaciğer hasarında oksidan ve antioksidanlar ile radikal temizleyicilerinin rolleri

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ÖZET: *Etanolle oluşturulan hepatik hasarda, serbest radikaller ve süperoksid dismutaz (SOD), Katalaz, glutatyon (GSH), glutatyon peroksidaz (GSH-Px) ile serbest radikal temizleyicilerinin rolünü araştırdığımız çalışmada 50 sıçan, Kontrol; Etanol; E vitamini + Etanol; Allopurinol + Etanol; C vitamini + Etanol; olmak üzere 5 gruba ayrıldı. Bu grupların karaciğer dokuları histopatolojik yönden incelendi. Etanol grubu ratların doku ve eritrosit karaciğer ve eritrosit malondialdehid (MDA) düzeylerinin kontrol grubuna göre yüksek olduğu, doku ve eritrosit glutatyon düzeylerinin ise düştüğü saptandı. Etanol+allopurinol ve E vit+Etanol uyguladığımız gruplarda ise bütün bu parametrelerde kontrol grubuna göre bir farklılık olmadığı saptandı. Karaciğer GSH-Px ve eritrosit katalaz aktivitelerinde kontrol grubuna göre bir farklılık bulunmadı. Eritrosit SOD aktiviteleri ile serum transaminazlarının (Aspartat transaminaz ve alanin transaminaz) önemli düzeyde yükselmiş olduğu saptandı. Etanol + C vitamini uygulanan grupta kontrollere göre doku ve eritrosit glutatyon düzeylerinin düştüğü, karaciğer ve eritrosit MDA, karaciğer GSH-Px, eritrosit SOD, katalaz ve serum transaminaz düzeylerinin ise değişmediği saptandı. Serbest radikal giderici ajan allapurinol, antioksidan E ve C vitaminlerinin etanol ile oluşturulan hepatik doku hasarında histolojik olarak lezyon oluşumunu önledikleri ve lipid peroksidasyonunu bloke ettiklerini saptadık. Sonuç olarak, serbest radikallerin etanolle oluşturulan hepatik doku hasarının etiyopatojenezinde, tek başına değilse de, rol oynadıklarını ve bir kısım serbest radikal giderici ve antioksidan ajanın hepatik doku hasarının tedavisinde kullanılmasının yararlı olabileceğini düşünmekteyiz. Elde edilen sonuçlarla oksijen radikallerinin uzaklaştırılması bu açıdan hastalara yaklaşımda yeni boyutlar getirmektedir.*

Anahtar Kelimeler: **Lipid peroksidasyonu, katalaz, süperoksid dismutaz, glutatyon, glutatyon peroksidaz, allopurinol, E vitamini, C vitamini, alkole bağlı karaciğer hasarı**

SUMMARY: *In this study, we investigated the role of free radicals, superoxide dismutase (SOD), catalase, glutathione (GSH), glutathione peroxidase (GSH-Px) and free radical scavengers in ethanol-induced liver injury. 50 rats were divided into five groups: Control; Ethanol; Vitamin E + Ethanol; Allopurinol + Ethanol; Vitamin C + Ethanol. The liver tissues of rats in these groups were investigated histopathologically. In the ethanol group, glutathione levels were decreased, malondialdehyde (MDA) levels in erythrocyte and liver were higher, compared to those in the control group. No differences were found in liver GSH-Px and erythrocyte catalase activities compared to the control group. However, a significant increase was observed in erythrocyte SOD activites and the levels of Aspartat transaminase (AST) and Alanin transaminase (ALT). In the groups treated with allopurinol and vitamin C plus ethanol, it was noticed that there were no differences in all these parameters, when compared to the control group. It was also observed that glutathione levels decreased in the vitamin C plus ethanol administered group. However, the levels of MDA, GSH-Px, SOD, catalase, ALT and AST did not exhibit differences in comparison with the controls. We also observed that free radical scavenger agents, allopurinol, and antioxidants vitamin E and vitamin C reduced the occurence of histopathologically and blocked lipid peroxidation in ethanol-induced hepatic tissue injury. We are of the opinion that, free radicals may have a role in the etiopathogenesis of ethanol-induced tissue damage, and administering of some free radical scavengers and antioxidant agents may be effective in the treatment of tissue damage.*

Key Words: **Lipid peroxidation, catalase, superoxide dismutase, glutathione, glutathione peroxidase, allopurinol, vitamin E, vitamin C, alcohol-induced liver injury**

RECENT findings about the role of increased free oxygen radicals in the pathogenesis of many diseases have led to enhancement of interest in agents scavenging these radicals recently (2,3,4,5). It has been reported that agents such as vitamin E, allopurinol, β caroten and ascorbic acid (vitamin C) may prevent cell and tissue injury by scavenging free radicals and preventing lipid peroxidation (6).

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It has been shown that superoxide free radicals (O^{-}_{2}) may be generated by monoelectronic reduction of molecular oxygen through various enzymatic mechanisms (1). They include enzymes such as SOD, which enhances the generation of H_2O_2 from superoxide,and catalase and glutathione peroxidase,which destroy H_2O_2 and thus hinder the generation of OH. Another line of antioxidant defence is represented by substrates acting as chain-breaking antioxidants against the destructive process linked to prooxidants. The main membranous chain-breaking antioxidants is α-tocopherol (vitamin E), which is able to quench lipid peroxyradicals resulting from peroxidative attacks on membranous polyunsaturated fatty acids (1). During this reaction, (-tocopherol is converted into its free radical form. The regeneration of α-tocopherol from its radical form is mainly achieved by reduction through ascorbate (1). Owing to its localization close to the interface of the membranes, α-tocopherol is able to react with the water-soluble ascorbate, which therefore contributes effectively to the regeneration of vitamin E (1).

Previous research carried out in the laboratory demonstrated a protective effect of allopurinol, a xanthine oxidase inhibitor, against myocardial tissue damage and renal hypoxia. This concept implies the preservation of normal metabolic constituents during stressful situations which otherwise would cause metabolic decay. This is achieved through the preservation of the nucleotide pool by preventing the irreversible degradation of these compounds at the xanthine-hypoxanthine level (2).

It this study, we aimed to eliminate these free radicals by different free radical scavengers and antioxidant agents. We discussed the comparative results obtained from the probable effects of allopurinol, nonenzymatic antioxidant (as chain broker donor) α-tocopherol (vitamin E), and vitamin C which are the specific inhibitors of xanthine dehydrogenase leading to formation of free oxygen radicals and superoxide radical, on liver damage induced by 96% ethanol by intragastric route.

MATERIALS AND METHODS

50 adult male wistar albino rats, mean 180-200 gr, provided by DUSAM (Research Center for Health Sciences, Dicle University) were divided into five groups (each containing ten rats).

1- Controls; 2- Absolute ethanol; 3- Vitamin E + ethanol; 4- Allopurinol + ethanol; 5- Vitamin C + ethanol

Laparotomy was performed under ether anesthesia to the control rats (first group). 1 ml of ethanol (96%) was to the rats in the second group following 24 hours' starvation, and a laparotomy was performed under ether anesthesia after 120 minutes. To the rats in the third group, vitamin E (25 unit/kg, in olieve oil) was administered by orogastrically for a week; following 24 hours' starvation, vitamin E was administered again, and a laparotomy was performed by giving 1 ml of absolute ethanol after 120 minutes. The operations performed on the rats in the third group were applied in exactly the same way to the rats in the fourth and fifth groups for allopurinol (50 mg/kg, 0.1 M NaOH) and vitamin C (40 mg/kg, in the drinking water).

When the laparotomy was performed, liver tissue, heparinizied blood and blood samples were taken and blood samples were centrifuged; then, the liver tissues were washed with 0.9% NaCI. They were kept at -20°C till experiment time. MDA, GSH-Px and GSH levels in these tissues were measured. The erythrocytes package was prepared by washing the heparinized blood samples with 0.9% NaCI solution three times, by centrifuging them at 3000 rpm for each time, and by averting of supernatants. SOD, catalase, GSH and MDA levels were measured in erythrocyte, while serum transaminases (AST, ALT) in plasma. Liver tissues were put into a 10% formalin solution for histopathological investigation. Following paraffin blocking, thin layers were taken and stained by hematoxylen-eosin. Samples were evaluated with respect to hidropic degeneration, congestion, inflammation, oedema, and periportal fibrosis.

In the histopathologic assessment, the scoring was performed based upon the changes with respect to the control group. The severity degree of lesion is mentioned in the findings section. Pathologic findings were qualitatively assessed. Lesions were established by capitalizing on existing literature data and pathology text-books. (7,8,9)

Liver MDA levels were measured by Ohkawa's thiobarbituric acid method (10); erythrocyte MDA levels by Stocks and Dormandy's thiobarbituric acid method (11), and the measurements were calculated by using extinction coefficient. Results were determined as nmol/g tissue for liver MDA and as nmol/ml erythrocyte for serum MDA. Liver GSH levels were measured by Dithionitrobenzen (DTNB) method described by Ellman, and results were determined as mol/g tissue (12). GSH-Px ac-

tivity was measured by the method of Hafeman, Sunde and Hoekstra (13). When the decrease of log (GSH) per minute in enzymatic reaction was substracted from the decrease of log (GSH) per minute in non-enzymatic reaction, each calculated 0.001 unit reduction was identified as 1 enzyme unit, and results were determined as unit/100 mg tissue. Erythrocyte GSH levels were measured by dithionitrobenzen (DNTB) method as described by Beutler, and results were determined as mg GSH/100 ml erythrocytes (14). Erythrocytes SOD was measured by modified Winterbourn and Hawkin's method based on reduction of nitroblue tetrasolium, and data were evaluated as unit/gHb (15), and catalase levels were measured by colorimetric method of modified Aebi (16). Data were changed to k/gHb after "k" value was determined, taking suitable absorbans for each analysis according to calculated regression. AST and ALT contents in plasma were measured in Abbott spectrum autoanalyzer by enzymatic-colorimetric method. The "Student's" test was used for statistical evaluations.

RESULTS

Results obtained from our findings are as follows:

1-While MDA levels in liver and erythrocyte of the ethanol group increased relative to those of the control group, GSH level decreased ($p<0.001$). A significant difference was not found in the activities of liver GSH-Px and erythrocyte catalase, compared to the control group ($p>0.05$). Howewer, a significant difference was observed in erythrocyte SOD activities and serum transminases, when compared to controls ($p<0.001$) (Table 1).

2-It was observed that there were no differences in all parameters we measured in blood and liver tissues in vitamin E + ethanol and allopurinol + ethanol groups relative to those of controls ($p>0.05$) (Table 1).

3-The difference between the content of MDA, GSH-Px, SOD, catalase and transaminases of vitamin C + ethanol group and control group was found statistically insignificant. However, it was noticed that GSH levels in liver and erythrocyte decreased ($p<0.02$) (Table 1).

Table 1. Characteristics of patients included in the study

	Control Group (n=10)	*Ethanol Group* (n=10)	*E Vit. + Ethanol* (n=10)	*Allopurinol + Ethanol* (n=10)	*C Vit. + Ethanol* (n=10)
Liver MDA (nmol/g)	108.4±9.4	129.0±5.6[a*]	115.7±3.08[a]	113.0±4.5[a]	110.2±3.01[a]
Liver GSH-Px (U/100 mg)	519.8±6.35	516.5±3.40	513.4±6.7	522.5±4.7[b]	518.2±4.6
Liver GSH (Mmol/g)	7.07±0.28	5.88±0.23[a*]	7.05±0.40[a]	6.97±0.15[a]	6.82±0.12[a**]
Erythrocyte MDA (nmol/ml)	1.23±0.05	1.82±0.09[a*]	1.29±0.07[a]	1.27±0.06[a]	1.32±0.03[a]
Erythrocyte SOD (U/gHb)	2245±61.8	2597.5±197.4[a*]	2257.3±7.6[a]	2271.1±5.2[a]	2261.8±7.8[a]
Erythrocyte Catalase (k/gHb)	1479.9±20.8	1466.7±10.1	1463.1±8.1	1468.8±3.18	1466.8±9.9
Erythrocyte GSH (mg/100 ml)	78.1±2.5	64.1±5.1[a*]	73.4±4.4[a]	76.5±1.19[a]	74.8±3.15[a**]
Serum AST (IU/L)	26.8±4.2	53.0±4.4[a*]	29.6±4.6[a]	29.5±1.83[a]	29.4±1.71[a]
Serum ALT (IUL)	30.0±4.7	65.5±2.8[a*]	34.5±3.1[a]	32.0±3.29[a]	30.9±3.83[a]

Values are mean±SD

*$p<0.001$; **$p<0.002$ (Compared to control group)

[a]$p<0.001$; [b]$p<0.005$ (Compared to ethanol group)

MDA (Malodialdehyde); GSH (Glutathione); SOD (Superoxide dismutase); GSH-Px (Glutathione peroxidase); AST (Aspartat transaminase); ALT (Alanin transaminase)

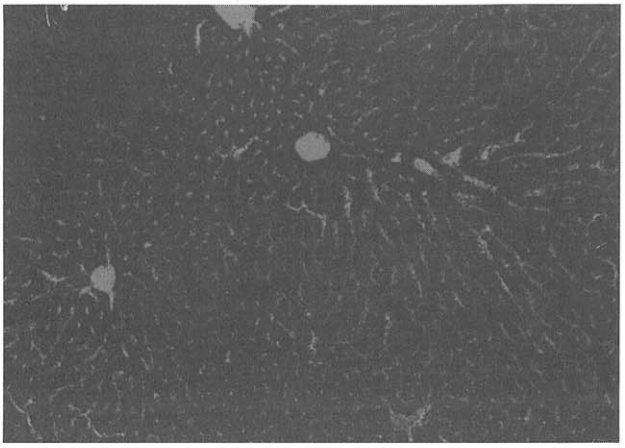

Figure 1. *Normal histologic appearance of rats liver in the control group (X16, H-E)*

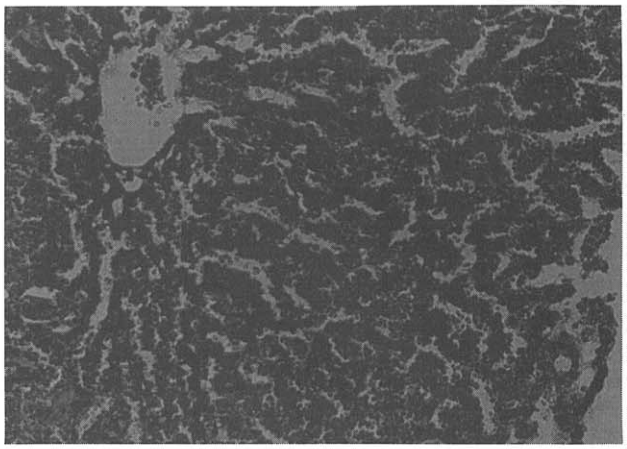

Figure 2. *Appearance of rats liver in the absolute ethanol group (X41, H-E)*

4-It was observed that MDA in liver and erythrocyte ($p<0.001$), erythrocyte SOD and serum transaminases ($p<0.001$) increased in only ethanol administered rats relative to those of vitamin E + ethanol, allopurinol + ethanol, vitamin C + ethanol groups. There were no differences in erythrocyte catalase levels ($p>0.05$) (Table I). Also, it was found that there were no significant differences in GSH-Px levels in the liver of vitamin E + ethanol and vitamin C + ethanol rats, when compared to controls ($p>0.05$). However, increase in of GSH-Px levels in allopurinol + ethanol administered rats was statistically significant ($P<0.005$).

In the histopathologically examination, injury levels in samples taken from all groups are shown in figures 1-5.

On histopathologic examination of the liver section of rats in the control group, no pathological finding was recorded no findings except it own histologic structure (Figure 1).

On liver samples of rats in the absolute ethanol group; and evident dilatation in sinuses, congestion, periportal and sinuses, periportal hydropic degeneration, slight focal inflammation were observed (Figure 2).

On histopathologic investigation of liver samples of rats in the vitamin E + ethanol group: No alterations were not found except a mild congestion in central venouses due to ethanol (Figure 3).

On histopathologic examination of liver samples of rats in the allopurinol + ethanol group: a mild congestion and oedema were detected (Figure 4).

On histologic investigation of liver samples of rats in the vitamin E + ethanol group: a mild congestion and oedema, and mild periportal fibrosis were observed (Figure 5).

DISCUSSION

There are a number of endogenous substances that are sources of free radical formation (17). Free radicals can damage numerous biochemical structures particularly lipids, proteins and nucleic acids. Detailed discussions about the role of oxygen metabolites in many important diseases have led to enhancement of interest in agents scavenging these radicals (2,3,4,5). Initially, Diluzio reported that the occurence of ethanol-induced liver damage could be prevented by administration of different antioxidants before the damage is produced (18) and that lipid peroxidation may have a role in pathogenesis of ethanol-induced liver damage (19). Several investigators have stressed that α-tocopherol taken by diet has a crucial role in the

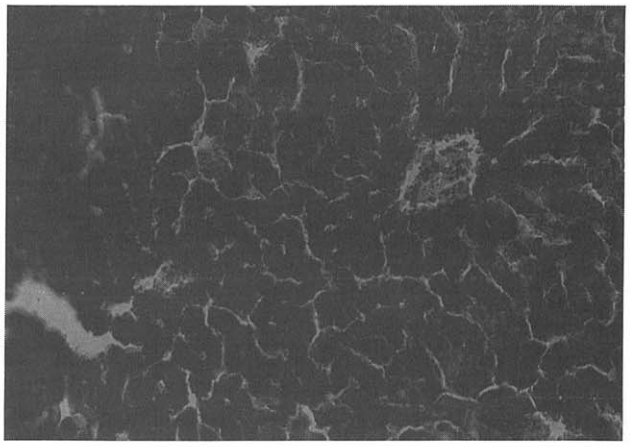

Figure 3. *Histologic appearance of liver of rats in the Vitamine E + Ethanol group (X16, H-E)*

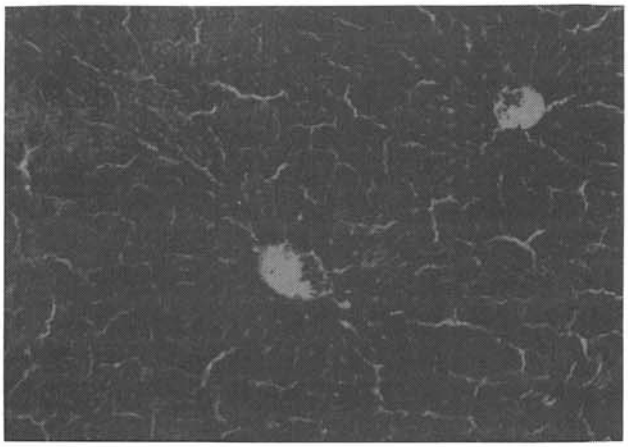

Figure 4. *Histologic appearance of liver of rats in the Allopurinol + Ethanol group (X16, H-E)*

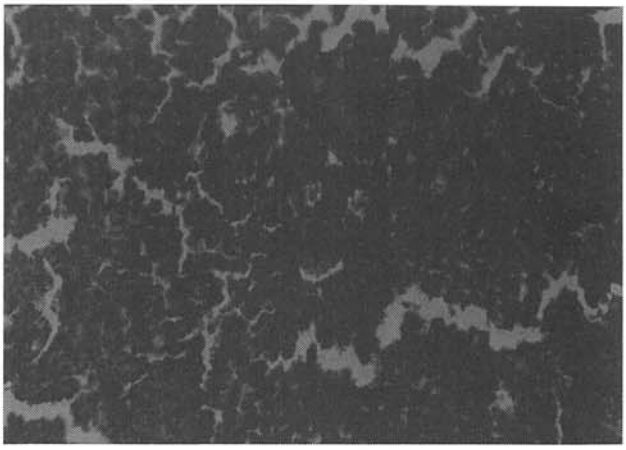

Figure 5. *Histologic appearance of liver of rats in the Vitamine C + Ethanol group (X41, H-E)*

events resulting from lipid peroxidation and oxdative stress, and on the damage in hepatic enzymatic and non-enzymatic antioxidant system following experimentally acute and chronic ethanol intake. Damaged antioxidant defense systems associated with ethanol administration were reported in the studies carried out on blood and liver biopsies (1). It has been accepted that the factors held responsible for influencing the mechanism of alcohol on the liver are associated with toxic effects of alcohol and its metabolites, alterations in intracellular redox potential, water and protein retantions in cells of liver, hypermetabolic condition, nutrition condition, enhancement of rats in liver, immunologic liver damage and fibrosis. Lipid peroxidation is one of the important factors about which several investigators have been made (20). It has been suggested that there is an increase in the formation of free radicals following acute ethanol administration (21).

The hypothesis that ethanol can induce hepatic injury through a mechanism involving free radical induced lipid peroxidation is not new (18). Diluzio et al (18,19) have detected that lipid peroxidation needed to excess dose of ethanol nearly 5-6 g per body weight. It did not form with lower dose of ethanol nearly 3 g per body weight; namely, lipid peroxidation may hardly begin to form with pharmacologic doses. However, it has caused a rigorous evaluation of the administering of lower doses of ethanol chronically and without eating led to glutathione depletion, increase in (-amino-n-butiric acid generation and lipid peroxidation reported by the latter studies (20).

Hepatic antioxidant status may play a critical role in the defence against oxidative stress. GSH functions with the enzyme GSH-Px to reduce free lipid hydroperoxides and H2O2. Furthermore, it is a cosubstrate for the peroxidation inhibiting protein which is capable of destroying hydroperoxide fatty acids located in phospholipids (22).

In the present study our purpose was to eliminate the free radicals by various free radical scavengers and antioxidant agents. Hepatic tissue damage and malondialdehyde levels significantly increased when we compared those of ethanol administered rats to those of control rats.

When, particularly, the group prophylacted by vitamin E was compared to only the ethanol administered rats, it was found that vitamin E was a defender, both at histologic level and lipid peroxidation. The results of MDA which are an indicator of lipid peroxidation in tissue in allopurinol and vitamin C administered groups were found similar the same as those of ethanol free group that was used as the control group. It was observed that free radical scavenger could rather significantly eliminate the free radical formations. The results that we obtained are concordance with the other investigators (6,17,18,19).

It was detected that SOD activity increased in liver homogenates of acute ethanol administered rats (21). It has been suggested that this increase is a defence mechanism against accumulation of superoxide anion during ethanol or asetaldehyde oxidation. Conversely, no alterations were found in activities of catalase and GSH-Px metabolizing H_2O_2, which occur by dismutation of superoxide anion (21). There are several reports supporting the results that stimulation of lipid peroxidation

by ethanol depend on enhancement of hydroxyl radical (21, 23). Similarly, we detected SOD activity in erythrocyte of ethanol treated rats increased relative to that of the control group, while activities of catalase liver GSH-Px of the rats prophylacted by allopurinol, vitamin E and vitamin C was statistically insignificant compared to control group.

Initially, Comporti et al (24) have suggested that a relation may be existent between the alterations in liver lipid peroxidation and liver GSH levels following acute ethanol administration, but, it has still not been enlighted what kind of a relation there is between lipid peroxidation and GSH levels after acute ethanol ingestion. Reduction in GSH levels following acute ethanol administration was reported by several investigators (23,25,26). However, it has been detected that this reduction was of short duration, and returned to its normal levels within 9-12 hours depending upon the dose of given ethanol (23,25). We observed both liver GSH and erythrocyte GSH level decreased in ethanol given rats, when compared to the control group.

Reduction in liver GSH levels following acute ethanol ingestion is thought to depend on;

a) Stimulation in lipid peroxidation, b) Conjugation between acetaldehyde and GSH (23). It is not known if both factors are effective together or individually. No differences were found in liver and erythrocyte GSH levels in allopurinol, vitamin E and vitamin C plus ethanol administered rats relative to those of the control group.

AST and ALT activities were measured to observe how ethanol-induced liver damage exhibits influence serum transaminases. It was detected that these enzymes levels increased in the acute ethanol treated group compared to controls, while there were no significant changes in the free radical scavengers group.

These results suggest that inhibiting antioxidant activities may have a role in the prevention of lipid peroxidation stimulation following acute ethanol intake. In addition, these results indicate that lipid peroxidation stimulation occuring in liver is parallel to lipid peroxidation stimulation within the erythrocytes. Remarkable damage within erythrocytes membrane, structure and function following chronic alcohol intake have been reported (27). Although the factors held responsible for these damages are not completely enlighted, it has been suggested that lipid peroxidation may be a factor in these damages because of the fact that erythrocyte membrane is rich with polyunsaturated fatty acids, and its being in close connection with molecular oxygen (28).

As a result, we observed that;

a) Inhibition that ethanol exhibits on antiperoxidative enzymes, may have a role on stimulation of lipid peroxidation,

b) Acute ethanol administration significantly increases lipid peroxid levels in erythocyte and liver of rat.

c) Free radical scavenger agent, allopurinol and antioxidants, α-tocopherol and ascorbic acid reduce the occurence of lesion histologically, and blocked lipid peroxidation in ethanol-induced hepatic tissue damage. We suggest that free radicals may have a role in etiopathogenesis of ethanol-induced tissue damage, and administering of some free radical scavengers and antioxidant agents may be efficient in prevention of tissue damage.

REFERENCES

1. Nordmann R. Alcohol and antioxidant system. Alcohol & Alcoholism 1994; 29: 513-522
2. Vasko KA, Dewall RA, Rıley AM. Effect of allopurinol in renal ischemia. Surgery 1972;71:787-790
3. Gürsoy MA, Kayabalo M, Hazar H, Kotiloğlu E. Role of free oxygen radicals and radical sacavengers in stress ulcer occurred on rats. Ulusal Cerrahi Dergisi 1992;8: 22-28.
4. Bast A, Haenen GR, Doelman CJA. Oxidants and antioxidants: State of the art. Am J Med 1991;91 (Suppl 3C):2S-13S.
5. Parks D, Bulkley GB, GRanger DN. Role of oxygen derived free radicals in digestive tract diseases. Surgery 1993; 94:415-422.
6. Mozsik G, Suto G, Garamszegi M, et al. Oxygen free radicals and gastric mucosal damage in rats treated with ethanol and HCI. Eur J Gastroenterol Hepatol 1991; 3:757-761.
7. Kniepert E. and Seffner W.Histological and histometrical changes of liver damage in rats after treatment with ethanol and carbon tetrachloride. Exp Pathol1989; 36: 185-188
8. Sato C, Matsuda Y and Lieber CS. Increased hepatotoxicity of acetoaminophen after chronic ethanol consumption in the rat. Gastroenterology 1981; 80:140-8
9. Walker RM, McElliott TF, Power EM, et al. Increased acetaminophen-induced hepatotoxicity after chronic ethanol consumption in mice. Toxicology, 1983; 28: 193-206.
10. Ohkawa H, Ohishi N and Yagi N. Assay for lipid peroxidose in animal tissues by thiobarbituric acid reaction. Analyt Biochem 1979; 95:351-358.

11.Stocks J and Dormandy TL.The autoxidation of human red cell lipids induced by hydrogen peroxide. Br J Hematol 1971; 20:95-111

12.Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys 1959; 82:70-77.

13.Hafeman DG, Sunde RA and Hoekstra WG. Effect of dietary selenium on erythrocyte and liver glutathione peroxidase in the rat. J Nutr 1974;104:580-587.

14.Beutler E, Duron O and Kelly BM. Improved method for the determination of blood glutathione. J Lab Clin Med 1963; 61: 882-888.

15.Winterbourn CC, Hawkins RE, Brian M, Carrell RW. The estimation of red cell superoxide dismutase activity J Lab Clin Med 1975;85 (2):337

16.Aebi H. Catalase in vitro. Methods in enzymology 1984; 105:121.

17.Reilly PM, Sciller HJ, Bulkley GB. Pharmacological approach to tissue injury mediated by free radicals and other reactive oxygen metabolites. Am J Surg 1991; 161:488-503.

18.Di Luzio NR. Prevention of the acute ethanol-induced fatty liver by antioxidants. Physiologist 1963;6:169.

19.Di Luzio NR. A mechanism of the acute ethanol- induced fatty and the modification of liver injury by antioxidants. Lab Invest 1966;15:50-63

20.Batur Y. Serum selenium level in chronic liver diseases and alcoholism. Journal of Medical Faculty, Aegean University. 1988; 27:1499-1512.

21.Valenzuela A, Fernandez N, Fernandez V, et al. Effect of acute ethanol ingestion on lipoperoxidation and on activity of the enzymes related to peroxide metabolism in rat liver. FEBSL 1980;111:11-13.

22. Situnayake RD, Crump BJ, Thurnham DI, et al. Lipid peroxidation and hepatic antioxidants in alcoholic liver disease. Gut 1995; 31:1311-1317.

23.Videla LA and Valenzuela A. Alcohol ingestion, liver glutathione and lipoperoxidation: metabolic interrelations and pathological implications. Life Sci 1982; 31: 2395-2407.

24.Comporti M, Benedetti A, Chieli E. Studies on in vitro peroxidation of liver lipids in ethanol-treated rats. Lipids 1973; 8:498-502.

25.Mac Donald CM,Dow J, Moore MR. A possible protective role for sulphydryl compounds in acute alcoholic liver injury. Biochem Pharmacol 1977; 26:1529-1531.

26.Chiarpotto E, Biasi F, Aragno M, et al. Ethanol-induced potentiation of rat hepatocyte damage due to 1,2-dibromo ethane.-Alcohol 1995; 30::37-45

27-Wing DR, Harvey DJ, Hughes J, et al. Effects of chronic ethanol administration on the composition of membrane lipids in the mouse. Biochem Pharmacol 1982; 31:3431-3439.

28.Bidder TG, Jaeger PD. Malondialdehyde production by erythrocytes from alcoholic and non-alcoholic subjects. Life Sci 1982; 30:1021-1027.