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• BRIEF REPORTS •

# Potential effects of L-NAME on alcohol-induced oxidative stress

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## Abstract

**AIM:** Nitric oxide (NO) is a highly reactive oxidant synthesized from L-arginine by nitric oxide synthase (NOS). NO may cause injury through the generation of potent radicals. Nw-nitro-L-arginine methyl ester (L-NAME) is a non-selective inhibitor of NOS. We aimed to evaluate whether L-NAME treatment had protective effects against oxidative stress in rats intragastrically fed with ethanol during a 4 wk-period.

**METHODS:** Thirty-six male Wistar rats were divided into 3 equal groups: group 1 (control group-isocaloric dextrose was given), group 2 (6 g/kg·d ethanol-induced group) and group 3 (both ethanol 6 g/kg·d and L-NAME 500 mg/L in drinking water-given group). Animals were sacrificed at the end of 4 wk-experimental period, and intracardiac blood and liver tissues were obtained. Biochemical measurements were performed both in plasma and in homogenized liver tissues. Alanine amino transferase (ALT), aspartate amino transferase (AST), malondialdehyde (MDA), NO, superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH) levels were measured by spectrophotometry.

**RESULTS:** ALT and AST in group 2 (62 U/L and 128 U/L, respectively) were higher than those in group 1 (24 U/L and 38 U/L) and group 3 (37 U/L and 81 U/L) ($P$<0.001 for both). Plasma and tissue levels of MDA in group 2 (4.66 μmol/L and 0.55 nmol/mg protein) were higher than in group 1 (2.65 μmol/L and 0.34 nmol/mg protein) and group 3 (3.43 μmol/L and 0.36 nmol/mg protein) ($P$<0.001 for both). Plasma and liver tissue levels of NO in group 2 (54.67 μmol/L and 586.50 nmol/mg protein) were higher than in group 1 (34.67 μmol/L and 435.33 nmol/mg protein) and group 3 (27.50 μmol/L and 412.75 nmol/mg protein ) ($P$<0.001 for both). Plasma and liver tissue SOD activities in group 2 (15.25 U/mL and 5.38 U/ mg protein, respectively) were lower than in group 1 (20.00 U/mL and 8.13 U/ mg protein) and group 3 (19.00 U/mL and 6.93 U/ mg protein) ($P$<0.001 for both). Plasma and liver tissue CAT activities in group 2 (145 U/mL and 37 U/ mg protein, respectively) were lower than in group 1 (176 U/mL and 73 U/mg protein) and group 3 (167 U/mL and 61 U/mg protein) ($P$<0.001 for both). Meanwhile, erythrocytes and liver tissue levels of GSH in group 2 (4.12 mg/g Hb and 5.38 nmol/mg protein, respectively) were lower than in group 1 (5.52 mg/g Hb and 4.49 nmol/mg protein) and group 3 (5.64 mg/g Hb and 4.18 nmol/mg protein) ($P$<0.001 for both).

**CONCLUSION:** Our findings show that L-NAME may produce a restorative effect on ethanol-induced liver damage via decreasing oxidative stress and increasing antioxidant status.



**Key words:** Oxidative stress; Ethanol-induced liver damage; L-NAME

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## INTRODUCTION

Normal cellular metabolism involves the production of reactive oxygen species (ROS)[1]. Low level of ROS is vital for many cell signaling events and essential for proper cell functioning[2-4], while excessive *in vivo* generation of ROS can adversely affect cell functioning.[3]. In some clinical setting, such as obesity, liver transplantation, hepatic surgery, and hemorrhagic shock, as an outgrowth of ischemic-reperfusion injury in liver, microcirculatory derangement, energy depletion, production of ROS and lipid peroxidation occur[5-7]. ROS causes inflammation and cell death through modulation of signal transduction pathways by affecting redox-sensitivity enzyme and transcription factors, by supporting protease activity, and by stimulating the expression of inflammatory mediators and adhesion molecules[7,8]. In the recovery of hepatocellular function after severe traumas, free oxygen radicals should be kept in the normal ranges[9].

The chronic consumption of alcoholic beverages is the major cause of liver injury, and the development of serious liver diseases[10,11]. The mechanisms of liver injury may include the effects of oxygen radicals on hepatocytes[12]. Increased oxygen radical production leads to lipid peroxidation by inducing cytochrome P4 502E[13,14].

Oxidative stress is known to play an important role in the pathogenesis of ethanol-induced liver injury[15,16]. Oxidative damage correlates with the amount of ethanol consumed[17]. Recently, it has been demonstrated that nitric oxide (NO) is an important mediator of hepatotoxicity, and the changes in its generation or actions may contribute to pathologic states[18,19]. It has been proposed that the high production of NO causes injury, perhaps through the generation of potent radicals[20]. An increase in NO production has been reported in monocytes of patients with chronic liver disease[21] and in the livers of rats chronically fed with ethanol[22]. However, in some models of

inflammation, it has been shown that inhibition of NO increases tissue dysfunction or injury[20].

The role of NO seems to be controversial, and furthermore, the prooxidant and/or antioxidant effects of NOS inhibition in alcoholism have not been studied before. In the present study, we tested whether nitric oxide synthase (NOS) inhibition attenuated alcohol-induced oxidative stress in a rat model. For NOS inhibition Nw-Nitro-L-arginine methyl ester (L-NAME)-a nonselective inhibitor- was used.

## MATERIALS AND METHODS

### *Experimental procedure*

Thirty-six male Wistar-Albino rats weighing 240-300 g were used. Animals were fed *ad libitum* on a standard diet and had free access to water. All studies were performed in accordance with the National Institutes of Health Criteria for Care of Laboratory Animals.

The rats were divided into three groups, and were given isocaloric dextrose (group 1), ethanol (ETOH) (group 2), or both ethanol and L-NAME (ETOH + L-NAME) (group 3) for 4 wk. Ethanol was given intragastrically at a dose of 6 g/( kg/d) L-NAME was added to drinking water of the rats (500 mg/L).

All rats were sacrificed after 1 mo with Na-pentobarbital anesthesia (35 mg/kg i.p.). After exploration of the thorax, blood was taken by intracardiac puncture. Then, a laparotomy was done, liver tissue was excised, and stored at -70 ℃. Serum alcohol levels were measured on the day the rats were sacrificed.

### *Biochemical analysis*

Blood samples collected in heparinized vacutainer tubes were immediately transported to the laboratory in a cooler with ice. Upon arrival, plasma was separated by centrifugation (+4 ℃, 3 000 r/min, 10 min), and divided into 0.5-1.0 mL aliquots, placed in cryovials, and stored at -70 ℃ until analyzed. Erythrocytes were washed three times in 5 mL saline, hemolyzed by diluting 4-fold with water and glutathione (GSH) was studied in erythrocytes on the same day. Each plasma sample was divided into 4 aliquots; alcohol, ALT and AST were studied immediately in 1st aliquot; 2nd aliquot was saved until analysis of plasma NO within 2 wk, the other two aliquots were used for estimation of plasma MDA, SOD, CAT on a later date (within 1 mo).

The liver tissues were weighed, washed in 0.9 % NaCl, and homogenized in ice-cold 0.15 M KCl 100 g/L. Homogenates of 20% were obtained and sonicated twice at 30 s intervals at 4 ℃. Homogenates were centrifuged at >10 000 *g* for 15 min at 4 ℃. All biochemical parameters in homogenates were studied on the same day.

**ALT and AST activities** Plasma ALT and AST activities were measured by enzymatic methods using commercial kits (Olympus, Hamburg, Germany) on Olympus AU800 analyzer.

**Lipid peroxidation** MDA, an end product of fatty acid peroxidation, was measured in plasma and liver homogenates by the thiobarbituric acid reactivity assay as previously described[23]. The total protein concentration was measured by the method of Lowry *et al*[24].

**Nitric oxide** Plasma and tissue concentrations of NO were measured through its stable metabolites nitrate and nitrite. Nitrate was first reduced by nitrate reductase to nitrite and then nitrite was determined spectrophotometrically by the Griess reaction[25]. Griess reagent, the mixture (1:1) of 0.2% N-(1-naphthyl)-ethylene-diamine and sülfanilamide in 5% phosphoric acid, gave red-violet diazo dye with nitrite, and was measured in the visible range at 540 nm.

**Cu-Zn- superoxide dismutase (Cu-Zn SOD)** Plasma and tissue Cu-Zn SOD activities were determined by the method of Sun *et al*[26] by inhibition of nitroblue tetrazolium reduction with Xanthine/Xanthine oxidase used as a superoxide generator. One unit of SOD was defined as the amount of protein that inhibits the rate of NBT reduction by 50%.

**Catalase:** Catalase activity was measured by the breakdown of hydrogen peroxide catalysed by catalase enzyme[27].

**Glutathione** Erythrocyte and tissue glutathione (GSH) concentrations were determined according to the method of Beutler *et al*[28] using metaphosphoric acid for protein precipitation and 5'-5'- dithiobis-2-nitrobenzoic acid for color development.

**Alcohol** Serum alcohol level was measured by fluorescent polarizing immunoassay using commercial kits (Abbot TDx, CatNo:378190100).

### *Statistical analysis*

All results are expressed as mean±SD. The groups were compared with Anova-Tukey HSD. $P<0.05$ was considered to be statistically significant.

## RESULTS

Values of the analysed parameters and the statistical significances in the groups are shown in Tables 1 and 2. Plasma alcohol levels of groups 2 and 3 were comparable (210±42 mg/100 mL and 196±30 mg/100 mL). ALT and AST in group 2 were higher than those in group 1 and group 3 ($P<0.001$ for both). Similarly, the values were higher in group 3 in comparison with group 1 ($P<0.001$).

Lipid peroxidation levels as assessed by MDA in plasma and tissue were found to be significantly higher ($P<0.001$) in group 2 in comparison to groups 1 and 3. Plasma MDA level was significantly higher in group 3 in comparison to group 1 ($P<0.001$); however, there was no difference between these groups in tissue MDA level ($P>0.05$).

Plasma and tissue levels of NO in group 2 were higher than in groups 1 and 3 ($P<0.001$ for both). Plasma NO level was lower in group 3 in comparison with group 1 ($P<0.001$).

Each of plasma and tissue antioxidant components (SOD , CAT and GSH) in group 2 was lower than in groups 1 and 3

**Table 1** Plasma transaminase activities, prooxidant and antioxidant status in groups

| | Control (Group 1) | ETOH (Group 2) | ETOH + L-NAME (Group 3) | $P^1$ | $P^2$ | $P^3$ |
|---|---|---|---|---|---|---|
| ALT (U/L) | 24.25±4.07 | 62.00±6.73 | 37.00±4.73 | <0.001 | <0.001 | <0.001 |
| AST (U/L) | 38.92±6.42 | 128.42±16.98 | 81.33±14.90 | <0.001 | <0.001 | <0.001 |
| MDA (μmol/L) | 2.65± 0.30 | 4.66±0.64 | 3.43±0.35 | <0.001 | <0.001 | <0.001 |
| NO (μmol/L) | 34.67±4.69 | 54.67±9.19 | 27.50±4.34 | <0.001 | <0.001 | <0.001 |
| SOD (U/mL) | 20.00±1.86 | 15.25±0.97 | 19.00±1.54 | <0.001 | <0.001 | NS |
| CAT (U/mL) | 176.0±11.23 | 145.33±9.23 | 166.83±7.27 | <0.001 | <0.001 | <0.05 |
| GSH (mg/g Hb) | 5.52±0.41 | 4.12±0.32 | 5.64±0.52 | <0.001 | <0.001 | NS |

[1]Group 1 *vs* group 2; [2]Group 2 *vs* group 3; [3]Group 1 *vs* group 3. NS: Not-significant.

**Table 2** Hepatic prooxidant and antioxidant status in groups

| | Control (Group 1) | ETOH (Group 2) | ETOH + L-NAME (Group 3) | $P^1$ | $P^2$ | $P^3$ |
|---|---|---|---|---|---|---|
| MDA (nmol/mg protein) | 0.34±0.05 | 0.55±0.08 | 0.36±0.07 | <0.001 | <0.001 | NS |
| NO (nmol /mg protein) | 435.33±35.64 | 586.50±34.79 | 412.75±38.67 | <0.001 | <0.001 | <0.001 |
| SOD (U/mg protein) | 8.13±0.79 | 5.38±0.75 | 6.93±0.81 | <0.001 | <0.001 | <0.01 |
| CAT (U/mg protein) | 72.58±7.37 | 36.50±6.65 | 61.17±7.81 | <0.001 | <0.001 | <0.01 |
| GSH (nmol/mg protein) | 4.49±0.51 | 3.55±0.36 | 4.18±0.26 | <0.001 | <0.001 | NS |

[1]Group 1 *vs* group 2; [2]Group 2 *vs* group 3; [3]Group 1 *vs* group 3. NS: Not-significant.

($P$<0.001). There was no significant difference in plasma SOD, and erythrocyte and tissue GSH levels between groups 3 and 1 ($P$>0.05). However, tissue SOD, CAT, and plasma CAT levels were significantly higher in group 3 in comparison with group 1 ($P$<0.01, $P$<0.01, and $P$<0.05, respectively).

## DISCUSSION

It is now generally accepted that oxidative stress plays an important role in the pathogenesis of ethanol toxicity[15,16]. The close relation between ethanol and liver is due to the fact that more than 80% of ingested alcohol is metabolized in the liver without a feedback mechanism. In early phase, oxygen and NO-radicals derive from the complete oxidation of ethanol, and acetaldehyde in excess markedly alters the intracellular redox status, induces fat deposits, and triggers the inflammatory and immune response[29]. The progression of liver damage is also affected by generation of additional products between acetaldehyde and cytochrome c oxidase and/or P450 2E1[30,31]. Excessive production of reactive oxygen species (ROS), depletion of GSH and ATP, adducts with acetaldehyde, rise of lipid peroxidation markers, are all documented findings in alcoholics[31-33].

In this study, we found that chronic ethanol administration causes a significant rise in plasma and hepatic MDA, and fall in plasma SOD, erythrocyte GSH levels, hepatic GSH levels, SOD and catalase activity. Some authors have reported an increase in various parameters of lipid peroxidation such as MDA, diene conjugates and lipid hydroperoxidates after alcohol intake[34,35], whereas others have shown opposite effects of alcohol drinking[36,37]. We also observed the presence of increased oxidative stress and decreased antioxidant status in the plasma and liver of ethanol-treated rats in our previous study[38].

In the present study, alcohol-induced hepatotoxicity was manifested as an increase in the activities of ALT and AST enzymes and a decrease in GSH level, SOD and catalase activities. These findings pointed out an obvious change in prooxidant-antioxidant balance in the liver of rats following chronic ethanol administration. However, Husain *et al*[39] have reported that GSH levels decreased, and SOD activity increased in liver in chronic alcoholism.

NO is a highly reactive oxidant. It is produced both by parenchymal and nonparenchymal cells in the liver[40,41]. Supplementation of the NO precursor L-arginine has been shown to exacerbate damage in models of inflammation and injury[42]. *In vitro* and *in vivo* studies have also shown NO down-regulates cytochrome P-450 and suppresses liver protein and DNA synthesis, and induces apoptosis and necrosis, all of which may contribute to liver failure[19,43]. NO also inhibits catalase activity, suggesting that it may alter the detoxification of cytotoxic free radicals, and react with superoxide anions to form peroxynitrite, which can react with sulphydryl residues in cell membranes leading to lipid peroxidation[40].

In this study, we found that there was a significant increase in plasma and liver NO concentrations in ethanol-induced rats. Our findings suggest that NO may have an important role in cellular damage seen in alcoholism. Wang *et al* have shown that serum NO concentration increased in cirrhotic rats. Also in some studies, it has been shown that there is an increase in NO production in monocytes of patients with chronic liver disease[44], and in livers of rats chronically fed with ethanol[22]. On the other hand, Sergent *et al*[45] reported that NO biosynthesis in hepatocytes protects them from ethanol-induced oxidative stress. Joshi *et al*[46] suggested that low-level NO acts as an antioxidant and higher level as a pro-antioxidant. They proposed that the mechanism of low concentration of NO's protection may involve diminished metal- catalyzed lipid peroxidation and the high concentration of NO's potentiation of oxidative stress may involve mitochondrial dysfunction. The significant rise in NO concentration that was seen in our study might be the reason for oxidative stress-stimulated lipid peroxidation.

TNF-α administration could lead to hepatocyte apoptosis and liver failure[47] TNF-α has been considered a mediator of cell injuries in liver caused by alcoholism, reperfusion, primary graft nonfunction, graft rejection and endotoxic insult[48,49]. Yt is expressed by both infiltrating inflammatory cells and hepatocytes in chronic liver injuries, and has been proposed to play an important role during tissue damage. The role of IL-6 during chronic liver injuries and fibrogenesis remains to be clarified. Some reports provided evidences for an important role of IL-6 in reducing $CCl_4$- induced acute and chronic liver injury and fibrosis.[50,51]. However, some other data showed that the serum level of IL-6 was associated with hepatic necroinflammatory activity in patients with chronic hepatitis and cirrhosis[52]. It has been suggested that IL-6 might be vitally involved in fibrotic changes, partly by modulating intrahepatic expression of other cytokines[53,54]. On the other hand, another study has suggested that proinflammatory cytokines increase as a result of alcohol-induced cellular damage[55], and this causes an increase in NO production by stimulating inducible NOS (iNOS)[55]. Hink *et al*[56] have claimed that $H_2O_2$ actively stimulates iNOS expression. In this case, we can assume that the increase in NO concentration and the contribution of NO to cellular damage could be secondary; however, simultaneous administration of ethanol and L-NAME in our study was shown to decrease oxidative stress parameters, and increase antioxidant parameters. These findings suggest that the oxidative damage in alcoholism can be mediated by NO.

The results of our study also show that co-administration of L-NAME diminishes oxidative stress, by increasing antioxidant enzymes. This restoration of oxidant/antioxidant balance is reflected by lower levels of transaminases.

Liver contains different forms of NO-synthase: the neuronal form (nNOS) in the peribiliary plexus; the iNOS form in hepatocytes, cholangiocytes, Kupffer cells, and stellate cells; and the endothelial form (eNOS) in the endothelial cells[56,57]. Over-production of NO in the liver from L-arginine via iNOS[43] has been implicated as an important part of the cascade of events that takes place in the pathogenesis of septic shock and in various forms of hepatic injury, inflammation and acute

hepatic failure.

In conclusion, NO can be held responsible for alcohol-induced free radical damage, and NOS inhibition can decrease oxidative stress seen in alcoholism. However, further studies should be done to detect which type of NOS is stimulated by ethanol. Findings of this study suggest a role of NOS inhibition in the management of ethanol-induced liver damage.

## REFERENCES


1 **McCord JM.** Human disease, free radicals, and the oxidant/antioxidant balance. *Clin Biochem* 1993; **26:** 351-357
2 **Harabin AL,** Braisted JC, Flynn ET. Response of antioxidant enzymes to intermittent and continuous hyperbaric oxygen. *J Appl Physiol (1985)* 1990; **69:** 328-335
3 **Gregorevic P,** Lynch GS, Williams DA. Hyperbaric oxygen modulates antioxidant enzyme activity in rat skeletal muscles. *Eur J Appl Physiol* 2001; **86:** 24-27
4 **Fattman CL,** Schaefer LM, Oury TD. Extracellular superoxide dismutase in biology and medicine. *Free Radic Biol Med* 2003; **35:** 236-256
5 **Uzun H,** Zengin K, Taskin M, Aydin S, Simsek G, Dariyerli N. Changes in leptin, plasminogen activator factor and oxidative stress in morbidly obese patients following open and laparoscopic Swedish adjustable gastric banding. *Obes Surg* 2004; **14:** 659-665
6 **Chen MF,** Chen HM, Ueng SW, Shyr MH. Hyperbaric oxygen pretreatment attenuates hepatic reperfusion injury. *Liver* 1998; **18:** 110-116
7 **Bilzer M,** Gerbes AL. Preservation injury of the liver: mechanisms and novel therapeutic strategies. *J Hepatol* 2000; **32:** 508-515
8 **Jaeschke H.** Reactive oxygen and mechanisms of inflammatory liver injury. *J Gastroenterol Hepatol* 2000; **15:** 718-724
9 **Jarrar D,** Wang P, Cioffi WG, Bland KI, Chaudry IH. Critical role of oxygen radicals in the initiation of hepatic depression after trauma hemorrhage. *J Trauma* 2000; **49:** 879-885
10 **Tuma DJ**. Role of malondialdehyde-acetaldehyde adducts in liver injury. *Free Radic Biol Med* 2002; **32:** 303-308
11 **Griffon B**, Cillard J, Chevanne M, Morel I, Cillard P, Sergent O. Activated macrophages increase the susceptibility of rat hepatocytes to ethanol-induced oxidative stress: conflicting effects of nitric oxide. *Alcohol Alcohol* 2000; **35:** 230-235
12 **McDonough KH.** Antioxidant nutrients and alcohol. *Toxicology* 2003; **189:** 89-97
13 **Dai Y**, Rashba-Step J, Cederbaum AI. Stable expression of human cytochrome P4502E1 in HepG2 cells: characterization of catalytic activities and production of reactive oxygen intermediates. *Biochemistry* 1993; **32:** 6928-6937
14 **Castillo T**, Koop DR, Kamimura S, Triadafilopoulos G, Tsukamoto H. Role of cytochrome P-450 2E1 in ethanol- carbon tetrachloride-and iron-dependent microsomal lipid peroxidation. *Hepatology* 1992; **16:** 992-996
15 **Lindros KO.** Alcoholic liver disease: pathobiological aspects. *J Hepatol* 1995;**23** Suppl 1**:** 7-15
16 **Balkan J**, Dogru –Abbasoglu S, Kanbagli O, Çevikbaþ U, Aykaç-Toker G, Uysal M. Taurine has a protective effect against thioacetamide-induced liver cirrhosis by decreasing oxidative stress. *Hum Exp Toxicol* 2001; **20:** 251-254
17 **Clot P**, Tabone M, Arico S, Albano E. Monitoring oxidative damage in patients with liver cirrhosis and different daily alcohol intake. *Gut* 1994; **35:** 1637-1643
18 **Gardner CR**, Heck DE, Yang CS, Thomas PE, Zhang XJ, DeGeorge GL, Laskin JD, Laskin DL. Role of nitric oxide in acetaminophen-induced hepatotoxicity in the rat. *Hepatology* 1998; **27:** 748-754
19 **Diez-Fernandez C**, Sanz N, Alvarez AM, Zaragoza A, Cascales M. Influence of aminoguanidine on parameters of liver injury and regeneration induced in rats by a necrogenic dose of thioacetamide. *Br J Pharmacol* 1998; **125:** 102-108
20 **Kubes P**, McCafferty DM. Nitric oxide and intestinal inflammation. *Am J Med* 2000; **109:** 150-158
21 **Hunt NC**, Goldin RD. Nitric oxide production by monocytes in alcoholic liver disease. *J Hepatol* 1992; **14:** 146-150
22 **Wang JF**, Greenberg SS, Spitzer JJ. Chronic alcohol administration stimulates nitric oxide formation in the rat liver with or without pretreatment by lipopolysaccharide. *Alcohol Clin Exp Res* 1995; **19:** 387-393
23 **Angel MF**, Ramasastry SS, Swartz WM, Narayanan K, Kuhns DB, Basford RE, Futrell JW. The critical relationship between free radicals and degrees of ischemia: evidence for tissue intolerance of marginal perfusion. *Plast Reconstr Surg* 1988; **81:** 233-239
24 **Lowry OH,** Rosenbrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951; **193:** 265-275
25 **Green LC,** Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR. Analysis of nitrate, nitrite, and [15N] nitrate in biological fluids. *Anal Biochem* 1982; **126:** 131-138
26 **Sun Y,** Oberley LW, Li Y. A simple method for clinical assay of superoxide dismutase. *Clin Chem* 1988; **34:** 497-500
27 **Yasmineh WG,** Kaur TP, Blazar BR, Theologides A. Serum catalase as marker of graft-vs-host disease in allogeneic bone marrow transplant recipients: pilot study. *Clin Chem* 1995; **41:** 1574-1580
28 **Beutler E,** Duran O, Kelly MB. Improved method for the determination of blood glutathione. *J Lab Clin Med* 1963; **61:** 882-888
29 **Zima T**, Fialova L, Mestek O, Janebova M, Crkovska J, Malbohan I, Stipek S, Mikulikova L, Popov P. Oxidative stress, metabolism of ethanol and alcohol-related diseases. *J Biomed Sci* 2001; **8:** 59-70
30 **Chen J**, Robinson NC, Schenker S, Frosto TA, Henderson GI. Formation of 4-hydroxynonenal adducts with cytochrome c oxidase in rats following short-term ethanol intake. *Hepatology* 1999; **29:** 1792-1798
31 **Dupont I,** Bodenez P, Berthou F, Simon B, Bardou LG, Lucas D. Cytochrome P-450 2E1 activity and oxidative stress in alcoholic patients. *Alcohol Alcohol* 2000; **35:** 98-103
32 **Rolla R,** Vay D, Mottaran E, Parodi M, Traverso N, Arico S, Sartori M, Bellomo G, Klassen LW, Thiele GM, Tuma DJ, Albano E. Detection of circulating antibodies against malondialdehyde-acetaldehyde adducts in patients with alcohol-induced liver disease. *Hepatology* 2000; **31:** 878-884
33 **Loguercio C,** Blanco FD, De Girolamo V, Disalvo D, Nardi G, Parente A, Blanco CD. Ethanol consumption, amino acid and glutathione blood levels in patients with and without chronic liver disease. *Alcohol Clin Exp Res* 1999; **23:** 1780-1784
34 **Aykac G,** Uysal M, Yalcýn SA, Kocak-Toker N, Sivas A, Oz H. The effect of chronic ethanol ingestion on hepatic lipid peroxide, glutathione, glutathione peroxidase and glutathione transferase in rats. *Toxicology* 1985; **36:** 71-76
35 **Shaw S,** Jayatilleke E, Lieber CS. Lipid peroxidation as a mechanism of alcoholic liver injury: role of iron mobilization and microsomal induction. *Alcohol* 1988; **5:** 135-140
36 **Speisky H**, Bunout D, Orrego H, Giles HG, Gunasekara A, Israel Y. Lack of changes in diene conjugate levels following ethanol induced glutathione depletion or hepatic necrosis. *Res Commun Chem Pathol Pharmacol* 1985; **48:** 77-90
37 **Coudray C,** Richard MJ, Faure H, Favier A. Blood and liver lipid peroxide status after chronic ethanol administration in rats. *Clin Chim Acta* 1993; **219:** 35-45
38 **Ozaras R**, Tahan V, Aydin S, Uzun H, Kaya S, Senturk H. N-acetylcysteine attenuates alcohol-induced oxidative stess in rats. *World J Gastroenterol* 2003; **9:** 791-794
39 **Husain K,** Scott BR, Reddy SK, Somani SM. Chronic ethanol and nicotine interaction on rat tissue antioxidant defense system. *Alcohol* 2001; **25:** 89-97
40 **Wang JH,** Redmond HP, Wu QD, Bouchier-Hayes D. Nitric oxide mediates hepatocyte injury. *Am J Physiol* 1998; **275:** G1117 -G1126
41 **Reutov VP**, Sorokina EG. NO-synthase and nitrite-reductase components of nitric oxide cycle. *Biochemistry (Mosc)* 1998; **63:** 874-884
42 **Gardner CR**, Heck DE, Yang CS, Thomas PE, Zhang XJ, DeGeorge GL, Laskin JD, Laskin DL. Role of nitric oxide in

acetaminophen–induced hepatotoxicity in the rat. *Hepatology* 1998; **27:** 748-754

43 **Milosevic N**, Schawalder H, Maier P. Kupffer cell-mediated differential down-regulation of cytochrome P450 metabolism in rat hepatocytes. *Eur J Pharmacol* 1999; **368:** 75-87

44 **Hunt N**, Goldin RD. Nitric oxide production by monocytes in alcoholic liver disease. *J Hepatol* 1992; **14:** 146-150

45 **Sergent O,** Griffon B, Morel I, Chevanne M, Dubos MP, Cillard P, Cillard J. Effect of nitric oxide on iron-mediated oxidative stress in primary rat hepatocyte culture. *Hepatology* 1997; **25:** 122-127

46 **Joshi MS,** Ponthier JL, Lancaster JR. Cellular antioxidant and pro-oxidant actions of nitric oxide. *Free Radic Biol Med* 1999; **27:** 1357-1366

47 **Roberts RA,** James NH, Cosulich SC. The role of protein kinase B and mitogen-activated protein kinase in epidermal growth factor and tumor necrosis factor alpha-mediated rat hepatocyte survival and apoptosis. *Hepatology* 2000; **31:** 420-427

48 **Crespo J,** Cayon A, Fernandez-Gil P, Hernandez-Guerra M, Mayorga M, Dominguez-Diez A, Fernandez-Escalante JC, Pons-Romero F. Gene expression of tumor necrosis factor alpha and TNF-receptors, p55 and p75, in nonalcoholic steatohepatitis patients. *Hepatology* 2001; **34:** 1158-1163

49 **Sakamoto T,** Liu Z, Murase N, Ezure T, Yokomuro S, Poli V, Demetris AJ. Mitosis and apoptosis in the liver of interleukin-6-deficient mice after partial hepatectomy. *Hepatology* 1999; **29:** 403-411

50 **Peters M,** Blinn G, Jostock T, Schirmacher P, Meyer zum Buschenfelde KH, Galle PR, Rose-John S. Combined interleukin-6 and soluble interleukin-6 receptor accelerates murine liver regeneration. *Gastroenterology* 2000; **119:** 1663-1671

51 **Cressman DE,** Greenbaum LE, DeAngelis RA, Ciliberto G, Furth EE, Poli V, Taub R. Liver failure and defective hepatocyte regeneration in interleukin-6-deficient mice. *Science* 1996; **274:** 1379-1383

52 **Natsume M,** Tsuji H, Harada A, Akiyama M, Yano T, Ishikura H, Nakanishi I, Matsushima K, Kaneko S, Mukaida N. Attenuated liver fibrosis and depressed serum albumin levels in carbon tetrachloride-treated IL-6-deficient mice. *J Leukoc Biol* 1999; **66:** 601-608

53 **Lancaster FE.** Alcohol and the brain: what's NO got to do with it? *Metab Brain Dis* 1995; **10:** 125-133

54 **Hink U**, Li H, Mollnau H, Oelze M, Matheis E, Hartmann M, Skatchkov M, Thaiss F, Stahl RA, Warnholtz A, Meinertz T, Griendling K, Harrison DG, Forstermann U, Munzel T. Mechanisms underlying endothelial dysfunction in diabetes mellitus. *Circ Res* 2001; **88:** E14-E22

55 **Loguercio C**, Federico A. Oxidative stress in viral and alcoholic hepatitis. *Free Radic Biol Med* 2003; **34:** 1-10

56 **Hwang TL,** Yang JT, Lau YT. Arginine-nitric oxide pathway in plasma membrane of rat hepatocytes during early and late sepsis. *Crit Care Med* 1999; **27:** 137-141

57 **Losser MR,** Payen D. Mechanisms of liver damage. *Semin Liver Dis* 1996; **16:** 357-367

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