Original Paper

Serum Nitrate Levels in Alcoholic Patients: A Study in an Urban Government Hospital and De-addiction Centre

Kulkarni SR*, Pratibha Ravindra K**, Dhume CY***, Rataboli PV+, Rodrigues EJ++

ABSTRACT

This study was designed to determine the nitric oxide metabolism measured as circulating nitrate levels in the serum of alcoholic patients. Nitric oxide (NO) plays a significant role in the inflammatory process and has been implicated in several autoimmune disorders. This study was carried out prospectively to estimate the levels of nitrate in the serum, as a surrogate marker of NO production, among alcohol-dependent patients. Serum nitrate concentrations as an index of plasma NO levels were assessed in alcohol-dependent patients attending the de-addiction centre of a major hospital.

Total sample size comprised 450 males. Age- and sex-matched non-alcoholic control population (n=90) was compared with alcoholics (n=360) of similar socioeconomic status. Serum nitrate concentration was assayed spectrophotometrically. Stable metabolites of nitrates were significantly higher in alcoholics compared with their nonalcoholic controls ($p<0.05$).

The results may support the involvement of oxidative damage due to high NO concentration, and may be linked to its excitotoxicity and cytotoxicity in neurons, glia, and myelin. Generation of NO has been linked to an increased tendency towards tolerance to alcohol.

Key Words: Serum nitrate, Ethanol, Alcoholism

Introduction

Excessive consumption of alcohol (ethanol) by a large section of the population is still an important medical and social problem in many countries. It leads to various physical, mental, social, and psychological changes. Alcoholism is well known to cause fatty liver disease and cirrhosis over a period of time. In addition, many neurological lesions and even cerebral atrophy may develop in alcoholics.[1-5]

Ethanol is extensively metabolized in the liver, leading to the generation of acetaldehyde by the enzymatic activity in cytosol, microsomes, and peroxisomes. Acetaldehyde is further oxidized to acetate by acetaldehyde dehydrogenase in the mitochondria, which results in the generation of free radicals/reactive oxygen species (ROS).[1-6] Additionally, ethanol is metabolized more selectively in brain microsomes by cytochrome P-450IIE1, which may result in the generation of ROS.[2,3]

Oxidation of ethanol by alcohol dehydrogenase generates NADH, and NADH-dependent production of ROS by various organelles increases after chronic ethanol consumption.[5,7] These ROS can cause cellular damage until they are removed by the antioxidant system.

*Dept of Biochemistry, Grant Medical College & Sir J.J.Groups of Hospital, Byculla, Mumbai-8.
**Dept of Life Sciences, University of Mumbai, Kalina, Mumbai-400 098.
***Dept of Biochemistry, Goa Medical College, Bambolim Complex, Goa 403 202.
+Dept of Pharmacology, Goa Medical College, Bambolim Complex, Goa 403 202.
++Dept of Forensic Medicine, Goa Medical College, Bambolim Complex, Goa 403 202.
*(*Author for correspondence*): 17: B-Kaupineshwar, Natu Paranjpee Complex, Mith Bandar Road, Thane (East) 400 608 Maharashtra. Email: srk_178@yahoo.co.in

The antioxidant system includes antioxidant enzymes and antioxidant substances.[2,3,5,6] Nitric oxide (NO) is a highly diffusible, lipid-soluble, short-lived free radical gas generated from arginine by NO synthase (NOS, EC1. 14.13.39). It is associated with both physiological and pathological events in the body, including the brain.[8, 9] One of the most important interactions in biological fluids is the reaction of NO with O_2 to form nitrite and nitrate, and its rapid reaction with the superoxide anion (O_2^-) to form the highly reactive peroxynitrite ($ONOO^-$).[8,9] $ONOO^-$ is a strong oxidizing agent that is capable of hydroxylating and nitrating aromatic compounds, and inducing cellular injury by lipoprotein oxidation, DNA fragmentation similar to that of apoptosis, damaging proteins and plasma lipids, depleting important plasma antioxidants, and nitration of proteins leading to cellular dysfunction that damage the endothelium, and induce thickening of the intima in the arterial walls.[6,10] Nitric oxide has important bioregulatory functions in the immune, cardiovascular, and central nervous systems (CNS). Its role in physiological and pathological events may be modulated by alcohol.[8,9,11] Kahkonen and Zvartau[12] showed that NO may modulate the cardiovascular system in alcohol withdrawal syndrome. NO synthesized in the CNS produces important effects. It is implicated in, among other things, the control of blood flow, learning and memory, neurotransmitter release, gene expression, immune responsiveness, and cell survival.[9] Alcohol is lipophilic, and readily crosses the blood-brain barrier, and therefore enters the CNS. Alcohol produces numerous effects in the CNS, some of which overlap with those thought to be modulated or mediated by NO.[8,13] The acute and chronic effects of ethanol on the CNS are complex, involving a range of cell types and signaling systems.

Acute and chronic ethanol exposures have been reported to lead to excitotoxicity, partially due to increased levels of NO.[5,9,11] Increased free radical formation in the brain has been shown to be one of the manifestations of acute and chronic ethanol intoxication.[2,3,14] Brain mitochondria are very susceptible to oxidative injury caused by alcohol-induced free radicals.[2] Investigators have obtained variable data concerning the effects of ethanol on brain and blood total antioxidant activity (AOA), and NO levels. Therefore, the present study was undertaken to investigate the effect of alcohol consumption on NO levels in the sera of alcohol dependent patients.

Materials & Methods

This study was carried out after getting clearance from the Institutional Ethical Review Committee, Grant Medical College & Sir J.J. Groups of Hospitals, Byculla, Mumbai. Patients were selected as per the flow chart depicted in **Fig 1**.

A discrimination procedure was designed to separate alcoholics from controls and patients with non-alcoholic hepatic diseases, by using a combination of the most powerful tests. The discrimination model was constructed with a battery of screening instruments for detecting alcohol problems: CAGE,[15,16,17] Michigan Alcohol Screening Test (MAST),[18,19] Alcohol Use Disorder Identification Test (AUDIT),[20,21] and Severity of Alcohol Use Disorder Data (SADD).[22]

Patients between 25 and 45 years of age, willing to participate in the study, and with no history of undergoing long-term medical intervention for conditions such as cancer, diabetes, advanced alcohol liver disorder, acute respiratory distress syndrome (ARDS), chronic renal failure (CRF), cardiovascular diseases (CVS), and other serious medical, surgical, or neurological conditions, were included in the study. Patients with acute psychotic states were excluded.

Alcoholic patients (n=450) attending the deaddiction center who met the inclusion criteria, and gave their informed consent were included in the study. These patients were matched for age, sex and socio-economic status with normal controls (n=90) who were participating in a screening programme. These controls were, to their knowledge, healthy, and had no reason to consult their local doctors during the preceding 12 months. Further, their nutritional anthropometry (age-independent anthropometric indices) was evaluated by the method of Rao.[23]

Exclusion criteria for patients and controls comprised:

1. Patients below 25 and above 45
2. Patients undergoing long-term medical intervention for cancer, diabetes, advanced alcohol liver disorder, acute respiratory distress syndrome (ARDS), chronic renal failure (CRF), cardiovascular diseases (CVS), and other serious medical, surgical, or neurological conditions.
3. Excessive smoking, evaluated as per Fagerstrom Test for Nicotine Dependence, with a score more than 15.[24, 25, 26]

4. Substance abuse involving cannabis, opiates, and other psychotropic substances.
5. Patients taking vitamins, antioxidants, or any other significant nutritional supplements.
6. Immunocompromised patients, and those in an acute infectious state.
7. Patients with acute psychotic states.
8. Patients unwilling to participate in study.

A dietary survey of study population was conducted by oral questionnaire method, to assess the daily consumption of calories, fats, and protein. The daily food intake was recorded on a proforma, and the values were computed on the basis of standard charts for "Recommended Dietary Allowances for Indians,"[27] and by estimating dietary antioxidant vitamins in the blood of study population. Further assessment of their socio-economic status was done based on per capita income and education, with the help of personal interview.[28]

Within 24 hours of admission, and overnight fasting conditions, a total of 10ml of venous blood samples were collected. From blood samples collected in plain tubes, serum was separated by centrifuging at 2500 rpm for 7 minutes at room temperature, and was used for estimation of serum gamma glutamyl transferase (GGT), serum glutamic-oxaloacetic transaminases (SGOT), serum glutamic-pyruvic transaminases (SGPT), and beta carotene, vitamins E and C, malondialdehyde (MDA), and serum nitrite concentration. Haemolysed or turbid samples were discarded. Blood samples collected in EDTA tubes were used for estimating MCV.

All the chemicals used in this study were procured from Sigma Company (St Louis, MO, USA), while other reagents were obtained from E-Merck, India Ltd.

Total alcohol content in the liquor samples collected from the patients were analyzed by gas chromatography.[29] Determination of inorganic nitrate in serum was done by a kinetic cadmium-reduction method.[30] Nitrate in serum was assayed by a modification of the cadmium-reduction method; the nitrite produced was determined by diazotization of sulfanilamide and coupling to naphthyle-

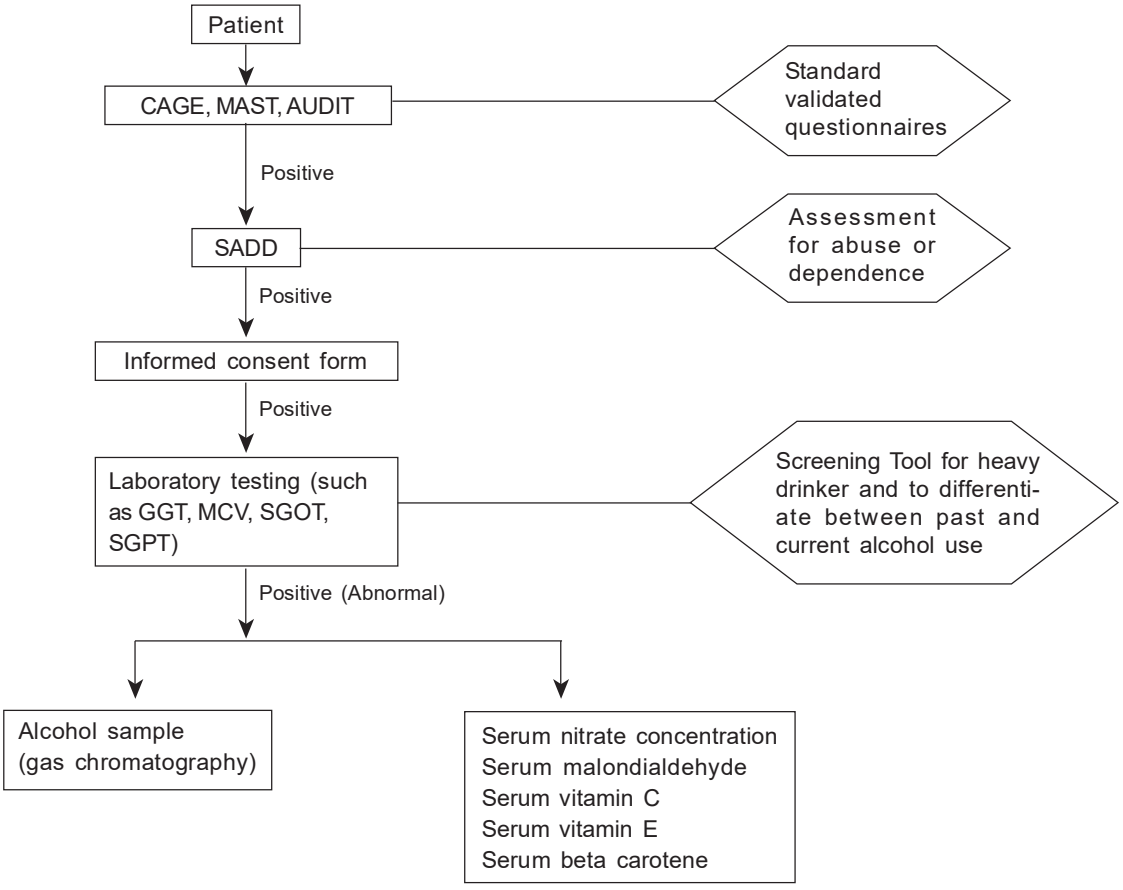

Fig. 1 Screening and Diagnosis of Alcoholism

thylene diamine. After the samples were deproteinized with somogyl reagent, the nitrate was reduced by Cu-coated Cd in glycine buffer at pH 9.7 (2.5 to 3g of Cd granules for a 4 ml reaction mixture).The reduction followed pseudo-first order reaction kinetics, a convenient time interval for assay being 75 to 90 min. Maximum reduction (85%) occurred at about 2h. Detection limits in serum were 2 to 250 micromoles/L. This method does not require the reaction to go to completion, does not require expensive reagents or equipments, and can be used to assay several samples simultaneously.

The serum lipid peroxidation was estimated by thiobarbituric acid (TBA) reactivity.[31,32] Malondialdehyde (MDA) and end-product of fatty acid peroxidation, reacts with TBA to form a coloured complex that has maximum absorbance at 532 nm. MDA values were calculated from the absorbance coefficient of MDA-TBA complex at 532 nm, 156,000 cm^{-1} mol^{-1}. Serum vitamin E was estimated by the method of Baker et al.[33] Vitamin E from serum was extracted into *n*-heptane, which reacts with ferric chloride, and reduces ferric to ferrous ions. Ferrous ions then form a red coloured complex with 2,2-dipyridyl, which was read at 520 nm. The method proposed by Ayekyaw was used to estimate serum vitamin C.[34] Vitamin C from serum reacts with phosphotungstate to give a blue colour that has maximum absorbance at 600 nm.

All spectrophotometric readings were taken on Shimadzu UV-160A, UV-Visible Recording Spectrophotometer.
All the samples were run in duplicate, and were statistically assessed using student t-Test,[35] by using statistical software MINITAB, and were subjected to ONE-WAY ANOVA. The results obtained were expressed as Mean ± Standard Deviation (SD).

Results

1. Demographic and clinical data (Mean ± SD) of controls and alcoholic patients from three different socioeconomic backgrounds consuming alcohol of three different qualities, exhibited significantly lower values of parameters such as Anthropometric Index, indicative of malnutrition.
2. The results, concerning the scores of rating scales and markers of alcoholism in alcoholics versus control group showed a statistically significant increase in the rating scales such as CAGE, MAST, AUDIT and SADD of alcoholics consuming alcohol of different qualities from different socioeconomic background.
3. The results demonstrated statistically significant increase in the GGT, MCV, SGOT, and SGPT values of all alcoholic patients ($p < 0.001$) as compared to their respective controls.
4. A significant increase was observed in the levels of serum nitrite, and malondialdehyde (MDA), while a significant decrease in the levels of serum vitamin C, vitamin E, and vitamin A (beta carotene) concentration, in alcoholic patients.

Discussion

The study data showed that alcohol-dependent patients displayed significantly lower levels of dietary antioxidant vitamins such as vitamin E, vitamin C, and beta carotene, and significantly higher levels of serum MDA, than their non-alcoholic healthy control group. This is an important finding in view of the simultaneous deleterious effects of free radicals on serum constituents. It is well known that decreased antioxidant activity results in increased lipid peroxidation, which in turn plays a significant role in the pathogenesis of various diseases.[2,5,14] Many investigators have shown that alcohol decreases the activity of various antioxidant enzymes and vitamins, which results in decreased total antioxidant activity. Indeed, it has been reported that both brain[36] and serum[37,38] α-tocopherol levels, and brain[3, 39, 40,41] and serum[6, 14 ,41-44] activity of some significant antioxidant enzymes were reduced after ethanol exposure. Husain et al [6] and Aydýn et al[44] reported that chronic ethanol consumption increased plasma malondialdehyde (MDA) levels, and decreased antioxidant enzymes activities. One explanation for the observed lower level of antioxidant vitamins in our alcohol-dependent patients may be due to the increased utilization of antioxidants in the scavenging of free radicals. Mounting evidence points to oxidative stress as an important mechanism in alcohol toxicity, because alcohol induces peroxidation of membrane lipids, and oxidation of proteins and nucleic acids.[36,40,45]

As evident from the foregoing reported findings, many investigators have measured different antioxidant substances or enzymes in evaluating the effect of alcohol on the antioxidant defense system, since it may be particularly useful for monitoring alterations in antioxidant levels.[46] Thus, we are of the opinion that our data provide additional, practical, and applicable information for evaluating the effect of alcohol on the antioxidant defense system.

In the present study serum NO levels in the alcohol-dependent patients were significantly higher than their respective controls. This finding is in accordance with the findings of many other investigators.[5,44,45,47,48] One mechanism proposed for this change is an increase in the level of free radicals due to alcohol consumption,[1-7,36] as free radicals induced by ethanol stimulate the synthesis of some cytokines (such as interleukin-1,-2,-6, and -8, and tumour necrosis factor-alpha), which in turn stimulate the synthesis of NO.[5,45]

Two additional mechanisms have been proposed for this increase. One mechanism is to do with the overproduction of NO in alcoholics via inducible NOS (iNOS), resulting from chronic inflammation and stimulation of the immune system. Increased production of NO, and increased production of superoxide, with vasoconstrictive effects can play a role in cardiovascular instability and the evolution of hypertension in alcoholics.[5, 6] The other proposed mechanism concerns an increase in vascular endothelial growth factor expression, as well as endothelial NOS (eNOS) activation, leading to NO generation in response to mild to moderate concentrations of ethanol *in vitro* and *in vivo*.[6,49] In addition, from our results it can be postulated that since NO is also a free radical, lower levels of dietary antioxidant vitamins such as vitamin E, vitamin C, and beta carotene, and enhanced levels of serum MDA in alcohol-dependent patients might be another significant cause of increased serum NO levels, or vice versa.

Studies on human alcoholic liver disease reveal that the high haemodynamic state of patients with advanced cirrhosis is possibly due to either increased endothelial synthesis of NO,[50,51] or smooth-muscle derived NO.[52] Peripheral blood monocytes from patients with alcoholic hepatitis secrete more nitric oxide than monocytes from patients without alcoholic hepatitis.[53] But this was refuted in another study.[54] Peripheral blood monocytes from patients with alcohol-induced cirrhosis however clearly produce higher levels of NO compared to monocytes from healthy controls.[54,55] Binge drinking has been experimentally shown to influence metabolic pathways in liver cells.[56,57] Gluconeogenesis has cbeen found to be delayed in isolated perfused rat liver,[58] and F-actin content was found to be unregulated in leucocytes of female rats.[59] Acute ethanol intoxication has been reported to stimulate superoxide anion production in the perfused rat liver,[60] and ethanol-induced oxidative stress has been shown to cause massive mitochondrial DNA degradation.[61] Acute alcohol intoxication may increase free radical release due to activation of Kupffer endothelial cells,[62,-65] and hepatocytes.[66] Acute ethanol intoxication also influences expression of interleukin-6 surface receptors in both Kupffer cells and hepatocytes.[67] Chemokine production by Kupffer cells is also modulated by binge drinking.[68,69]

In conclusion, our findings of reduced dietary antioxidant vitamins such as vitamin E, vitamin C and beta carotene, and increased MDA levels in the alcohol-dependent patients constitute evidence that alcohol weakens the body's defense mechanism against free radical attacks. Furthermore, decreased dietary antioxidant vitamin levels in the alcohol-dependent patients may be another significant cause of increased levels of serum NO, or vice versa, which could lead to exitotoxicity, and subsequent cytotoxicity in neurons, glia, and myelin. Formation of NO has been linked to an increased tendency towards tolerance to alcohol. Nonetheless, a better understanding of the effects of alcohol on NO levels requires further investigation.

REFERENCES

1. Mayes PA. Lipid transport and storage. In: Murray RK, Granner DK, Mayes PA, Rodwell VW (eds). Harper's Biochemistry. 23rd edn, 1993. London: Lange Medical Publication. p.260.
2. Reddy SK, Husain K, Schlorff EC, Scott RB, Somani SM. Dose response of ethanol ingestion on antioxidant defense system in rat brain subcellular fractions. Neurotoxicol 1999; 20: 977-987.
3. Somani SM, Husain K. Interaction of exercise training and chronic ethanol ingestion on antioxidant system of rat brain regions. J Appl Toxicol 1997; 7: 329-336.
4. Charness ME. Brain lesions in alcoholics. Alcohol Clin Exp Res 1993; 17: 2-11.
5. Zima T, Fialova L, Metsek O, Janebova M, Crkovska J, Malbohan I, et al. Oxidative stress, metabolism of ethanol and alcohol-related diseases. J Biomedical Sci 2001; 8: 59-70.
6. Husain K, Mejia J, Lalla J, Kazim S. Dose response of alcohol-induced changes in BP, nitric oxide and antioxidants in rat plasma. Pharmacol Res 2005; 1: 337-343.
7. Kukielka E, Dicker E, Cederbaum AI. Increased production of reactive oxygen species by rat liver mitochondria after chronic ethanol treatment. Arch Biochem Biophys 1994; 309: 377-386.
8. Davis RL, Syapin PJ. Interactions of alcohol and nitric-oxide synthase in the brain. Brain Res Rev 2005; 49: 494-504.

9. Shih CL, Chi SI, Chiu TH, Sun GY, Lin TN. Ethanol effects on nitric oxide production in cerebral pial cultures. Alcoholism Clin Exper Res 2001; 25: 612-618.
10. Cakatay U, Telci A, Kayali R, Tekeli F, Akcay T, Sivas A. Relation of oxidative protein damage and nitrotyrosine levels in the aging rat brain. Exper Gerontol 2001; 36: 221-229.
11. Lancaster FE. Alcohol and the brain: What's NO got to do with it? Metabol Brain Dis 1995; 10: 125-133.
12. Kahkonen S, Zvartau EE. Altered cardiovascular responses to nitrosorbide in alcohol withdrawal. Neuropsychobiol 2003; 48: 124-130.
13. Dizon ML, Brown LA, Black SM. Brain nitric oxide synthase levels increase in response to antenatal ethanol exposure. Alcohol Alcoholism 2004; 39: 101-105.
14. Mihailescu I, Mohora M, Dinu V. Preliminary evidence for lipid peroxidation and changes in pro-oxidant status in brain tissue and blood in ethanol intoxicated rats. Revue Roumaine De Physiologie 1991; 28: 133-137.
15. Ewing JA. Detecting Alcoholism: The CAGE Questionnaire. JAMA 1984; 252: 190-197.
16. Ewing JA, Rouse BA. Identifying the hidden alcoholic. Proceedings, 29th International Congress on Alcohol and Drug Dependence, Sydney, Australia, Feb 3, 1970.
17. Mayfield D, Mcleod G, Hall P. The CAGE Questionnaire: Validation of a new Alcoholics Screening Instrument. Am J Psychiatry 1974; 131: 1121-1123.
18. Selzer ML The Michigan Alcoholism Screening Test: The quest for a new diagnostic instrument. Am J Psychiatry 1971; 127:1653-16519.
19. Selzer ML, Vinokur A, Van Rooijen L. A self-administered short Michigan Alcoholism Screening Test (MAST). J Studies Alcohol 1975; 36: 117-126.
20. Babor TF, De La Fuente JR, Saunders J, et al. AUDIT, the Alcohol Use Disorders Identification Test guidelines for use in primary health care. 1992. World Health Organization, Geneva.
21. Bohn MJ, Babor TF, Kranzler HR. The Alcohol Use Disorders Identification Test (AUDIT): Validation of a screening instrument for use in medical settings. J Studies Alcohol 1995; 6: 423-432.
22. Stockwell T, Sitharathan T, McGrath E. The measurement of alcohol dependence and impaired control in community sample. Addiction 1994; 69: 167-174.
23. Ghai and Gupta. Essential Preventive Medicine. 1989. p105-175.
24. Fagerstrom KO. Measuring degree of physical dependence to tobacco smoking with reference to individualization of treatment. Addict Behav 1978; 3: 235-241.
25. Pomerleau CS, Carton SM, Lutzke ML, et al. Reliability of the Fagerstrom Tolerance Questionnaire and the Fagerstrom Test for Nicotine Dependence." Addict Behav 1994; 19: 33-39.
26. Heatherton TF, Kozlowski LT, Frecker RC, et al. The Fagerstrom Test for Nicotine Dependence: A revision of Fagerstrom Tolerance Questionnaire. Br J Addict 1994; 86: 1119-1127.
27. Mahajan BK, Gupta MC. Textbook of Preventive and Social Medicine. 2nd edn, 1991. 134-135.
28. Kuppuswamy B. Revised .Manual of Socioeconomic Status Scale (Urban). 1981. Delhi: Manasayan.
29. Bruce W. Determination of Alcohol Content. In: Wine Analysis and Production, Laboratory Procedures. 1995. 407-408.
30. Cortas NK, Wakid NW. Determination of inorganic nitrate in serum by a kinetic cadmium-reduction method. Clin Chem 1990: 1440-1443.
31. Wilbur KM, Frederick B, Shapiro OW. The thiobarbituric acid reagent as a test for the oxidation products of unsaturated fatty acids by various agents. Arch Biochem Biophys 1949; 305-313.
32. Buege A. Microsomal lipid peroxidation. Methods Enzymol 1978; 105: 302-310.
33. Baker H, Frank O. Determination of serum alpha tocopherol. In: Gokenlock AH, McMurray JR, Mehauchian DM. Varley's Practical Clinical Biochemistry. 6th edn, 1968. London: William Heinemann Medical Books Ltd. 902-903.
34. Ayekyaw A simple colorimetric method for ascorbic acid determination in blood plasma. Clinica Acta 1978; 86: 153-157.
35. Stell RGU, Torrie JH. Principles and Procedures of Statistics. 1960. New York: McGraw Hill. 78.
36. Saravanan R, Rajendra Prasad N, Pugalendi KV. Effect of piper betel leaf extract on alcoholic toxicity in the rat brain. J Med Food Sci 2003; 6: 261-265.
37. Bjorneboe GA, Johnsen J, Bjorneboe A, Morland J, Drevon CA. Effect of heavy alcohol consumption on serum concentrations of fat-soluble vitamins and selenium. Alcohol Alcoholism 1987; 1: 533-537.
38. Bjorneboe GE, Johnsen J, Bjorneboe A, Marklund SL, Skylv N, Hoiseth A, et al. Some aspects of antioxidant status in blood from alcoholics. Alcoholism Clin Exper Res 1988; 12: 806-810.
39. Ledig M, M'Paria JR, Mandel P. Superoxide dismutase activity in rat brain during acute and chronic alcohol intoxication. Neurochem Res 1981; 6: 385-390.
40. Pushpakiran G, Mahalakshmi K, Anuradha CV. Taurine restores ethanol-induced depletion of antioxidants and attenuates oxidative stress in rat tissues. Amino Acids 2004; 27: 91-96.

41. Carmiel-Haggai M, Cederbaum AI, Nieto N. Binge ethanol exposure increases liver injury in obese rats. Gastroenterol 2003; 125: 1818-1833.
42. Seo DO, Rivier C. Interaction between alcohol and nitric oxide on ACTH release in the rat. Alcoholism Clin Exper Res 2003; 27: 989-996.
43. Schisler NJ, Singh SM. Effect of ethanol *in vivo* on enzymes which detoxify oxygen free radicals. Free Radical Biol Med 1989; 7: 117-123.
44. Aydin S, Ozaras R, Uzun H, Belce A, Uslu E, Tahan V, et al. N-acetylcysteine reduced the effect of ethanol on antioxidant system in rat plasma and brain tissue. Tohoku J Exp Med 2002; 198: 71- 77.
45. Soardo G, Donnini D, Varutti R, Moretti M, Milocco C, Basan L, et al. Alcohol-induced endothelial changes are associated with oxidative stress and are rapidly reversed after withdrawal. Alcohol Clin Exp Res 2005; 29: 1889-1898.
46. Erel O. A new automated colorimetric method for measuring total oxidant status. Clin Biochem 2005; 38: 1103-1111.
47. Li YM, Chen SH, Yu CH, Zhang Y, Xu GY. Effect of acute alcoholism on hepatic enzymes and oxidation/antioxidation in rats. Hepatobil Pancreat Dis Int 2004; 3: 2414.
48. Oekonomaki E, Notas G, Mouzas IA, Valatas V, Skordilis P, Xidakis C, et al. Binge drinking and nitric oxide metabolites in chronic liver disease. Alcoholism Alcohol 2004; 39: 106-109.
49. Bennett KR, Gu JW, Adair TH, Heath BJ. Moderate levels of ethanol induce expression of vascular endothelial growth factor and stimulate angiogenesis. Am J Physiol Regul Integr Comp Physiol 2001; 281: 365-372.
50. Calver A, Harris A, Maxwell JD, Vallance P. Effect of local inhibition of nitric oxide synthesis on forearm blood flow and dorsal hand vein size in patients with alcoholic cirrhosis. Clin Sci 1994; 86: 203-208.
51. Campillo B, Chabrier PE, Pelle G, et al. Inhibition of nitric oxide synthesis in the forearm arterial bed of patients with advanced cirrhosis. Hepatol 1995; 22: 1423-1429.
52. Ryan J, Jennings G, Dudley F, Chin-Dusting J. Smooth muscle-derived nitric oxide is elevated in isolated forearm veins in human alcoholic cirrhosis. Clin Sci 1996; 91: 23-28.
53. Hunt NC, Goldin RD. Nitric oxide production by monocytes in alcoholic liver disease. J Hepatol 1992; 14: 146-150.
54. Criado-Jimenez M, Rivas-Cabanero L, Martin-Oterino JA, Lopez-Novoa JM, Sanchez-Rodriguez A. Nitric oxide production by mononuclear leukocytes in alcoholic cirrhosis. J Mol Med 1995; 73: 3331-3355.
55. Sanchez-Rodriguez A, Criado M, Rodriguez-Lopez AM, Esteller A, Martin DA, Lopez-Novoa JM. Increased nitric oxide synthesis and inducible nitric oxide synthase expression in patients with alcoholic and non-alcoholic liver cirrhosis. Clin Sci 1998; 94: 637-643.
56. Arteel GE, Raleigh JA, Bradford BU, Thurman RG. Acute alcohol produces hypoxia directly in rat liver tissue *in vivo*: Role of Kupffer cells. Amer J Physiol 1996; 271: G494–G500.
57. Rivera CA, Bradford BU, Seabra V, Thurman RG. Role of endotoxin in the hypermetabolic state after acute ethanol exposure. Amer J Physiol 1998; 275: G1252–G1258.
58. Deaciuc IV, D'Souza NB, Lang CH, Spitzer JJ. Effects of acute alcohol intoxication on gluconeogenesis and its hormonal responsiveness in isolated, perfused rat liver. Biochem Pharmacol 1992; 44: 1617–1624.
59. Zhang P, Spitzer JA. Acute ethanol administration modulates leukocyte actin polymerization in endotoxic rats. Alcohol Clin Exper Res 1997; 21: 779–783.
60. Bautista AP, Spitzer JJ. Acute ethanol intoxication stimulates superoxide anion production by *in situ* perfused rat liver. Hepatol 1992; 15: 892-898.
61. Mansouri A, Gaou I, De Kerguenec C, et al. An alcoholic binge causes massive degradation of hepatic mitochondrial DNA in mice. Gastroenterol 1999; 117: 181-190.
62. Bautista AP, Elliott KE. Acute ethanol intoxication regulates chemotaxis and superoxide release by neutrophils and Kupffer cells through modulation of the formyl peptide receptor in the rat. Life Sci 1994; 54: 721–730.
63. Bautista AP, Spitzer JJ. Post-binge effects of acute alcohol intoxication on hepatic free radical formation. Alcohol Clin Exper Res 1996; 20: 502-509.
64. Bautista AP, Spitzer JJ. Role of Kupffer cells in the ethanol-induced oxidative stress in the liver. Frontiers Biosci 1999; 4: D589–D595.
65. Yokoyama H, Fukuda M, Okamura Y, et al. Superoxide anion release into the hepatic sinusoid after an acute ethanol challenge and its attenuation by Kupffer cell depletion. Alcohol Clin Exper Res 1999; 23: 71S-75S.
66. Bailey SM, Cunningham CC. Acute and chronic ethanol increases reactive oxygen species generation and decreases viability in fresh, isolated rat hepatocytes. Hepatol 1998; 28: 1318–1326.
67. Deaciuc IV, Alappat JM, D'Souza NB. Effect of acute and chronic alcohol administration to rats on the expression of interleukin-6 cell-surface receptors of hepatic parenchymal and non-parenchymal cells. Alcohol Clin Exper Res 1994; 18: 1207-1214.
68. Bukara M, Bautista AP. Acute alcohol intoxication and gadolinium chloride attenuate endotoxin-induced release of CC chemokines in the rat. Alcohol 2000; 20: 193-203.
69. Bautista AP. Acute alcohol intoxication and endotoxemia desensitize HIV-1 gp120-induced CC-chemokine production by Kupffer cells. Life Sci 2001; 68: 1939-1949.