PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

Pakistan Journal of Nutrition 5 (6): 507-511, 2006
ISSN 1680-5194

The Hepatoprotective Effect of Vitamin C and E on Hepatotoxicity Induced by Ethanol in Sprague Dawley Rats

C.A. Oyinbo[1], W.N. Dare[1], G.R.A. Okogun[2], L.C. Anyanwu[1],
N.M. Ibeabuchi[3], C.C. Noronha[3] and O.A.Okanlawon[3]
[1]Department of Anatomy, College of Medicine,
Ambrose Alli University, P.M.B 14, Ekpoma, Edo State, Nigeria
[2]Department of Medical Laboratory Sciences, College of Medicine,
Ambrose Alli University, P.M.B 14, Ekpoma, Edo State, Nigeria
[3]Department of Anatomy, College of Medicine, University of Lagos, Lagos State, Nigeria

Abstract: Ethanol liver disease is a major health problem worldwide. One aspect of ethanol toxicity that has received increasing attention in recent years is the role of free radical species in the etiology of liver injury. Vitamins C and E are antioxidants that scavenge for free radicals. The effect of 200mg Vitamin C + 200mg Vitamin E as food supplement administered for 7 days was studied on hepatic damage induced by ethanol (40% v/v, 2.0ml/100g body weight per oral for 21 days) in male Sprague-Dawley rats. The supplements were administered for the last week of 21 days administration of ethanol. Serum transaminases, Lipid peroxidase and Bilirubin were estimated to access liver damage. Our results showed that ethanol induce increased serum Transaminases, Lipid peroxidase and Bilirubin levels. The biochemical findings were supplemented by histopathological examination of the liver sections. The study confirmed the hepatoprotective effects of Vitamins C and E on the hepatocytes of rats as demonstrated by previous investigators.

Key words: Hepatoprotective, free radicals, vitamins C and E

Introduction

The nutritional importance of ethanol has been extensively studied (Berger et *al*., 1999). The availability of alcoholic beverages is also on the increases (Garfield *et al*., 2003; Treno *et al*., 2003). There is no hope that social drinking would ever be out fashioned. In man excess alcohol consumption is associated with liver disease and cirrhosis. Alcohol liver disease is a major health problem worldwide (Befrits *et al*., 1995). One aspect of alcohol toxicity that has received increasing attention in recent years is the role of free radical species in the etiology of liver injury (Normann *et al*., 1992). It is not unlikely that hepatic toxicity due to ethanol is probably multifactorial; nonetheless, evidence for the role of free radicals in the pathogenesis of liver disease has been reported (Diehi *et al*., 1988; Lecomte *et al*., 1994). Animal experimentation has demonstrated that either acute or chronic alcohol administration increases the rate of lipid peroxidation (Diehi *et al*., 1988), which is a classical biochemical feature in hepatotoxic poisoning (Dianzani *et al*., 1991, Lieber, 1993). A self-propagating chain of free radicals absorbs electrons from cell membrane to attain electrochemical stability (Stohs, 1995, Levine and Kidd, 1985). This alters the integrity of cell membranes. They do not only affect the macromolecules but also DNA and cell organelles (Halliwell and Gutteridge, 1984). This massive destructive chain reaction continues unless a sufficient supply of antioxidant is available to stop it (Li and Friedman, 1999). Biological antioxidants prevent free radical oxidative processes by free radical energy reduction, interruption of its chain reaction and prevention of its formation (Traber, 1999). Pathological conditions due to free radical occur when the body's antioxidant mechanism can not keep pace with the rate of free radicals and other oxidant formation (Bland, 1986).

This work on the effects of vitamins C and E on hepatotoxicity induced by ethanol in Sprague-Dawley Rats is aimed at determining the biological and histological effects of alcohol administration on animal hepatocytes and the possible ameliorating effect of antioxidants.

Materials and Methods

Experimental animals: Fifteen male Sprague-Dawley rats of 150-200g-body weight were grouped into three, of five animals per group. They were kept under standard laboratory conditions and were allowed access to water and commercial pellet diet *ad libitum*. The room temperature was maintained at 26±1°C. Group 1 served as saline control, groups 2 and 3 served as experimental.

Drugs and chemicals: Alcohol (sigma U.K.) Vitamins C and E (sigma U.K.) were provided as gift samples by the Anatomy and Physiology laboratories of the College of Medicine, University of Lagos, Lagos- Nigeria.

Treatment: Group 1 rats were given normal saline 2ml/100g-body weight per oral for 21 days. On the 22nd day, they were sacrificed in a chloroform chamber. The

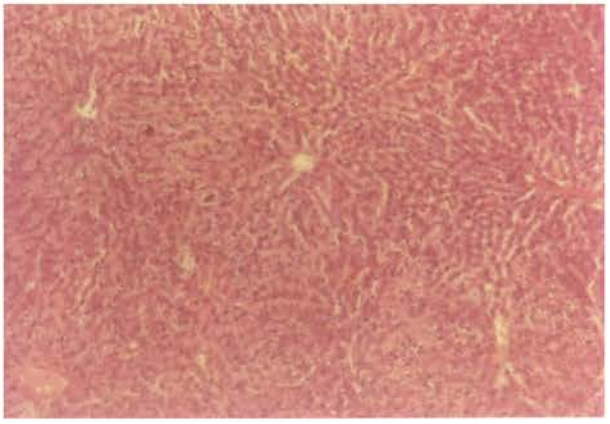

Fig. 1: Control rat: LM Section of liver showing the normal lobular histological picture. (H &E x100).

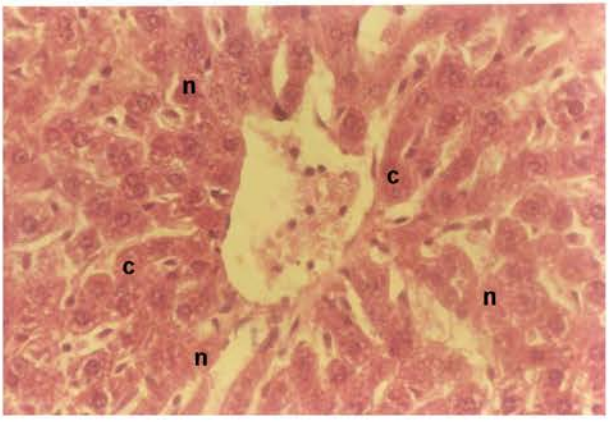

Fig. 2: Control rat: LM Section of liver showing a normal histological picture. Normal hepatocytes with brought out nuclei (n), cytoplasm (c) and a well distinct hepatic laminae. (H & E x 400).

hearts were exposed and 1- 2ml of blood samples were taken through a hypodermic syringe and transferred into universal bottles. Portions of the liver were also excised. A portion was fixed in 10% Formalin and another portion was blended as tissue homogenate. Biochemical and histopathological analyses were carried out on the blood and liver samples.

Group 2: Hepatotoxicity was induced by the administration of 40 % ethanol v/v, 2.0ml per 100g-body weight, per oral for 21 days (Vivek *et al.*, 1994). On the 22nd day, they were sacrificed and treated as described earlier.

Group 3: In addition to the treatment described above, rats were given 200mg vitamin C plus 200mg vitamin E orally for one week as food supplements. On the 22nd day, they were sacrificed and treated as described above.

Biochemical analysis: Serum levels of Glutamate Pyruvate Transaminase [SGPT] and Glutamate Oxaloacetate Transanimase [SGOT] (Retiman and Frankel, 1957), and Bilirubin, [total and direct] (Malloy and Evelyn, 1937), were determined by colorimetric methods using Randox diagnostic kits (U.K.) in Pathology laboratory of the College of Medicine, University of Lagos.

Tissue homogenate: Thiobarbituric acid reactive substance (TBARS) (Ohkawa and Ohishi, 1979, Zdeneka *et al.,* 1966) conc. [Lipid peroxides as nmol of MDA/mg of protein] of 10% liver homogenate (in 0.15M KCl) by the calorimetric reaction of 0.8% Thiobarbituric acid (TBA) with Malondialdeyhde (MDA), a secondary product of lipid peroxidation was measured at 540nm. Amount of protein / mg of tissue were measured (Lowry *et al.,* 1951) to determine the level of lipid peroxidation.

Histopathological analysis: Portions of the preserved liver (10% Formalin) were processed and embedded in paraffin wax. Sections of 5-6 Microns were made and stained routinely with hematoxylin and eosin (Luna, 1966).

Statistical analysis: The mean ± S.D. values were calculated for each group and a one-way analysis of variance (ANOVA) was done for each quantitative parameter to determine the significance of inter-group differences (Armitage and Berry, 1985)

Results

Histopathological

Group 1 (Control): The normal lobular architectural pattern of the liver section is as shown in Fig. 1. The lobulation is modest as a result of the low content of interstitial tissues, and can be determined only with reference to the central vein. Sinusoids at the periphery or the lobule are fused into a reticulum. The hepatocytes are arranged in a series of branching and anastomosing perforated laminae to form a labyrinth, between which were sinusoidal spaces. The cytoplasm of the hepatocytes was clearly eosinophilic, with prominent nuclei (Fig. 2).

Group 2 (Ethanol only): The normal lobular architectural pattern of the liver section cannot be discerned, though several central veins are seen (Fig. 3). It was observed that the hepatocytes were swollen and the sinusoidal spaces occluded. The cytoplasm of the hepatocytes appeared cloudy poorly stained (hydropic degeneration) and with necrotic signs (Fig. 4).

GROUP 3 (Ethanol + Vitamins C and E): The photomicrograph of the liver section of the vitamins treated group, (Fig. 5) showed a histological picture that closely approximate that of the control group (Fig. 2). The

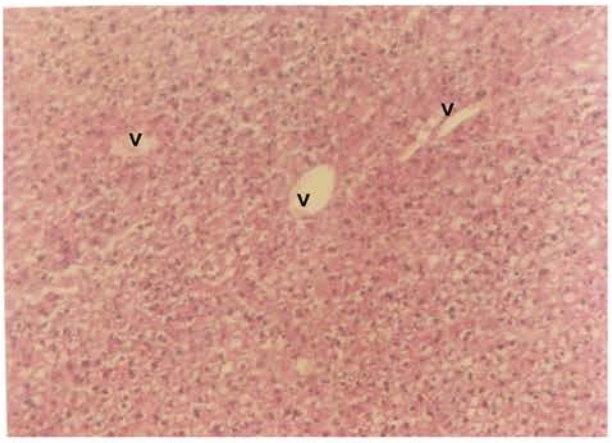

Fig. 3: Alcohol treated rat: LM Section showing several central veins (v), with no observable lobular histological picture. (H&Ex100).

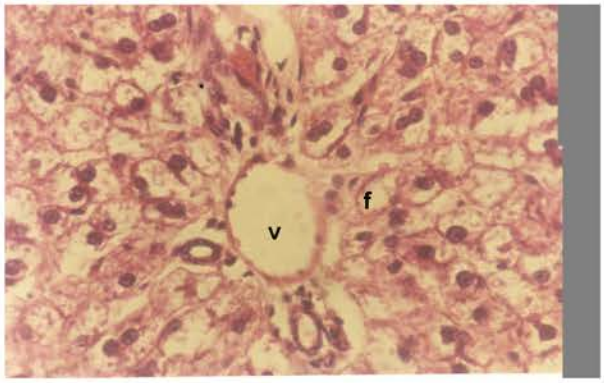

Fig. 4: Alcohol treated rat: LM Section showing (I) a central vein (v), (ii) occluded sinusoidal spaces, (iii) hydropic degeneration, (iv) swollen and necrotic hepatocytes, (v) mild fatty degeneration (f). (H&Ex400).

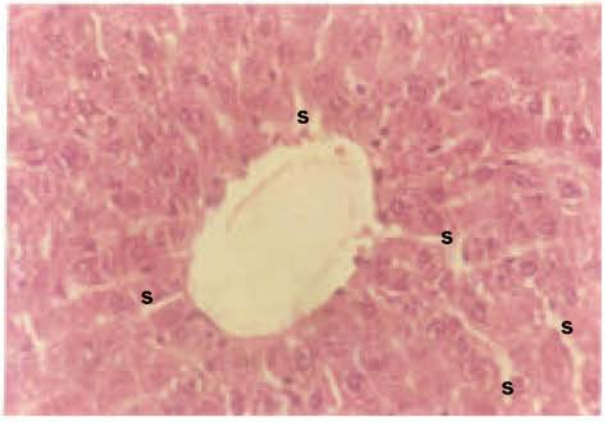

Fig. 5: Alcohol + Vitamins E and C treated rat: LM Section showing mild closure of sinusoidal spaces and less conspicuous hepatic laminae. (H&E x 400).

plate-like arrangements of the hepatocytes were seen, the sinusoidal spaces were also visible, but not as prominent as in the control group. The cytoplasms of the hepatocyte were clearly eosinophilic as in the control group.

Biochemical: The results of the biochemical analysis are depicted in (Table 1). It shows the average (n=5) levels of these biochemical parameters for liver function tests (LFT). A statistical analysis of the results was done as mentioned earlier and was also depicted on the table. Laboratory analysis of blood samples revealed a significantly elevated levels of serum transaminases and serum bilirubin in the experimental groups (2, and 3), ($p < 0.05$) as compared with the control (group 1). Elevations in values tend to be relative to the amount of liver damage. From Table 1, it is observed that the level of serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) were higher in the animals fed with ethanol (group 2); and the levels were seen to be significantly lower in group 3 animals. ($P<0.05$). The levels of these transaminases were absolutely lower in group 3. The total bilirubin and the direct bilirubin levels were significantly higher in the experimental group 2 ($p<0.05$) when compared with the control. The effect of ethanol was less in group 3 rats. There was no significant difference between the control and group 3 rats ($P>0.05$). There was a significant effect of ethanol on serum lipid peroxidases levels of group 2 rats (mean ± S.D. 1.20 ± 0.243) when compared with control group (mean ± S>D 0.62 ± 0.123). This effect was still significant in the vitamins C and E treated rats (group 3) ($P<0.05$), but the effect of ethanol on this group of rats was less marked.

Discussion

The etiology of free radical in alcohol liver disease is long established (Befrits *et al.*, 1995; Normann *et al.*, 1992). This work shows that consumption of vitamins C and E as food supplement protects the hepatocytes, and reduces the severity of damage due to ethanol toxicity.

The photomicrograph of the liver section of group 2 rats differs from those seen in normal liver sections (Underwood, 1992). The sinusoidal spaces were obscured, the hepatocytes swollen and necrotic, as earlier reported (Vivek *et al.*, 1994). Steatosis was seen in the liver sections of experimental animals that received ethanol without antioxidants, suggesting hepatoprotective effect of antioxidants as earlier reported (Li and Friedman *et al.*, 1999; Levine and Kidd, 1985). Histopathological analysis of group 3 photomicrographs revealed appreciably normal liver section and healthy hepatocytes. Hence, it could be inferred that the level of ethanol toxicity is much lower in-group 2. The administration of vitamins C and E mops up free radicals generated in alcohol metabolism, and

Table 1: Effect of vitamins E and C on Biochemical parameters in rats subjected to Ethanol

Group	SGOT (U/l)	SGPT (U/l)	Bilirubin (µmol/l)		Lipid peroxides (nmoles MDA/ mg protein)
			Direct	Total	
I- Control	13.4±0.65	17.8±1.48	2.60±0.35	4.50±0.65	0.62±0.30
II-Ethanol	29.9±1.02	23.7±1.20	4.98±0.50	7.80±0.36	1.20±0.34
III-Ethanol+Vitamins E & C	15.6±0.56	20.8±1.86	3.20±0.63	4.70±0.44	0.95±0.42
F ratio*	132.7	9.605	20.31	32.52	9.933
Significant Differences	1 & 2	1& 2	1 & 2	1 & 2	1 & 2
Between various groups	2 & 3		2 & 3	2 & 3	2 & 3

P<0.05. Values are; Mean ± S.D., n=5, Critical value = 3.89

is responsible for the healthy state of liver cells, as earlier reported (Befrits *et al*., 1995, Underwood, 1992) Our biochemical investigations revealed elevated serum liver enzymes in alcohol treated rats. The greater the values of these parameters the greater the damage on the liver cells. Elevated level of bilirubin is an indication of biliary obstruction and haemolysis; this may be sequel to reduced blood supply to hepatocytes. The elevation of total and indirect bilirubin suggests that the hepatocyte degeneration is gradual; hence, it might require long time exposure for necrosis to take place. Elevated levels of serum glutamic-oxaloacetic transaminase (SGOT) and serum glutamic-pyruvic transaminase (SGPT) are indications of hepatocellular injury. Excessive ethanol consumption activates a number of systems that generate oxygen free radical and reactive aldehydic species. It has been demonstrated that hydroxyl-ethyl radicals are generated during ethanol metabolism by microsomal mono-oxygenase system involving the alcohol inducible cytochrome. Hepatitis and Cirrhosis may also occur as a result of enhanced lipid peroxidation from the microsomal metabolism of ethanol. Lipid peroxidative degradation of biomembrane is one of the principal causes of hepatotoxicity.

Fatty infiltration in liver cells is a relatively benign abnormality. In alcoholic liver disease, essential metabolic pathways for fats are disrupted and diverted to the metabolism of alcohol, so fat accumulates in the liver as earlier reported that alcohol stimulates collagen synthesis in the liver leading to fibrosis and eventually cirrhosis (Voehringer *et al*., 2000, Olaso and Friendman, 1988). There is evidence that in both processes, the productions of reactive oxygen specie and 1-hydroxyethyl radicals are involved.

In conclusion, the authors in this report confirmed the hepatotoxic effect of ethanol by free radical mechanism and observed that treatment with antioxidants protects the hepatocytes, and reduce the severity of damage due to ethanol toxicity.

References

Armitage, P. and G. Berry, 1985. In Statistical methods in medical research. 2nd Ed. London. Blackwell scientific publications, 201- 3.

Befrits, R., M. Hedman and L. Blomquist, 1995. Chronic hepatitis C in alcoholic Patients; Prevalence, genotypes, and correlations of liver disease. Scand. J. Gastroenterol., 30: 1113-1118.

Berger, K., U.A. Ajani and C.A. Kase, 1999. Light-to-moderate alcohol consumption and risk of Stroke among US malephysicians. New Engl. J. Med., 341: 1557-1564.

Bland, J., 1986. The nutritional effect of free radical pathology: 1986/A year in year in nutritional medicine, Keats Publishing Inc. New Cannon, CT, P: 16.

Dianzani, M.U., G. Muzio, M.E. Biocca and R.A. Canuto, 1991. Lipid peroxidation in fatty liver induced by caffeine in rat. Int. J. Tiss. Reac., 13: 79-85.

Diehi, A.M., Z. Goodmann and K.G. Ishak, 1988. Alcohol like liver disease in non-alcoholics; a clinical and histologic comparison with alcohol-induced Liver injury. Gastroenterology, 98: 1056-62.

Garfield, C.F., P.J. Chung and P.J. Rathouz, 2003. Alcohol advertising in magazines and adolescent readership. JAMA, 14 289(18): 2424-9.

Halliwell, B. and J. Gutteridge, 1984. Oxygen toxicity, oxygen-radicals Transition metals disease. J. Biochem., 219: 1-14.

Lecomte, F., B. Herberth, P. Pirrolet, Y. Chancerelle , J. Arnaud, N. Musse, F. Paille, G. Siest and Y. Artu,.1994. Effect of alcohol consumption in blood Antioxidant nutrients and oxidative stree indicators. Am. J. Clin. Nutr., 60: 255-61.

Levine, S.A. and P.M. Kidd, 1985. Antioxidant Adaptation. Its role in free Radical pathology. San Leandro, C.A: Biocurrents, 171-218.

Li, D. and S.L. Friedman, 1999. Liver fibrogenesis and the role of hepatic stellate cell; new insights and prospects for therapy. J. Gast. Hep., 14: 618-33.

Lieber, C.S., 1993. Biochamical factors in alcoholic liver disease. Semin Liver Dis., 13: 136-53.

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with folin phenol reagent. J. Bio. Chem., 193: 265-75.

Luna, L.G., 1966. Manual of histological staining. Methods of Armed Forces Institute of Pathology, London, 1-31.

Malloy, H.T., E.A. and Evelyn, 1937. The determination of bilirubin with the photoelectric colorimeter. J. Bio. Chem., 119: 481-5.

Normann, R., C. Ribiere and H. Rouach, 1992. Implication of free radical mechanism in ethanol induced cellular injury. Free Rad. Biol. Med., 12: 219-40.

Ohkawa, H. and N. Ohishi, 1979. Yashk Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction Anal Biochem., 95: 351-8.

Olaso, E. and S.L. Friendman, 1998. Liver fibrogenesis and hepatic fibrogenesis. J Hepatol., 29: 837-47.

Retiman, S. and A.S. Frankel, 1957. A Colorimetric method for the determination of serum glutamic oxaloacetic and glutay pyruvic transaminases. Am. J. Clin. Pathol. 28: 53-6.

Stohs, S.J., 1995. The role of free radical in toxicity and disease. J. Basic Clin. Physiol. Pharmcol., 6: 205-28.

Traber, M.G., 1999. Utilization of vitamin E. Biofactors., 10: 115-20.

Treno, A.J., J.W. Grube and S.E. Martin, 2003. Alcohol availability as a predictor of youth drinking and driving: a hierarchical analysis of survey and archival data. Alcohol Clin. Exp. Res., 27: 835-40.

Underwood, J.C.E., 1992. Text book of General and systematic pathology. 1st edition, UK: Churchill Livingstone, 390-401.

Vivek, K., K.K. Pillai, S.Z. Hussiah and D.K. Balani, 1994 Hepatoprotective activity Of “Jigrine” on liver damage caused by alcohol-carbon tetrachloride and paracetamol in rats. Ind. J. Pharmacol., 26: 35-40.

Voehringer, D.W., D.L. Hirschberg, J. Xiao, Q. Lu, M. Roederer, C.B. Lock and L.A. Herzenberg. 2000. Gene microarray identification of redox and mitochondrial element that control resitance or sensitivity to apoptosis. Proc. Nat. Acad. Sci., 97: 2680-85.

Zdeneka, P., L.L. Cushman and B.C. Johnson, 1966. Estimation of product of lipid peroxidation (MDA) in biochemical systems. Anal. Biochem., 16: 359-64.