

Effects of Chronic Ethanol Administration on Body Weight, Reduced Glutathione (GSH), Malondialdehyde (MDA) Levels and Glutathione-s-transferase Activity (GST) in Rats

Ighodaro Osasenaga Macdonald¹, Omole Johnson Olusola¹ and Uwaifo Anthony Osaigbovo^{1,2}

¹.Department of Biochemistry, Lead City University, Ibadan, Oyo State, +234, Nigeria

².Cancer research & molecular biology unit, Department of Biochemistry, University of Ibadan, Ibadan, Oyo State, +234, Nigeria. macigho@yahoo.com

Abstract: The etiology of some oxidative-stress based pathological conditions in the liver has implicated excessive alcohol consumption. The present study therefore, is directed at investigating the effect of ethanol (found in alcoholic drinks) on selected markers of oxidative stress/damage. The effect was assessed on the basis of comparative levels of MDA, GSH and GST in rats vis-a-vis experimental group treated with ethanol. Ten male albino rats of Wister strain, body weight range of (166 ó 170) grams were treated with 5g ethanol (20% v/v) per kg body weight per day for 28 days along side with ten male untreated rats. Both groups were fed normal feeds and water ad libitum. The administration of ethanol was done orally using oral catheter. Biochemical analysis at the end of the administration of ethanol at a dose of 5g/kg body weight, caused significant increase ($P < 0.05$) of the hepatic, renal and intestinal concentrations of malondialdehyde (MDA) by 109.96%, 84.42% and 37.60% respectively, compared with control. Similarly, the hepatic, renal and intestinal GST activities were significantly increased ($p < 0.05$) by 112.29%, 85.76% and 80.96% respectively. The levels of reduced glutathione (GSH) in the liver, kidney and intestine of ethanol-treated animals significantly decreased by 66.72%, 50.36% and 56.67% relative to the control group. A lower mean body weight gain was observed in rats treated with ethanol as compared with control. Overall, the results of the study suggest that alcohol in chronic doses induces oxidative stress in the rats with implication of dangerous effects on humans if consumed at chronic doses. [New York Science Journal. 2010;3(4):39-47]. (ISSN: 1554-0200).

Key words: Ethanol, malondialdehyde (MDA), reduced glutathione (GSH), glutathione-s-transferase (GST.) and rats

1. Introduction

Alcohol is widely consumed in alcoholic drinks all over the world. More than ever before, there is an upsurge in alcohol abuse and as a result, alcohol-related disorders are becoming increasingly important causes of morbidity and mortality globally (Rukkumani *et al.*, 2004). Several reports on the association between chronic alcohol consumption and variety of pathological conditions varying from simple intoxication to severe life-threatening pathological states have been published. (Tsukamoto and Lu, 2001; Griffon *et al.*, 2000; McDonough, 2003; Maher, 1997; Tuma, 2002; Pratt, 1992; Lieber, 2003; Gruchow *et al.*, 1985; Roy *et al.*, 1991; Korsten, 1989; Stephen *et al.*, 1996; Charness, 1993; & Molina *et al.* 2002). Alcohol is absorbed into the blood stream in the gastrointestinal tract (GIT) (Bode and Bode, 1997) and is primarily metabolized in the liver (Maher, 1997).

The molecule is metabolized via two pathways: The alcohol dehydrogenase (ADH) pathway and the microsomal ethanol oxidizing system (MEOS) pathway. Both pathways generate potentially dangerous by-products such as acetaldehyde and highly reactive molecules called free radicals or reactive oxygen species (ROS) which cause oxidative stress and are capable of attacking cell membranes and biomolecules (Lindros, 1995; Balkan, *et al.*, 2001). Oxidative stress occurs when the production of ROS exceeds the level the body's natural antioxidant defense mechanisms can cope with; causing damage to macromolecules such as DNA, proteins and lipids (Bartsch and Nair, 2000). This is often characterized by high level of malondialdehyde (MDA), increased GST activity and reduction of reduced GSH molecules.

High MDA level is a marker of lipid peroxidation which is a fallout of oxidative damage. Reduced

glutathione (GSH) is an endogenous antioxidant which plays a vital role in the detoxification of xenobiotics and scavenging of free radicals or reactive oxygen species (ROS) in cells (Albano et al., 1998). GST is a detoxifying enzyme that primarily functions in conjugating functionalized p450 metabolites with endogenous ligand (reduced

glutathione) (Hartman *et al.*1990). In this study therefore, the effects of ethanol on the levels of malondialdehyde (MDA) and reduced glutathione (GSH), as well as GST activity in the liver, kidney and intestine of rats have been investigated and the results obtained evaluated with respect to possible implication for humans

2. Materials and Methods

Twenty male albino rats of the Wister strain were used for the experiment. They were purchased from the Institute for Advance Medical Research and Training (IMRAT), at the University College Hospital (UCH), Ibadan. The animals were handled humanely, kept in a plastic suspended cage placed in a well ventilated and hygienic rat house under suitable conditions of temperature and humidity. They were provided rat pellets and served water ad libitum and subjected to natural photoperiod of 12h light and 12h dark cycle. The rats attained a body weight range of 166g to 170g before being used for this study. The animals were randomly assigned into two (2) groups of ten (10) rats each.

Group A (Control): Rats fed with rat pellets and water only ad libitum for 28 days.

Group B (ET): Ethanol treated rats in a dose of 5g per kg body weight per day for 28 days along with rat pellets and water ad libitum.

2.1. Tissue Preparation for Biochemical Analysis

The animals (Control and ethanol-treated) were fasted overnight, weighed and sacrificed by cervical dislocation 24h after the last treatment and target organs (liver, intestine and kidney) were quickly excised from each rat. Each organ was separately hywashed in ice-cold 1.15% KCl solution, blotted and weighed. Each organ from different rat was separately homogenized in a volume of the homogenizing buffer (ice-cold Tris-Hcl buffer, 0.1M, pH 7.4) four times its weight, using a potter Elvehjem type homogenizer. The resulting homogenate in each case was centrifuged at 10,000g for 30 minutes in a

Beckman L5-50B ultra centrifuge with a 220.78 V02 rotor at 4°C. The resultant supernatant was collected and used for different biochemical analysis. Storage was done between 0°C to 4°C to preserve enzyme activity.

2.2. Estimation of Reduced Glutathione

The estimation of the reduced glutathione (GSH) in the different isolated organs were carried out using the method of Jollow *et al.*(1974). Non-Protein GSH was estimated by a calorimetric method using Ellman's reagent as described by Gunnet and Phillips (1951), and modified by Ellman (1951). The reduced form of glutathione comprise in most instance, bulk of cellular non-protein sulfohydryl group. This method is therefore based upon the development of a relatively stable yellow colour when 5,5-dithiobis (2-nitrobenzoic acid), Ellman reagent is added to sulfohydryl compound. The absorbance was read at 412nm and the equivalent GSH was estimated from the standard GSH curve supplied in the kits.

2.3. Estimation of Glutathione-s-transferase Activity

The glutathione-s-transferase activity was estimated spectrophotometrically at 37 °C according to the procedure of Habig et al., (1974). The method involves the production of a complex formed from the enzymatic conjugation of reduced glutathione with the aromatic substrate, 1-chloro-2,4 nitrobenzene. The complex formed has a characteristic absorption at 340nm. The spectrophotometric readings are considered indices of enzyme activity. The specific activity of glutathione-s-transferase is expressed as $\mu\text{mole of GSH} = \text{CDNB}$

conjugate formed (1ml/mg protein), using an extinction coefficient of 9.6mm cm^{-1}

GST activity ($\mu\text{g}/\text{min}/\text{mg}$ protein) = $\text{O.D.}/\text{min}^{-1}/9.6 \times \text{T.V}/\text{mg}/0.03\text{ml}$ protein. Where T.V = total reaction mixture volume, O.D = Optical density. Protein estimation was carried out using biurette method.

2.4. Estimation of Lipid Peroxidation (LPO)

Lipid peroxidation in microsomes prepared from the different organs was estimated spectrophotometrically using Thiobarbituric acid-reactive substances (TBARS) method as described by Varshney and Kale (1990), and is expressed in terms of malondialdehyde (MDA) per mg protein. This method is based on the reaction between 2-Thiobarbituric acid (TBA) and malondialdehyde (MDA), an end product of lipid peroxides during lipid peroxidation (LPO). On heating in acidic pH, the product forms a pink complex which absorbs maximally at 532nm and fluoresces at 533nm. It is readily extractable into organic solvents such as butan-1-ol. This test is often Calibrated using MDA as the standard and thus, the results are expressed as the amount of free MDA produced.

The malondiadehyde (MDA) level of the sample was estimated from the absorbance using an extinction coefficient of $1.5 \times 10^5 \text{ m}^{-1} \text{ Cm}^{-1}$ Adam óvizi and sergi, 1982).

$\text{MDA (Molg}^{-1} \text{ tissue)} = \text{Absorbance}/ E_{532} \times V_s \times \text{g Tissue}$

Where T.V = Total volume of reaction mixture, V_s = Volume of sample. g = gram. E_{532} = Molar extinction coefficient

Statistical Analysis

The results obtained in this study are presented with descriptive statistics. In order to establish statistical differences or absence thereof between the mean

The levels of MDA, a marker of lipid peroxidation, in the target organs: liver, kidney and intestine increased significantly ($p < 0.05$) by 109.96%, 84.42% and 37.60% respectively, in rats treated with ethanol compared to control (Table 1, Fig.1). In like manner the hepatic, renal and intestinal GST activities were significantly increased ($p < 0.05$)

values of BW; GSH; MDA and GST of the ET and control rats, the data obtained were subjected to student's t-test, Pearson's correlation and Levene's test for equality of variance (SAS1988). A p-value of $p < 0.05$ was used as indicator of statistical significance.

3. Results and Discussion

The mean percentage increase in body weights of control and ethanol rats are presented in Table 4. The body weights of treated rats did not show significant changes (Table 4). There was a significant decrease ($p < 0.05$) in the mean body weight gain of rats treated with ethanol as compared with the control group (Table 4). Comparison between the initial and final weights of rats in each group showed that rats in the control group had a mean % increase of 27.24% in body weight while rats treated with 5g per Kg body weight of ethanol (20% v/v) only increased in body weight by a mean value of 3.48% (Table 4). This observation agrees with earlier reports on the effects of alcohol on digestion, absorption, storage, utilization and excretion of essential nutrients such as vitamins, minerals and proteins. (Gruchow *et al* 1985, Lieber, 2003). Alcohol impairs nutrient absorption by damaging cells lining the stomach and intestines, and disabling transport of some nutrients into the blood (Feinman, 1998). Alcohol also inhibits the breakdown of nutrients into usable substances, by decreasing the secretion of digestive enzymes from the pancreas (Korsten, 1989). Moreover, Pratt and Thomson (1992) reported that excessive alcohol intake can impair the utilization of nutrients by altering their storage and excretion. In summary, the relative decrease in mean body-weight gain (23.76%) recorded in ethanol treated rats may be adduced to malnutrition resulting from reduced absorption of nutrients from the intestine of treated rats

by 112.29%, 85.76% and 80.96% respectively (Table 3, Fig.3). The amount of reduced glutathione (GSH) in the liver, kidney and intestine of ethanol-treated animals significantly decreased by 66.72%, 50.36% and 56.67% relatively to the control group (Table 2, Fig. 2). A number of systems that generate reactive aldehydic species and reactive oxygen species are

activated by chronic consumption of alcohol (Maher, 1997). This observation is consistent with the results

The increase in the level of MDA observed in the various target organs could be linked to the generation of free radicals, resulting in the peroxidation of membrane lipids. Moreover, the main pathway for alcohol metabolism involves the enzyme alcohol dehydrogenase (ADH) (Maher, 1997; Pronkro *et al.*, 2001); which metabolizes alcohol into toxic acetaldehyde, whose interaction with cell proteins and lipids can result in free radical generation and cellular damage

According to Albano *et al.*, (1998) and Cederbaum, (2001), chronic alcohol consumption does not only activate free radical generation, but also alters the levels of both enzymatic and non-enzymatic endogenous antioxidant systems. This results in oxidative stress with cascade of effects, thus, affecting both the functional and structural integrity of cells and organelles membranes (De level *et al.*, 1996). This is exemplified by the significant decrease in reduced glutathion (GSH) level, and concomitant increase in hepatic GST activity observed in the present study. Furthermore, the obtained results agree with the findings of

of the present study.

Hussain *et al.*, (2001) and Molina *et al.*, (2003) who reported that chronic ethanol treatment caused a significant reduction in hepatic GSH level and increase in GST activity.

GSH plays a significant role in the detoxification of xenobiotics and maintenance of the redox status of the cells (Sen, 1997). A decline in cellular level has been considered to be indicative of oxidative stress. The decrease of this endogenous antioxidant is obviously connected with ethanol-induced oxidative stress, which is characterized by the generation of toxic acetadehyde and other reactive molecules in the cell. The observed increase in GST activity is likely a defensive response to detoxify the toxic metabolites produced in the course of ethanol metabolism. For all the parameters analyzed, the liver seems to be the most affected organ (Figs. 1, 2 & 3), perhaps, because it is the primarily site of ethanol metabolism. It should however be noted that response of the body cells to a particular toxin is determined by a variety of factors such as dose administration, age, sex, species, strain, physiological and nutritional status of the animal.

Table 1. Malondialdehyde (MDA) concentration (molg⁻¹tissue) in ethanol treated and control rats

Group	Hepatic MDA	Renal MDA	Intestinal MDA
Control	51.36 ± 4.09	11.08 ± 1.96	19.39 ± 2.21
ET	107.39 ± 12.11*	15.25 ± 1.45	35.76 ± 3.33*

Values are expressed as mean ± S.D for ten animals per group

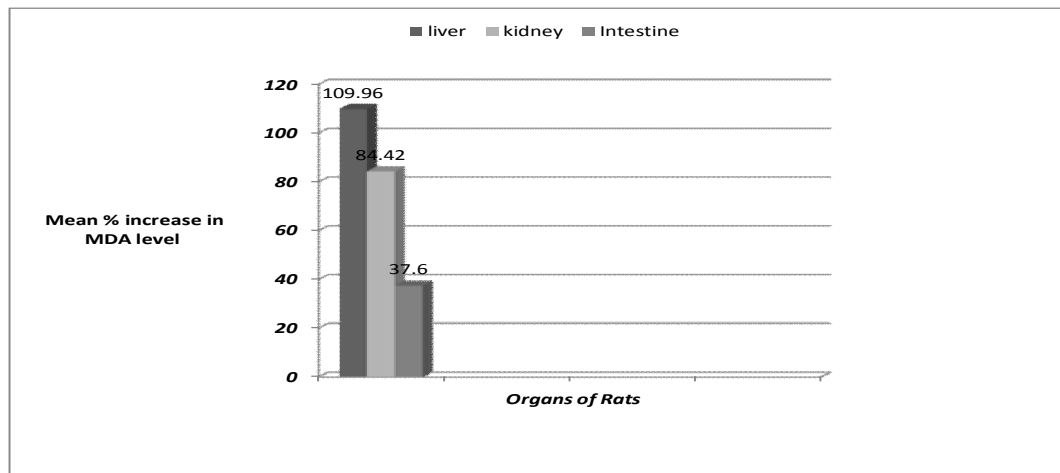


Fig.1: Effect of Ethanol (5g/kg bwt, 20% w/v) on MDA conc. in Liver, Kidney & Intestine of Rats after 28 days

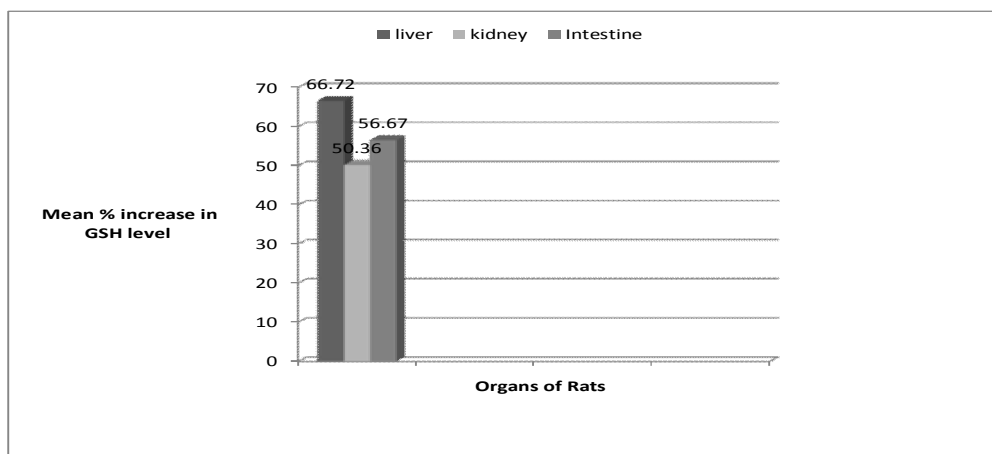


Figure 2. Effect of Ethanol (5g/kg bwt, 20% v/v) on GSH levels. in Liver, Kidney & Intestine of Rats after 28 days

Table 2: Level of reduced GSH ($\mu\text{g/g}$ tissue) in ethanol treated and control rats

Group	Hepatic GSH level	Renal GSH level	Intestinal GSH level
Control	12.38 ± 0.83	6.91 ± 1.81	7.43 ± 1.56
ET	$4.12 \pm 0.31^*$	$3.43 \pm 0.56^*$	$3.22 \pm 1.04^*$

Values are expressed as mean \pm S.D for ten animals per group. * $p < 0.05$ against control

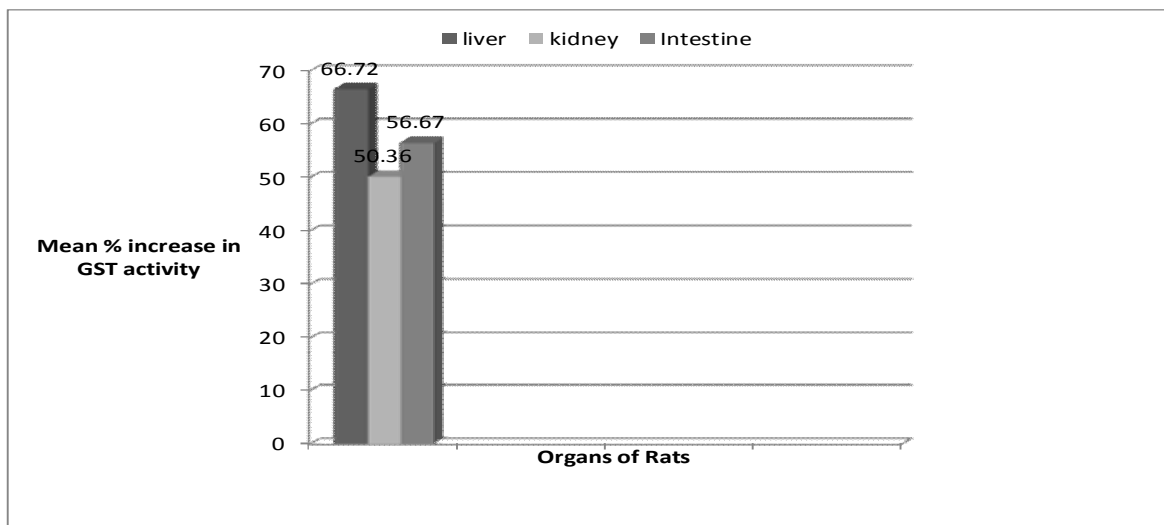


Figure.3: Effect of Ethanol (5g/kg bwt.) on GST activity in Liver, Kidney & Intestine of Rats after 28 days

Table 3. GST activity ($\mu\text{g}/\text{min}/\text{mg}$ protein) in ethanol treated and control rats

Group	Hepatic GST activity	Renal GST activity	Intestinal GST activity
Control	3.01 ± 0.43	2.95 ± 0.34	2.89 ± 0.31
ET	$6.36 \pm 0.19^*$	$5.48 \pm 0.21^*$	$5.23 \pm 0.28^*$

Values are expressed as mean \pm S.D for ten animals per group. * $p < 0.05$ against control

Table 4. Body weight (BW) and change in BW of control and ET rats before and after 28 days

Group	Initial weight (g)	Final weight (g)	Percentage Increase in weight (%)
Control	169.6 ± 4.02	215.8 ± 7.09	27.24
ET	166.9 ± 5.12	172.7 ± 6.08	3.48 *

Values are expressed as mean ± S.D for ten animals per group. * =significant difference * p<0.05 against control

Acknowledgement

The authors gratefully acknowledge the secretariat work of Miss Agness Ademola and the technical assistance of Mr Adelani Solomon

Correspondence to: Ighodaro O.M. Department of Biochemistry, Lead City University, Ibadan, Oyo State, +234, Nigeria. E-mail: macigho@yahoo.com
Tel: +2347031833938, +234823658692

References

Albano, E., French, S. and Ingelman-Sundberg, M. Cytochrome p450 2E1, hydroxyl ethyl radicals, and immune reaction associated alcoholic liver injury. *Alcoholism: Clinical and Experimental Research*. 1994.18:1057-1068

Balkan, J., Dogru-Abbasoglu, S., Kanbagli, O., Cevkbp, U., Aykac-Toker, G., Uysal, M. Taurine has a protective effect against thio-acetamide induced liver cirrhosis by decreasing oxidative stress. *Hum Exp Toxicol*. 2001. 20: 251-254

Bartsch, H., Nair, J. Ultrasensitive and specific detection methods for exocytic DNA adducts; markers for lipid peroxidation and oxidative stress. *Toxicology* .2000. 153: 105-114.

Bode, C., Bode, J.C. Alcohol's role in gastrointestinal Tract disorders. *Alcohol Health & Research World*. 1997. 21: 76-83

Charness, M.E. Brain lesions in alcoholics. *Alcoholism: Clinical and Experimental Research*. 1993. 17 : 2-11

Clot, P., Tabone, M., Arico, S. and Albano, E. Monitoring oxidative damage in patients with liver cirrhosis and different daily alcohol intake. *Gut*. 1994. 35:1637-1643

De Leve, L.D., Wang, X., Kuhlenkamp, J.F., Kaplowitz, N. Toxicity of azathioprine and monocrotaline in murine sinusoidal endothelial cells and hepatocytes: the role of glutathione and relevance to hepatic veno-occlusive disease: *Hepatology*. 1996. 23: 589-599

Feinman, L. and Lieber, C.S. Nutrition and diet in alcoholism. In: Shills, M.E., Oslon, J.A., Shike, M. and Ross, A.C; eds. *Modern Nutrition in Health and disease*. 9th ed. Baltimore: Williams and Wilkins. 1998. Pp1523-1542

Griffon, B., Cillard, J., Chevanne, M., Morel, I., Cillard, P. and Sergent, O. Activated macrophages increase the susceptibility of rats hepatocytes to induced oxidative-stress: Conflicting effects of nitric oxide. *Alcohol and Alcoholism*. 2000. 230-235

Gruchow, H.W., Sobocinski, K.A., Barboriak, J.J. Alcohol consumption, nutrient intake and relative body weight among US adults. *American Journal of Clinical Nutrition*. 1985. 42: 289-295

Habig, W.H., Pabst, M.J., Jacoby, W.B. Glutathion-s-transferase. The first step in mercapturic acid formation. *J. Bio chem*. 1974. 249: 7130-7139

Hartman, P.E. and Shankel, D.W. Antimutagens and Anticarcinogens a survey of putative interceptor molecules: *Environ. Mol Mutat Res*. 1990. 202: 285-306

- Hussain, k., Scott, B.R., Reddy, S.K. and Somani, S. *Alcohol* 2001. 25: 89-97
- Jollow, D.J., Michell, J.R., Zampaglionic and Gillete, J.R. Bromobenzene-induced Liver necrosis: Protective role of glutathione and evidence for 3,4-Bromobenzene oxide as hepatotoxic metabolite. *Pharmacology*. 1974. 11: 151-169
- Korsten, M.A. Alcoholism and Pancreatitis: Does nutrition play a role? *Alcohol Health & Research World*. 1989. 13 : 232-237.
- Lieber, C.S. Relationships between Nutrition, Alcohol Use, and Liver disease. *Alcohol Health & Research World*. 2003. 27 : 220-231.
- Lindros, K.O. Alcoholic liver disease: Pathobiological aspect. *J. hepatothology*, 1995. 23: 7-15
- Maher, J.J. Exploring Alcohol effects on Liver Functions. *Alcohol Health & Research World*. 1997. 21 : 5-12.
- McDonough, K.H. Antioxidant nutrients and alcohol. *Toxicology*. 2003. 189: 89-97
- Molina, P., Mclain, C., Villa, D. Molecular pathology and Clinical aspects of alcohol-induced tissue injury. *Alcoholism: Clinical and Experimental Research* 2003. 26 :120-128.
- Pronko, P., Bardina, L., Satanovskaya, V., Kuzmieh, A .and Zitmakinalcohol ,S.Effects of chronic alcohol consumption on ethanol and acetaldehyde metabolizing systems in rats gastrointestinal tract. *Alcohol and Alcoholism*. 2002 37 : 229-235
- Roy, A., DeJong, J., Lamparski, D., George, T. and LInnoila, M. Depression among alcoholics. *Archives of General psychiatry*. 1991.48 : 39-45
- Rukkumani, R., Aruna, K., Suresh Varma, P. and Menon, V.P. Influence of Ferulic acid on Circulatory Peroxidant-Antioxidant status during Alcohol and PUFA- induced toxicity. *Journal of physiology and pharmacology* 2004. 55 : 551-561
- Stephen, C; Roger, R. and Sandra, C. Sudden death due to metronidazole/Ethanol interaction. *American Journal of forensic medicine & pathology* 1996.17 : 343-346
- Tsukamoto, H. and Lu, C.S. Current concepts in the pathogenesis of alcoholic liver injury. *The FASEB Journal*. 2001. 15: 1335-1349
- Tuma, D.J and Casey, C.A. dangerous by-products of alcohol breakdown-focus on adduct *Alcohol Health & Research World*. 27:285-290.
- Vashney,R. and Kale, R.K. Effects of calmodulin Antagoniss. *Int. J Rad.Biol*.1990. 58:733-743

12/24/2009