ORIGINAL RESEARCH PAPER

Volume-7 | Issue-11 | November-2018 | PRINT ISSN No 2277 - 8179

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

THE ROLE OF OXIDATIVE STRESS AND ANTIOXIDATIVE STATUS AS SCREENING TEST FOR ALCOHOLIC LIVER DISEASE

Biochemistry

Nisha Chauhan* Demonstrator, Department of Biochemistry, Faculty of Medicine and Health Sciences, SGT University, Gurugram, Haryana *Corresponding Author

Dr Busi Karunanand Professor and Head, Department of Biochemistry, Faculty of Medicine and Health Sciences, SGT University, Gurugram, Haryana.

Dr D K Sharma Professor & Head, Department of Medicine, Faculty of Medicine and Health Sciences, SGT University, Gurugram, Haryana

Dr Sanjiv Kumar Bansal Professor, Department of Biochemistry, Faculty of Medicine and Health Sciences, SGT University, Gurugram, Haryana

Dr Arpita Suri Assistant Professor, Department of Biochemistry, Faculty of Medicine and Health Sciences, SGT University, Gurugram, Haryana

Dr Shikhaa Mahajan Associate Professor, Department of Biochemistry, Faculty of Medicine and Health Sciences, SGT University, Gurugram, Haryana

ABSTRACT

Background- The prevalence of Alcoholic Liver Disease (ALD) has increased in the developing countries including India. Alcohol consumption is associated with production of reactive oxygen species, lower cellular antioxidant levels and enhance oxidative stress in many tissues especially the liver. Therefore our study focusses on markers of oxidative stress which may have a role in etiopathogenesis of ALD and also their correlation with conventional markers of liver functioning.

Material and methods- 50 patients of alcohol liver disease, fulfilling the inclusion criteria, were enrolled for the study after taking written and informed consent as cases and 50 age and gender matched healthy individuals as controls from general population. Serum AST (SGOT), ALT (SGPT), ALP and Total protein were estimated by enzymatic methods on auto-analyzer EM-200(erba) and Plasma Superoxide dismutase (SOD), Malondialdehyde (MDA) were measured by ELISA.

Results- The mean age of the study population was found to be 53.2 ± 3.81 (cases) and 52.92 ± 2.73 (controls).The levels of AST,ALT,AST/ALT ratio, ALP, Malondialdehyde, Superoxide dismutase were statistically higher whereas the levels of serum total proteins were statistically lower in cases as compared to controls.The Pearson correlation between serum Total Proteins and Malondialdehyde was found to be statistically significant(r=0.445).

Conclusions- According to our study, excessive alcohol intake increases the hepatic oxidative stress. The measurement of these markers of antioxidant depletion (GSH) and hepatic damage (MDA) may be used as screening test for risk assessment in ALD patients.

KEYWORDS

INTRODUCTION

The excessive consumption of alcohol is an important cause of mortality and morbidity worldwide. In the recent years,alcohol abuse has increased exponentially in the developing countries.Although alcohol can damage several organs, alcoholic liver disease is the most common medical consequence of excessive alcohol intake.[1]

The pathogenesis of alcoholic liver disease (ALD) is a consequence of chronic alcohol abuse and approximately 44% of the 26,000 deaths from cirrhosis are due to ALD in the United States[3]. Alcohol consumption and consequently ALD is less prevalent in India in comparison to other countries, but in the last few years, the ALD was shown downward trend in the developed countries but the prevalence has increased in the developing countries including India.[4]

The spectrum of the ALD ranges from a simple steatosis to frank cirrhosis. Alcoholic hepatitis, the clinical presentation of ALD, remains to be a common life threatening cause of liver failure, especially when it is severe. Chronic alcohol consumption has long been associated with progressive liver disease from steatosis to inflammation, development of hepatic cirrhosis, and the subsequent increased risk of hepatocellular carcinoma. The exact amount of alcohol consumption which places, a person at risk of developing ALD is not known but most of patients of ALD give history of drinking more than 100 gm per day of alcohol which corresponds to 6-7 drinks per day.

Alcohol consumption is associated with the production of reactive oxygen species, lower cellular antioxidant levels and enhance oxidative stress in many tissues especially the liver.[2] Liver is a major organ attacked by ROS.[7] Parenchymal cells are primary cells subjected to oxidative stress induced injury in the liver. The mitochondrion, microsomes and peroxisomes in parenchymal cells can produce ROS, regulating peroxisome proliferator-activated receptor (PPAR), which is mainly related to the liver fatty acid oxidation gene expression. Cytokines like TNF-α can be produced in Kupffer cells induced by oxidative stress causing inflammation and apoptosis. The oxidative stress not only triggers hepatic damage by inducing irreversible alteration of lipids, proteins and DNA contents but also modulate pathways regulating genes transcription, protein expression, cell apoptosis, and hepatic stellate cell activation.

Protective actions against ROS are performed by several enzymes eg. Superoxide dismutase (SOD), catalase and glutathione peroxidase as well as non-enzymatic compounds eg. Tocopherol, β-carotene, ascorbate and glutathione (GSH).When the capacity of this antioxidant system decreases the level of ROS rises.[11]

SOD is very important enzyme that functions as a cellular antioxidant. It has many isoenzymes in different organelles such as copper, zinc SOD in cytoplasm and manganese SOD in mitochondria.This enzyme binds with superoxide radical and by removing an electron from the superoxide molecules converts it into less damaging species.[11]

Malondialdehyde (MDA) is a reactive dialdehyde formed from the non-enzymatic lipid peroxidation of unsaturated fatty acids during phagocytosis and from arachidonic acid catabolism in thrombocytes. MDA adducts have been found in the liver of people consuming alcohol.

Inflamed or injured liver cells leak high amounts of liver enzymes, into the blood stream, which can result in elevated serum liver enzymes. The liver enzymes most commonly found are alanine transaminase (ALT), aspartate transaminase (AST).Liver enzymes testing includes ALT, AST, ALP, true liver function tests which includes PT, INR,

albumin and bilirubin. ALT is almost exclusively found in the liver and AST enzyme is also found in muscles and many other tissues besides the liver.

MATERIALS AND METHODS

The present study was carried out in the Department of Biochemistry and Department of Medicine of SGT Hospital associated with SGT Medical College, Hospital & Research Institute, Budhera, Gurgaon. A total of 50 patients of alcohol liver disease fulfilling the inclusion criteria were enrolled for the study after informed consent and 50 healthy age and gender matched controls from general population. Patients aged >20years and <60years suffering from alcoholic liver disease undergoing treatment in the outpatient department (OPD) of Medicine Department, SGT Hospital, Gurugram, were taken as cases and were selected based on established and accepted clinical and biochemical criteria. Detailed history, clinical examination and laboratory investigations were done.TheCriteria for diagnosis of Alcoholic Liver Disease includedHistory of physical examination and lab tests, Amount of alcohol intake > 100gm/day [12], Duration of alcohol intake more than 10 years, USG of whole abdomen, AST level is raised as compare to ALT (2-6 times).Patients suffering from concomitant systemic disorders such as kidney disease, heart disease or endocrinal disorders and Patients on hormonal replacement therapy (oral pills) or steroids were excluded.6ml of venous blood after an overnight fast of 10-12 hours was for both case and controls. 3ml of blood was collected in plain vial and serum thus obtained was separated for the estimation of liver enzymes and total protein. 3ml of blood was collected in EDTA vial and plasma was separatedby centrifuging the blood at 3000 rpm for 10 minutes for the estimation of MDA,SOD. The following parameters were analyzed in serum by auto-analyzer EM-200(erba)-AST (SGOT), ALT (SGPT), ALPandTotal protein.Superoxide dismutase (SOD) and Malondialdehyde (MDA) were estimated by commercially provided kits by BIOVENDER R & D based on ELISA method.

STATISTICALANALYSIS

Data collected was entered in Microsoft Excel and analyzed using SPSS software version 21.The differences in the mean values of the parameters in the two groups was determined by using the Student's 't'-test for the unpaired data, the difference was considered as significant at a p value of <0.05. Correlation between different parameters was calculated was using Pearson correlation coefficient and its significance was analyzed using p value.

RESULTS

The mean age of the study population was found to be 53.2 ± 3.81 in cases and 52.92 ± 2.73 in controls. (Table 1)

TABLE 1: Age Distribution Of The Population

The levels of AST,ALT,AST/ALT ratio, ALP, Malondialdehyde, Superoxide dismutase were higher in cases whereas the levels of serum total proteins were higher in controls (Table 2)

Study subjects		N	Mean	Std. Deviation	Std. Error Mean	p value
Age	Cases	50	53.2000	3.81725	0.53984	.000
	Control	50	52.9200	2.73182	0.38634	.000

TABLE 2: Levels of AST,ALT,AST/ALT Ratio, ALP, Total Plasma Proteins,Malondialdehyde, Superoxide dismutase, Glutathione Peroxidase in study subjects

AST(IU/L)	Cases	50	62.8400	6.90271	0.97619	.000
	Control	50	18.2400	3.88382	0.54926	.000
ALT(IU/L)	Cases	50	35.9800	6.01186	0.85021	.000
	Control	50	21.3000	4.72186	0.66777	.000
AST/ALT Ratio	Cases	50	1.7870	0.31593	0.04468	.000
	Control	50	0.8688	0.15038	0.02127	.000
ALP(IU/L)	Cases	50	30.9800	4.37288	0.61842	.000
	Control	50	4.5232	1.38840	0.19635	.000
Total Proteins (gm/dl)	Cases	50	5.3608	.15529	0.02196	.000
	Control	50	7.3666	.15228	0.02154	.000
Malondialdehyde(mmol/L)	Cases	50	9.7970	.36982	0.05230	.000
	Control	50	3.8954	.45861	0.06486	.000
Superoxide dismutase(U/gmHb)	Cases	50	11.0140	.58554	0.08281	.000
	Control	50	5.7048	.33309	0.04711	.000

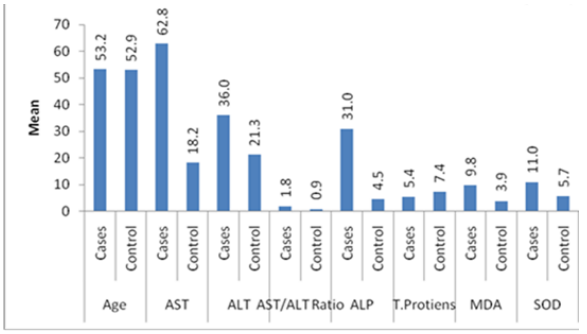

FIGURE 1: Age distribution and mean levels of AST,ALT,AST/ALT Ratio, ALP, T.Proteins, MDA, SOD in study subjects

MDA had strongest correlation(r=0.445) with total proteins shown in Table 3 and Figure 2 whereas SOD had the strongest correlation with AST/ALT and ALP(r=0.156) as compared to other parameters as shown in Table 4.

TABLE 3: Pearson correlation coefficients® between MDA and different parameters

Parameter	Pearson correlation coefficients(r) between MDA and different parameters
AST	.039
ALT	-.024
AST/ALT	.099
ALP	-.024
Total Protein	.445**

TABLE 4: Pearson correlation coefficients® between SOD and different parameters

Parameter	Pearson correlation coefficients(r) between SOD and different parameters
AST	.007
ALT	-.155
AST/ALT	.156
ALP	.156
Total Protein	-.079

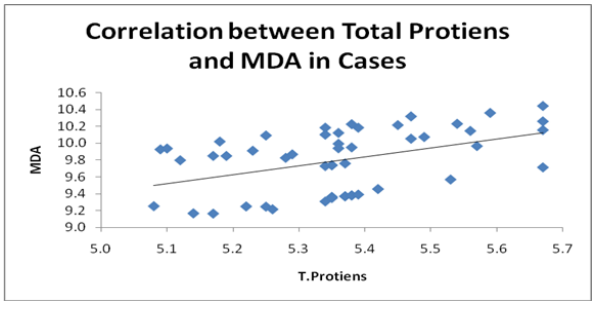

Figure 2: Scatter plot depicting correlation between total proteins and MDA.

DISCUSSION

Ethanol undergoes oxidative metabolism in the cytosol, peroxisome and microsomes. Liver injury due to acute or chronic abuse has been proved dependent on metabolic products of ethanol viz. acetaldehyde and ROS for the various functional derangements[48]. Induction of cytochrome P450 2E1 (CYP450 2E1) by ethanol increases the generation of ROS which initiates the oxidative stress. This is also potentiated by redox shift associated with ethanol oxidation by alcohol dehydrogenase. Acetaldehyde, a major metabolic product of ethanol by either alcohol dehydrogenase (ADH) or CYP450 2E1 catalyzed oxidation, promotes oxidative stress not only via consumption and inactivation of antioxidants but also by increased generation of free radicals[53].

In the present study, raised levels of serum transaminases observed in the present study may be due to increased permeability of cell membrane following the oxidative damage due to factors mentioned above. The ratio of AST/ALT (De Ritis ratio) was higher in cases (1.79) as compared to controls (0.87). The reversal of ratio may be because of

release of mitochondrial AST by alcohol itself or through its toxicity by its metabolites and/or oxidative stress[1]. In the present study there was significant increase in the levels of ALP in patients with ALD whereas the level of proteins in ALD patients was decreased. In 2006, Majhi S et al[53]also concluded th De Ritis ratio elevated in patients of ALD as compared to healthy control. The findings were similar to a study conducted on 20 male patients of ALD by Seema Gupta et al[1]who also reported reversal of AST/ALT ratio in ALD however both healthy smokers and non smokers were taken as control in this study.

The erythrocyte antioxidant enzymes i.e. Superoxide dismutase (SOD)and Malondialdehyde(MDA) activities have been increased significantly in ALD patients. Many authors have come across equivocal or controversial results regarding activities of these enzymes in alcoholics. Both increased and decreased SOD activities in alcoholics have been reported. [44,49,50] The increase in erythrocyte SOD activity may probable be an adaptive response towards oxidative stress. Our finding regarding SOD activity is supported by the work of DM Vasudevan and Subirkumar Das.[51]The probable reason of increase in SOD activity in our study may be that, in alcohol induced oxidative stress; there may be upregulation of enzyme activity due to oxidative stress. Evidence is accumulating that intermediates of oxygen reduction may in fact be associated with the development of alcoholic liver disease. The significant increase in MDA levels in alcoholics compared to controls suggests that alcoholics are subjected to more oxidative stress. In 2012 Ashok shinde were found that the level of MDA is increased in the patients of alcoholic liver disease[2]. Similar findings were observed by S Gupta of their study in cases of ALD where MDA is significantly increased in cases as compare to controls which had both healthy smoking and non smoking males[1]. The present study clearly demonstrates that due to alcohol induced oxidative stress the anti-oxidant defense system is compromised. It is reasonable to suggest that apart from the standard medical care for these patients, antioxidant supplement should form a part of the physician's prescription which will help in lowering the oxidative stress. It is expected that in future a more rational treatment plan for the patients with ALD can be devised.

CONCLUSIONS

According to the present study, excessive alcohol intake increases the hepatic oxidative stress. The use of the biological markers of hepatic damage (MDA) and antioxidant status (SOD) may be of help for the risk assessment in ALD. A better understanding of the pathophysiological mechanism and the correlation between the alcohol intake, biological markers and oxidative stress will help in better management of ALD.

The study needs further exploration taking more number of subjects to substantiate the results and arrive at a definite conclusion which would throw more light on oxidative stress & antioxidative status in patients with ALD.

REFERENCES

1. Gupta S, Pandey R, Katyal R, Agarwal HK, Agarwal SK. Lipid peroxide and antioxidant status in alcoholic liver disease. Ind J Clin Biochem 2005; 20(1): 67-71.
2. Shinde A , Ganu J , Naik P, et al. Oxidative stress and antioxidative status in patients with alcoholic liver disease. Biomedical Research 2012; 23 (1): 105-108.
3. M. Amini and B. A. Runyon, "Alcoholic hepatitis 2010: a clinician's guide to diagnosis and therapy," World Journal ofGastroenterology, vol. 16, no. 39, pp. 4905–12, 2010.
4. Basra S, Anand S B. definition, epidemiology and magnitude of alcoholic hepatitis. World journal of hepatology, 2011; 3(5): 108-13
5. Ray S. Alcoholic liver disease. Text book of hepatogasteroentrology medicine update 2007; 17(71).
6. Liber CS. Biochemical and molecular basis of alcohol induced injury to liver and other tissues. N Eng J Med 1988; 319:1639-50.
7. Sanchez-Valle, V., Chavez-Tapia, N.C., Uribe, M., Mendez-Sanchez, N. Role of oxidative stress and molecular changes in liver fibrosis: A review. Curr. Med. Chem. 2012; 19: 4850–60.
8. Feng, Y.,Wang, N., Ye, X., Li, H., Feng, Y., Cheung, F., Nagamatsu, T. Hepatoprotectiveeffect and its possible mechanism of Coptidis rhizoma aqueous extract on carbon tetrachloride-induced chronic liver hepatotoxicity in rats. J. Ethnopharmacol. 2011; 138: 683–90.
9. Singal, A.K., Jampana, S.C., Weinman, S.A. Antioxidants as therapeutic agents for liver disease. Liver Int. 2011; 31: 1432–48.
10. Medina, J.; Moreno-Otero, R. Pathophysiological basis for antioxidant therapy in chronic liver disease. Drugs 2005; 65: 2445–61.
11. Balkrishnan V, Mukherjee S, Vasudevan DM et al. Evaluation of blood oxidative stress related parameters in alcoholic liver disease and non alcoholic fatty liver disease. Scan J Clin Lab Invest 2008; 68(4): 323-34
12. Mandayam S, Jamal MM, Morgan TR. Epidemiology of alcoholic liver diseases. Semin Liver Dis 2004; 24: 217-32.
13. Cohen JA, Kaplan MM. The SGOT/SGPT ratio-an indicator of alcoholic liver disease. Dig Dis Sci 1979; 24: 835-38.
14. Black JM, Hawks JH, Medical-Surgical Nursing: Clinical Management for Positive Outcomes. 8th ed. St Louis, Mo: Saunders, 2005
15. O'Shea RS, Dasarathy S, McCullough AJ & Practice Guideline Committee of the AASLD & Practice Parameters Committee of ACG. AASLD practice guidelines-Alcoholic Liver Disease. AASLD. 2010
16. Sherlock S, Dooley J, Nemoci jater a žlučových cest (Disease of the liver and biliary system). Czech ed., Nadační fond ČHS, Hradec Králové, 2004.
17. Ishak KG, Zimmerman HJ, Ray MB, Alcoholic liver disease: pathologic, pathogenetic and clinical aspects. Alcohol Clin and Expl Res. 1991; 15: 45- 66.
18. Stickel F, Schuppan D, Hahn EG, Seitz HK, Cocarcinogenic effects of alcohol in hepatocarcinogenesis. Gut 2002; 51: 132- 39
19. Teli MR, Day CP, Burt AD, Bennett MK, James OF. Determinants of progression to cirrhosis or fibrosis in pure alcoholic fatty liver. Lancet 1995; 346: 987- 90.
20. Grant BF, Dufour MC, Harford TC, Epidemiology of alcoholic liver disease. Semin Liver Dis. 1988; 8: 12-25.
21. Chick J, Erickson CK, Conference summary: Consensus Conference on Alcohol Dependence and the Role of Pharmacotherapy in its Treatment. Alcohol Clin Exp Res.1996; 20: 391- 402.
22. Savolainen VT, Liesto K, Mannikko A, Penttila A, Karhunen PJ, Alcohol consumption and alcoholic liver disease: evidence of a threshold level of effects of ethanol. Alcohol Clin Exp Res. 1993; 17: 1112-17
23. Houglum K, Alcohol and iron: a radical combination? Hepatology 1996; 23: 1700-703.
24. Yin M, Wheeler MD, Kono H, Bradford BU, Gallucci RM, Luster MI, Essential role of tumor necrosis factor alpha in alcohol-induced liver injury in mice. Gastroenterology 1999; 117: 942-52.
25. Cunningham CC, Coleman WB, Spach PI, The effects of chronic ethanol consumption on hepatic mitochondrial energy metabolism. Alcohol Alcohol 1990; 25: 127-36
26. Bailey SM, Cunningham CC. Contribution of mitochondria to oxidative stress associated with alcoholic liver disease, Free Radic Biol Med. 2002; 32: 11-16
27. Thayer WS, Rubin E, Molecular alterations in the respiratory chain of rat liver after chronic ethanol consumption. J Biol Chem. 1981; 256: 6090- 97
28. French SW, The role of hypoxia in the pathogenesis of alcoholic liver disease. Hepatol Res. 2004; 29: 69-74.
29. Flora SJ. Role of free radicals and antioxidants in health and disease. Cell Mol Biol 2007; 53: 1-2
30. Hijioka T, Sato N, Matsumura T, Yoshihara H, Takei Y, Fukui H et al, Ethanol-induced disturbance of hepatic microcirculation and hepatic hypoxia. Biochem Pharmacol 1991; 41: 1551-57
31. Stewart S, Jones D, Day C P, Alcoholic liver disease: New insights into mechanisms and preventative strategies. Trends Mol Med. 2001; 7: 408-13.
32. Stewart SF, Leathart JB, Chen Y, Daly AK, Rolla R, Vay D et al, Valine-alanine manganese superoxide dismutase polymorphism is not associated with alcohol-induced oxidative stress or liver fibrosis. Hepatology 2002; 36: 1355- 60.
33. Halliwell B, Gutteridge JM. Free Radicals in Biology and Medicine, 4th ed. Oxford University Press Inc. New York, 2007.
34. Felicity J, Cecilia G. Superoxide dismutases and their impact upon human health. Molecular Aspects of Medicine, Molecular Aspects of Med. 2005; 26: 340-52
35. Ho YS, Xiong Y, Ma W, Spector A, Ho D, Mice lacking catalase develop normally but show differential sensitivity to oxidant tissue injury. J Biol Chem 2004; 279: 32804- 812
36. Luzzatto L, Metha A. The Metabolic Basis of Inherited Disease. 4th Ed. London, Churchill Livingstone 1995; p. 109-26.
37. Vulliamy T, Mason P and Luzzatto L, The molecular basis of glucose-6-phosphate dehydrogenase deficiency. Trends Genet 1992; 8: 138-43.
38. Beutler E, Vulliamy T, Luzzatto L, Hum Genet 1996; 29: 49-56
39. Shinde A , Ganu J , Naik P, et al. Oxidative stress and antioxidative status in patients with alcoholic liver disease. Biomedical Research 2012; 23 (1): 105-108.
40. Li Sha , Tan Hor-Yue , Wang Ning , et al. The Role of Oxidative Stress and Antioxidants in Liver Diseases. Int. J. Mol. Sci. 2015; 16: 26087–124.
41. Galicia-Moreno M, Gutiérrez-Reyes G et al. The role of oxidative stress in the development of alcoholic liver disease. Rev Gastroenterol Mex. 2014;79:135-44 - Vol. 79
42. Pradhan R, Lekharu R, Srivastava R, et al. A Study of Oxidative Stress in Alcoholic Liver Disease. GCSMC J Med Sci. 2014; 3(1) :16-17.
43. Singh M, Gupta S, Pandey R et al. oxidative stress and chronic alcoholic liver diseases: the current perspectives. Indo American journal of pharmaceutical research; 2014:4(03).
44. Chen Y, Chen J, Bair J M et al. Antioxidative status of patients with alcoholic liver disease in southeastern Taiwan. World journal of gastroenterology. 2011; 17(8): 1063-70
45. R M, AV J, R K et al. erythrocytes lipid peroxidation and antioxidants in chronic alcoholics with alcoholic liver disease. Asian journal of pharmaceutical and clinical research.2010; 3(3): 183-85.
46. Das S kumar, Vasudevan D. M., et al. Monitoring oxidative stress alcoholic liver diseases in patients with non-alcoholic and alcoholic liver diseases. Indian Journal of Clinical Biochemistry. 2005; 20 (2): 24-28.
47. Gupta S,Pandey R, Katyal RI, et al. lipid peroxidation levels and antioxidant status in alcoholic liver disease. Indian Journal of clinical biochemistry. 2005; 20 (1): 67-71.
48. Lieber, C.S.(1984). Metabolism and metabolic effects of alcohol. Med. Clin. NorthAm.68(1),3-31.
49. Chari S, Gupta M. Status of blood antioxidant enzymes in alcoholic cirrhosis. Ind J Physiology and Pharmacology 2003; 47(3): 343-46.
50. Janani AV, Suprapaneni KM. Antioxidant vitamins and enzyme status in patients with alcoholic liver disease. J Clic and Dia Res 2010 Aug ;(4): 2742-7.
51. Das S, Vasudevan DM. Effects on alcohol on liver antioxidant defense System: a dose dependent study. Ind J ClinBiochem 2005; 20(1):80-84.
52. Chandra R, Aneja R, Rewal C, Konduri R, Dass K, Agarwal S. An opaium alkaloid papaverine ameliorates ethanol-induced hepatotoxicity: diminution of oxidative stress. Ind J ClinBiochem 2000; 15(2): 155-60.
53. Majhi S, Baral N, Lamsal M, Mehta KD. De Ritis ratio as diagnostic marker of alcoholic liver disease. Nepal Medical College journal: NMCJ. 2006 Mar;8(1):40-2.