Report
International Journal of Integrative Biology
A journal for biology beyond borders
IJIB
ISSN 0973-8363

Fish lipid prevents alcohol induced damages in liver and kidney tissues of mice (*Mus musculus*)

Manju M Surliker, Ramaballav Roy*

Lipid Biochemistry Laboratory, Department of Zoology, Goa University, Goa, India

Submitted: 31 Aug. 2010; Revised: 16 Dec. 2010; Accepted: 9 Jan. 2011

Abstract
The beneficial effect of dietary lipid is hypothesized to depend upon its type and composition, dose and duration of intake. The present study has been aimed to evaluate the ameliorative effect of two fish lipid, extracted from *Sardinella longiceps* and *Rastrelliger kanagurta*, in alcohol induced damages in liver and kidney tissue of mice, *Mus musculus*. Concentration of various biochemical parameters viz., total protein, albumin, total carbohydrate, urea, triglyceride, total and conjugated bilirubin, reduced glutathione (GSH) and Thiobarbituric acid reactive substances (TBARS) along with the activities the of alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphates (ALP) and gamma glutamyl transpeptidase (GGT) were monitored in these tissues. Altered levels of tissue biochemical compositions and the increased activities of the studied enzymes due to alcohol toxicities were reverted with the supplementation of 10% fish lipids. Lipids from *Sardinella longiceps* brought the maximum changes in concentration of albumin, Bilirubin, triglycerides and in the activities of AST and GGT in comparison with the same of *Rastrelliger kanagurta*. Histological studies of liver and kidney confirms the protective effect of dietary fish lipid from alcohol toxicities.

Keywords: fish lipid; PUFA; alcohol; tissue damage; *Mus musculus*.

INTRODUCTION

Ethanol is the most frequently abused drug and is a preferred fuel once consumed. Ethanol ingestion produces wide variety of pathological disturbances affecting a number of organs. Being a small molecule and soluble both in water and lipids, ethanol permeates all tissues of the body and affects most vital functions of virtually all organs including liver, kidney, brain, heart and pancreas (Lieber, 1995). The increased production of reactive oxygen species (ROS) by the heavy intake of ethanol, lead to increase in lipid peroxidation and thus results in oxidative stress which have been extensively studied using human and animal cells as well as animal models (Lindros, 1995; Zima *et al.*, 2001). Metabolism of ethanol which takes place in liver produces specific metabolites that cause toxic disturbances and lead to severe liver damage (You-Gui *et al.*, 2008; Luis et *al.*, 2006 and Utu-Baku *et al.*, 2009). After ethanol administration, ethanol and its metabolites go through kidneys and are excreted into the urine, and its content in the urine is higher than that of the blood and liver (Das and Vasudevan, 2008). Kidney seems to be the only vital organ generally spared in chronic alcoholics without advanced alcoholic liver diseases or hepato-renal syndrome. However, due to regular alcohol consumption, the harmful product of alcohol metabolism passes through the kidney which *per se* may be a risk factor for renal damage (Heidland et *al.*, 1985). Alcohol consumption may also cause acute tubular necrosis (Hirsch *et al.*, 1994) and renal tubular dysfunction (De Marchi *et al.*, 1993).

**Corresponding author:*
R. Roy, Ph.D.
Lipid Biochemistry Laboratory,
Department of Zoology, Goa University,
Goa, 403 206, India
Email: rroy@unigoa.ac.in

Dietary lipids play an important role in various physiological processes and thus help in maintaining health. A large number of studies have shown positive health benefits associated with consumption of omega-3 polyunsaturated fatty acids (PUFAs) on cancer, infant development, cardiovascular diseases and various mental illnesses such as depression, hyperactivity disorder and dementia (Riediger *et al.*, 2009). However, studies also have suggested that total dietary fat intake is linked to an increased risk of obesity, diabetes (Astrup *et al.*, 2008). Diets containing higher amount of saturated fatty acids (SFA) or omega-6 PUFAs are correlated with an increased incidence of arthrosclerosis and coronary heart diseases (Hu *et al.*, 1997). Effects of changing the total fat intake and

©IJIB, All rights reserved

saturated/ unsaturated ratios are still controversial. The cosmopolitan distribution of omega-6 PUFA in nature makes it a predominant PUFA in our diet, thereby making the amount of omega-3 consumed very less. Fish lipids have been used as most effective means of correcting the balance between omega-6 and omega-3 fatty acids in terms of improvements of health. Therefore, we hypothesized that the beneficial effect of dietary fish lipid depends upon its type (the composition of fat, relative concentration of saturated and unsaturated fatty acids, relative concentration of omega-3 and omega-6 long chain polyunsaturated fatty acids), its quantity of intake and duration of its intake. Pujari *et al.* (2010) have shown that dietary intake of 10% fish oil, enriched with omega-3 polyunsaturated fatty acids, for a period of 30 days is quite beneficial to laboratory mice, *Mus musculus* in maintaining the sound health.

The severe liver diseases are associated with lower percentage of plasma fatty acid of omega-3 and omega-6 series. Long chain PUFA deficiency is observed in patients with liver cirrhosis (Okita and Watanabe., 1998). Habitual fish intake may protect hepatic encephalopathy (Cabre, 1996). Diets enriched with omega-3 polyunsaturated fatty acids are associated with the amelioration of D-galactosime induced hepatitis in *Mus musculus* (Pujari and Roy, 2010). The same might also be associated with amelioration of alcohol induced tissue damage. However, the route, dosage and safety of PUFA supplementation need further investigation. Hence, the objective of the present study is to test the hypothesis that the quality of the dietary fish lipid is more important in preventing the alcohol induced tissue damages in Swiss albino mice, *Mus musculus*. We have used two laboratory extracted fish lipid viz., Sardine fish, *Sardinella longiceps and* Mackerel fish, *Rastrelliger kanagurta* for our present study. These two fish lipids are having apparently higher amount of omega-3 PUFA but they do differ in their composition (Tables 1 *[Supplementary data]* and Table 2 *[Supplementary data]*).

MATERIALS AND METHODS

Prior permission of Animal Ethic Committee, Goa University was taken to use the Swiss albino mice for the present study. The animals were maintained according to the guidelines of CPCSEA (Committee for the Purpose of Control and Supervision on Experiments on Animals), Govt. of India.

Extraction of fish lipid

Muscle tissues of fish were sonicated with enough hexane at temperature 30ºC for 1 hr with a stop for 5 minutes after every 15 minutes to avoid the heat generation due to sonication. The filtrate was collected under vaccum and stored. The residue was resonicated once again with fresh hexane. The pull filtrate was concentrated under Rotatory Vaccum Evaporator to remove the excess hexane. BHT (butalated hydroxyl toluene) was added to the oil in order to prevent the oxidation. The concentration of BHT in the oil was maintained as 1mM. Finally the oil was stored in amber colored bottle and refrigerated. Lipid analyses of these fishes were carried out as per our own experiences (Pujari *et* al., 2010).

Protocol of experiments

Two months old Swiss albino mice of uniform weight (22 ± 0.5g) were selected for the present study. After acclimatization for a period of 2 weeks, the animals were divided into four different groups each containing 6 mice.

Group I

Served as control group [C] and were maintained with standard pellet diet and water *ad libitum* during the experimental period of 30 days.

Group II

Served as Alcohol treated group [AL] these group, mice were maintained with standard pellet diet like the control group of mice. However, they were supplied with 20% alcohol as a source of drinking for 30 days.

Group III

Served as Alcohol treated group with supplementation of Sardine lipid, [AS] Sardine lipid at a dose of 10% was freshly blended with the standard pellet diet and fed to mice *ad libitum* along with 20% alcohol as a source of drinking for the period of 30 days.

Group IV

Served as Alcohol treated group with supplementation of Mackerel lipid, [AR] Mackerel lipid at a dose of 10% was freshly blended with the standard pellet diet and fed to mice *ad libitum* along with 20% alcohol as a source of drinking for the period of 30 days.

After 30 days of feeding experiment, animals were starved for 24hr before sacrificing. Animals were anesthetized to collect the liver, kidney and blood samples for further analysis.

Biochemical analysis

Protein (using Lowy's method), Albumin (using Bromocresol green reagent), Urea (using Diacetyl monoxime reagent), total Carbohydrate (using Anthrone reagent) and total and conjugated Bilirubin (using Diazo reagent) were estimated by following routine analytical method as mentioned by (Godkar,

©IJIB, All rights reserved

1994). Thiobarbituric acid reactive substances (TBARS) were estimated by using TBA-TCA-HCL reagent following the method of Niehaus and Samuelsson, (1968). Reduced glutathione (GSH) was measured by using 5, 5'-Dithiobis, 2-nitrobenzoic acid (Moron *et a.l.*, 1979). Triglyceride was estimated by using Chromotropic acid reagent (Kates, 1986).

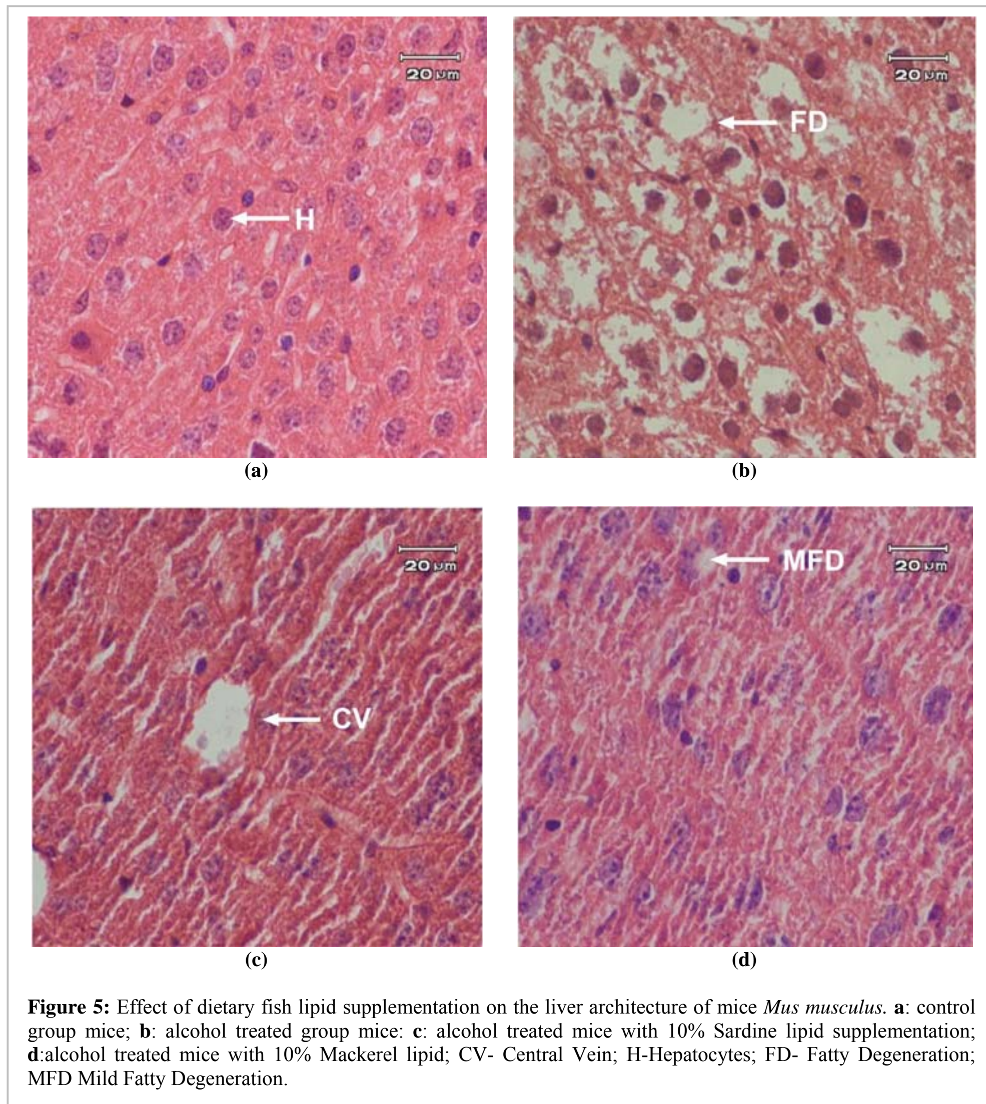

Figure 5: Effect of dietary fish lipid supplementation on the liver architecture of mice *Mus musculus.* **a**: control group mice; **b**: alcohol treated group mice: **c**: alcohol treated mice with 10% Sardine lipid supplementation; **d**:alcohol treated mice with 10% Mackerel lipid; CV- Central Vein; H-Hepatocytes; FD- Fatty Degeneration; MFD Mild Fatty Degeneration.

Enzymatic analysis

Alanine transaminase (ALT) [EC 2.6.1.2] and Aspartate transaminase (AST) [EC 2.6.1.1] activities were assayed by using dinitrophenyl hydrazine reagent (Reitman, 1957). Alkaline phosphates (ALP) [EC 2.6.1.2] activity was assayed by using p-nitro phenol reagent (King, 1934). Gammaglutamyl tranpeptidase (GGT) [EC 2.3.2.2] activity was measured by using glacial acetic reagent (Fiala *et al*., 1972).

Histopathological analysis

Routine laboratory method was followed for histological studies. Liver and kidney tissues were per fused with phosphate buffer saline (pH 7.0) and fixed in 10% formalin. The paraffin block was cut into uniform sections of 10µm thickness using a microtome and tissue sections were stained with hematoxylin and eosin for Histopathological examination (40 X) under a polarizing microscope (Olympus BX41).

Statistical analysis

Analyses were performed using the Statistical Package for the Social Sciences, Version 7.5 (SPSS Inc, Chicago III). Difference between the groups was analyzed by independent samples student *t* test. The level of significance was set to $p<0.05$. The results were represented as mean ± standard error.

RESULTS

It is revealed from Table 3 *[Supplementary data]* that alcohol consumption for a period of 30 days led to elevation of total protein (15-45%, $p<0.001$), albumin fractions of protein (2-fold, $p<0.001$), Bilirubin (70% - 6 fold, $p<0.001$) along with reduction in globulin fraction of protein (20-50%, $p<0.05$-0.01), total carbohydrate (20-60%, $p<0.01$-0.001) in different tissues of mice, *Mus musculus.* Augmentation of albumin fraction and reduction of globulin fractions of protein due to alcohol intoxication led to significant elevation in A/G ratio almost by 3 fold. The elevated level of protein was brought down by (10-20%, $p<0.01$-0.001) more prominently in liver and serum of mice when alcohol consumption was done along with the dietary intake of fish lipid. This was clearly reflected in albumin fractions of protein. Intake of Mackerel lipid along with alcohol consumption did not elevate the

©IJIB, All rights reserved

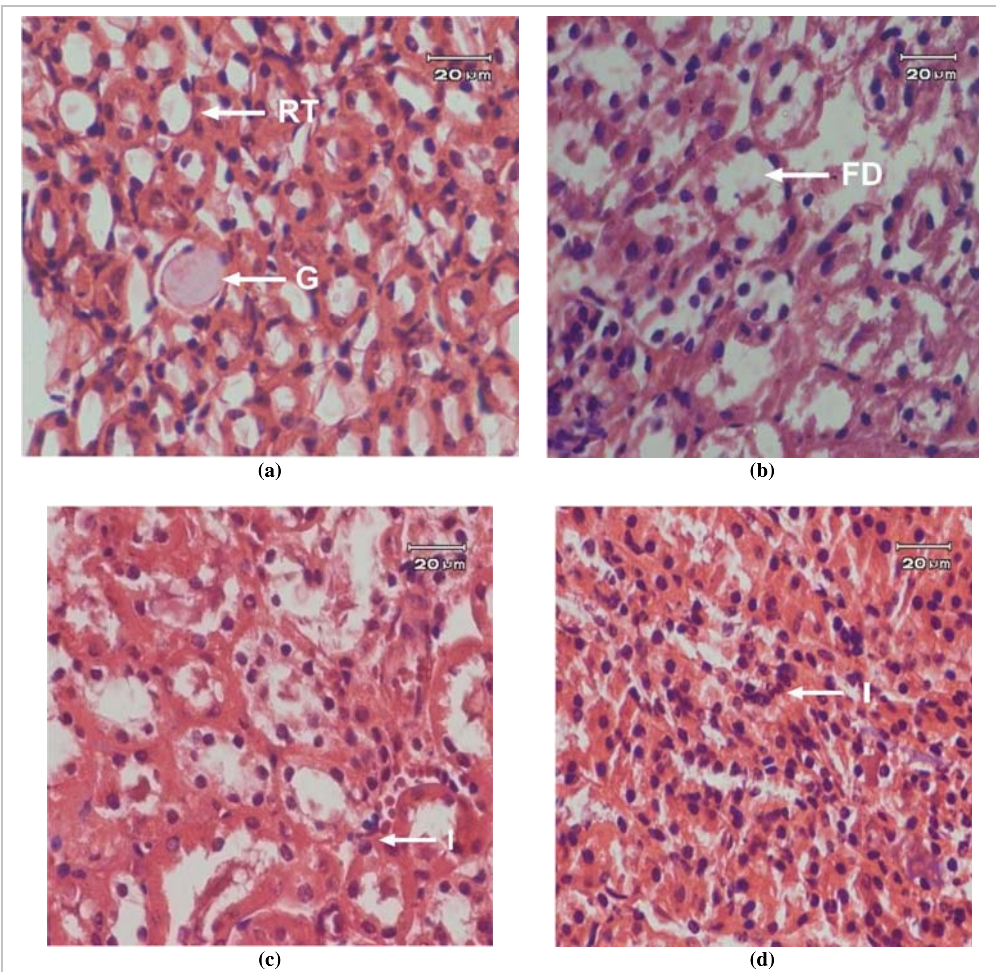

Figure 6: Effect of dietary fish lipid supplementation on the kidney architecture of mice *Mus musculus.* **a**: control group mice; **b**: alcohol treated group mice: **c**: alcohol treated mice with 10% Sardine lipid supplementation; **d**:alcohol treated mice with 10% Mackerel lipid; RT-Renal Tubules; G- Glomerulus; FD- Fatty Degeneration; I-Inflammation.

reduced level of globulin fractions; as a result A/G ratio was brought back almost to the normal level only with Sardine lipid intake along with alcohol consumption. Intake of fish lipid along with alcohol consumption elevated the reduced level of total carbohydrate. It is interesting to note that intake of Sardine lipid elevated the total carbohydrate level more than the normal values. The elevated level of bilirubin was significantly reduced by (10-70%, p<0.01-0.001) with dietary intake of fish lipid along with alcohol consumption. However, the level of bilirubin still remained higher than the same of control group of mice. The bilirubin level was much reduced in Sardine lipid treated group than the Mackerel lipid treated group of mice.

Consumption of alcohol over a period of 30 days led to elevation in the levels of urea concentration (25 to 2.4 fold, p<0.001), triglyceride (47%- 2.3 fold, p<0.001), TBARS (44%-3 fold, p<0.001), along with reduction in the levels of GSH (10-30%, p<0.02-0.001). Intake of fish lipid along with alcohol consumption reduced the elevated levels of urea by 10-40% (p<0.01-0.001), triglycerides by 10-50% (p<0.01-0.001), TBARS by 20-40% (p<0.001). However, the reduction of serum urea and TBARS in kidney was more with Sardine lipid supplementation in compare with Mackerel lipid supplementation (Table 4 *[Supplementary data]*). Dietary lipid intake brought the GSH concentration back to the almost normal level in all the three tissues of mice, namely liver, kidney and serum.

The biochemical changes are also reflected in the activity of some cellular enzymes like ALT, AST, ALP and GGT. Alcohol intake for the period of 30 days elevated the activity of all the enzymes tested by 25% to 3 fold (p< 0.01). The increased activity of ALT (Fig. 1 *[Supplementary data]*), AST (Fig. 2 *[Supplementary data]*), ALP (Fig. 3 *[Supplementary data]*) and GGT (Fig. 4 *[Supplementary data]*) were significantly reduced when alcohol consumption was along with dietary supplementation of Sardine or Mackerel lipid. This reduction effect was more with intake of Sardine lipid than the Mackerel lipid.

The control group of mice showed normal architecture of liver with normal hepatocytes (Fig. 5a) and of kidney with renal tubules and glomerulus's (Fig. 6a). Alcohol intoxication caused complete distortion of normal architecture of liver and kidney due to accumulation of fatty vacuoles leading to destruction of arrangement of granulocytes and certain degree of inflammation (Fig. 5b and Fig. 6b).While dietary intake of Sardine lipid along with alcohol consumption for 30 days restored the normal architecture of liver and kidney (Fig. 5c and Fig. 6c), fatty vacuoles and mild degenerative changes in the architecture of liver and

©IJIB, All rights reserved

kidney were still prevailed with the dietary supplementation of Mackerel lipid (Fig. 5d and Fig. 6d).

DISCUSSION

Fish lipid contains long-chain n-3 (omega-3) PUFA, particularly EPA (C20:5 n-3) and DHA (C22:6 n-3). Consumptions of these PUFAs have been perceived to be important in human nutrition, health and disease prevention. Due to cosmopolitan distribution of omega-6 fatty acids and restricted distribution of omega-3 fatty acid, fish oil and fish meal have been used as the most effective means of correcting the balance between omega-6 and omega-3 in order to increase the intake of omega-3 PUFA in terms of improvement seen in health. However, from the Table 1 it is very clear that Mackerel lipid contains lesser amount of cholesterol, phospholipids, glycolipid and triglycerides (per unit of lipid) in compare with same of Sardine lipid and thus maintain phospholipids to triglycerides ratio lower than 1.0. Although both of these lipids contained 17% of n3 PUFA but they differed in the amount of total saturated, total unsaturated and total n6 fatty acids (Table 2).

Alcohol is a fat-soluble non-electrolyte, which is readily absorbed from the gastrointestinal tract, diffuses rapidly into circulation and is distributed uniformly throughout the body. Metabolites of alcohol metabolism might reach to all the vital organs (like, liver and kidney) of *Mus musculus* due to the consumption of alcohol (20%) *ad libitum* for 30 days and cause some degree of tissue damage. These damages might lead to changes in the tissue biochemical composition. In the present study we observed that alcohol intoxication lead to 15-45% elevation in total protein ($p<0.001$). This elevation might be due to dramatic augmentation of albumin fraction protein and 20-50% reduction in the globulin fraction of the protein (Table 3). Increased level of tissue protein could result either from increased synthesis or from decreased export into the plasma or both. Investigators have reported that chronic ethanol administration increases the protein synthesis in ribosomes and microsomes (Renis *et al*., 1975), whereas acute administration produces opposite effects. 25% to 2.4 fold increase in urea concentration along with 20-60% reduction in total carbohydrate suggest the utilization of protein along with carbohydrate to meet the extra demand for the energy supply due to alcohol intoxication. Further experimental support is required to determine the degree of increased level of protein synthesis in mice upon alcohol intoxication. 70% to 6 fold augmentation in the level of Bilirubin concentration in liver, kidney and serum of mice due to alcohol consumption as observed supports the earlier reports. Available reports indicated that the alcohol intake led to more augmentation of Bilirubin which along with the increased activities of liver function enzymes led to fibrosis of liver in the patients suffering from the hepatitis virus or chemical induced hepatitis (Pessione *et al*., 1998 and Schmidt *et al*., 2002). The observed distortion in the architecture of liver and kidney tissues in terms of accumulation of fatty vacuoles (Fig. 4b and Fig. 5b) clearly indicates the beginning of necrosis in theses tissues. Continued oxidation of ethanol resulted in shifting of the intracellular NAD+ to NADH as a consequence the redox state of the membrane changes that led to hinder the fatty acid oxidation. This led to excess accumulation of triglycerides in liver cells (Lieber and Savolainen, 1984).

GSH is endogenous antioxidants which counter balance free radical mediated damage. Insufficiency in non-enzymatic antioxidant status in tissues of ethanol intoxicated mice could be the consequence of increased utilization for trapping free radicals. Acetaldehyde promotes peroxidation reaction by binding to cysteine and/ or glutathione, which causes depletion of GSH. In our present observation we noticed about 10-30% reduction in the GSH level along with 50% to 2 fold elevation in TBARS level in liver, kidney and serum of mice (Table 4) due to alcohol administration. Reduction of GSH also might be due to the increased utilization of GSH by antioxidant enzymes (per oxidase), which scavenge H_2O_2 (Anand *et al*., 1996).

The enzyme ALT is located in the cytoplasm and the soluble enzyme AST is located mainly in the organelles such as mitochondria (Senthil *et al*., 2003), increased levels of AST and ALT suggested damage of both hepatic cellular and mitochondrial membranes in alcohol treated mice. Increased activity of ALP can be due to the disturbance in secretary activity or the transport of metabolites (Sharma *et al*., 1995). Gamma glutamyl transpeptidase enzyme is most sensitive indicator of liver damage and a number of chemicals are known to increase its activity by the induction of microsomal enzyme (Trivedi *et al*., 2001). This may be due to the fact that depletion of GSH may induce hepatic GGT activity through an increased synthesis of its mRNA (Chikhi *et al*., 1999). A marked increase (40%- 2 fold, $p<0.02$-0.001) in liver function test and GGT activity in the alcohol treated group confirmed the tissue damages.

Dietary intake of fish oil brought down the total protein level by 10-40% ($p<0.01$-0.0001) and enhanced GSH, TBARS levels were also brought down. Similar results were seen by (Pushpakiran *et al*., 2004) were Fish oil supplementation reduced lipid peroxidation in serum, liver and kidney tissues of ethanol treated mice as evidenced by reduction in TBARS. Recent studies in our lab (Pujari and Roy 2010) also showed the similar results with fish oil and D-gain administration. The higher triglyceride level was also reduced by 10-45% ($p<0.01$-0.0001) after dietary fish oil supplementation.

©IJIB, All rights reserved

The enzyme activity of all the 4 enzymes i.e. ALT, AST, ALP and GGT was reduced with fish oil supplementation. Dietary fatty acid modifies the lipid metabolism. Alcohol intoxication might alter the post primordial gene expression by up regulation or down regulation of some mRNA which are yet to be verified. The changes in the gene expression also might reprogram the tissue lipid profiles. PUFA present in the fish oil try to ameliorate the above mentioned abnormal changes. We observed that Sardine lipid offered better protection against alcohol induced damage which once again confirmed the protective effect of omega-3 PUFA in experimental animals (Sachan *et al.,* 2002). Thus Consumption of fish oil does the betterment of pathological damage caused by alcohol intoxication. Nandini *et al.* (1993) reported that the higher content of omega-3 fatty acid in contrast to omega-6 has a better protective effect on alcohol induced liver damage in rat. In the present study, the fish lipids used are having 17% fatty as n3PUFA and 6% fatty acid as n6PUFA (as in case of Sardine lipid) 8% fatty acid as n6PUFA (as in case of Mackerel lipid). This small difference may not be sufficient to give a better protective status with dietary intake of Sardine lipid. It perhaps might be due to the differences in their lipid composition (Table 1) or some other factors present in the fish lipid.

Acknowledgement

Financial support from the Department of Biotechnology, Govt. of India vides the sanction letter no. BT/PR11635/AAQ/03/448/2008 dated September 9, 2009 is acknowledged.

References

Anand CV, Anand U, et *al.* (1996) Antioxidant enzymes, gammaglutamyl Transpeptidase and lipid peroxidation in kidney of rats exposed to cigarette smoke. *Indian Journal of Experimental Biology.*, **34**(5): 486-488. [PMID: 9063083].

Astrup A, Dyeberg J, et *al. (*2008) Nutrition transition and its relationship to the development of obesity and related chronic diseases. *Obesity Review*, **9**(1): 48-52. [PMID: 18307699].

Cabre E, Periago JL, *et al*. (1990) Plasma fatty acid profile in advanced cirrhosis: unsaturation deficit of lipid fractions. *Am J Gastroenterol*., **85**(12)**:** 1597-604. [PMID: 2252024].

Chikhi N, Holic N, *et al*. (1999) Gamma glutamyl Transpeptidase gene organization and expression: a comparative analysis in rat, mouse, pig and human species. *Comparative Biochem Physiol B Biochem Mol Biol.*, **122**(4)**:** 367-380. [PMID: 10392451].

Das SK and Vasudevan DM, (2008) Alcohol induced effects on kidney. *Indian Journal of Clinical Biochemistry*, **23**(1): 4-9. [ISSN: 0970-1915].

De Marchi S, Cecchin E, *et al.* (1993) Renal tubular dysfunction in chronic alcohol abuse-effects of abstinence. *N. Engl. J. Med.*, **329**(26): 1927-34. [PMID: 8247056].

Fiala S, Fiala AE, *et al.* (1972) Gamma glutamyl Transpeptidase in chemically Induced rat hepatomas and spontaneous mouse hepatomas. *J. Nall. Cancer Inst*., **48:** 1393-1409.

Godkar PB (1994) Clinical Biochemistry Principle and Practice. *India: Bhalani Publishing House, Delhi.*

Heidland A, Horl WH, *et al.* (1985) Role of alcohol in clinical nephrology. *Klin Wochenschr*., **63**(18): 948-58. [PMID: 4057921].

Hirsch DJ, Jindal KK, *et al.* (1994) Acute renal failure after binge drinking. *Nephrol Dial Transplant.*, **9**(3): 330-1. [PMID: 8052446].

Hu FB, Stampfer MJ, *et al*. (1997) Dietary fat intake and the risk of coronary heart disease in women. *N Engl J Med*., **337**(21): 1491-9. [PMID: 9366580].

Kates M (1986) Techniques of lipidology isolation. Analysis and identification of Lipids. *El eseviour., New York*. pp: 112-185.

King EJ, Armstrong AR, *et al.* (1934) A convenient method for determining serum and bile phosphates activity. *Can Med Assoc J.*, *31*(4): 376-381. [PMID: 20319659].

Lieber CS and Savolainen M (1984) Ethanol and lipids. *Alcoholism-Clinical and Experimental Research*, **8**:409-423. [ISSN: 1530-0277].

Lieber CS (1995) Medical disorders of alcoholism. *N Engl J Med.*, **333**(16): 1058-1065. [PMID: 7675050].

Lindros KO (1995) Alcoholic liver diseases: pathobiological aspects. J *Hepatology*, **23**(1)**:** 7-15. [PMID: 8551014].

Luis B, Maria GB, *et al*. (2006) Effect of resveratrol on alcohol-induced mortality and liver lesions in mice. *BMC Gastroenterology*, **6**: 35-43. [PMID: 17105669].

Nandini M, Rahiman MA, *et al*. (1993) Effect of dietary fish on antioxidants parameters of normal and cholesterol stresses rats. *Indian Journal of Experimental Biology*, **37**(12): 1187-1191. [PMID: 10865885].

Niehaus WG, Samuelsson B, *et al*. (1968) Formation of malondialdehyde from phospholipids arachidonate during microsomal lipid peroxidation. *Eur J Biochem.*, **6**(1): 126-130. [PMID: 4387188].

Moron MS, Depierre JW, *et al*. (1979) Levels of glutathione S-transferase activities in rat Lung and liver. *Biochem Biophys Acta.*, **582**(1): 67-78. [PMID: 760819].

Okita M, and Watanabe A, (1998) Nutritional treatment for abnormal lipid metabolism in cirrhotic patients. In: *Progress in Hepatology*, Elsevier Science, Amsterdam, **14**:101-114.

Pessione F, Degos F, *et al.* (1998) Effect of alcohol consumption on serum hepatitis C virus RNA and histological lesion in chronic hepatitis C. *Hepatology*, **27**(6): 1717-22. [PMID: 9620348].

Pujari P, *et al.* (2010) Effect of dietary manipulation of lipids on mice, *Mus musculus*. *Advances in Biological Research*, **4**(3): 146-153. [ISSN 1992-0067].

Pujari P, and Roy R (2010) Diets rich in n-3 polyunsaturated fatty acids are associated with amelioration of D- galactosamine induced hepatitis in mice. *Recent Research in Science and Technology*, *2*(1)**:** 2076-5061. [ISSN: 2076-5061].

Pushpakiran G, Mahalakshmi K, *et al.* (2004) Taurine restores ethanol-induced depletion of antioxidants and attenuates oxidative stress in rat tissues. *Amino acids*, **27**(1): 91-96. [PMID: 15309576].

©IJIB, All rights reserved

Renis M, Giovine A, *et al*. (1975) Protein synthesis in mitochondrial and microsomal fractions from rat brain and liver after acute or chronic ethanol administration. *Life sciences*, **16**(9): 1447-1457. [PMID: 1134202].

Riediger ND, Othman RA, et *al.* (2009) A systemic review of the roles of n-3 fatty acids in health and disease. *J. Am. Diet Association*, **109**(4): 668-79. [PMID: 19328262].

Reitman S and Frankel S (1957) Colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. *Am. J. Clin. Pathol.,* **28**(1): 56-63. [PMID: 13458125].

Sachan SD, Yatim MA, *et al.* (2002) Comparative effects of dietary corn oil, safflower oil, fish oil and palm oil on metabolism of ethanol and carnitine in the rat. *Journal of the American College of Nutrition*, **21**(3): 233-238. [PMID: 12074250].

Schmidt LE, Dalhoff K, *et al*. (2002) Acute versus chronic alcohol consumption in acetaminophen-induced hepatotoxicity. *Hepatology*, **35**(4): 876-82. [PMID: 11915034].

Senthil KR, Ponmozhi M, *et al*. (2003) Activity of cassia auriculata leaf extract in rats with alcoholic liver injury. *Journal of Nutritional Biochemistry*, **14**(8): 452- 458. [PMID: 12948875].

Sharma A, Mathur R, *et al*. (1995) Hepatoprotective action of a proprietary herbal formulation against carbon tetrachloride intoxication. *Indian Drugs*, **32**: 120-124. [ISSN: 0974-4304].

Trivedi NP and Rawal UM, (2001) Hepatoprotective and antioxidant property of Andrographis paniculata in BHC induced liver damage in mice. *Ind. J. Exp. Biol.*, **39**(1): 41-6. [PMID: 11349524].

Utu-Baku AB, *et al*. (2009) The influence of alcohol kolanut constituents on liver Aspartate amino transferase and Alanine amino transferase enzyme activities on albino wistar rats. *International journal of biological chemistry*, **3**: 25-29. [ISSN 1819-155X].

You-Gui LI, Dong-Feng JI, *et al*. (2008) Protective effects of sericin protein on alcohol- mediated liver damage in mice. *Alcohol and Alcoholism*, **43**(3)**:** 246-253. [PMID: 18263900].

Zima T, Fialova L, *et al.* (2001) Oxidative stress, metabolism of ethanol and alcohol-related diseases. *Journal of Biomedical Science*, **8**(1)**:** 59-7. [PMID: 11173977].

©IJIB, All rights reserved