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Hepatoprotective property evaluation of fish oil from *Sardinella fimbriata* over fatty liver disease

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ABSTRACT

The present study was designed to investigate the effect of fish oil extracted from Sardinella fimbriata against fatty liver diseases in rat experimental models and regenerative property. Ethanol and Methotrexate were administered to eight weeks old animals for 4 weeks to induce Alcoholic fatty liver disease and Non Alcoholic fatty liver disease, respectively. After experimental period, the parameters such as liver weight, food intake, liver histology and liver markers alanine amino transferase, aspartate amino transferase, alkaline phosphatase, direct bilirubin, total bilirubin and gamma- glutamyl transpeptidase were studied. Along with liver markers, lipid profile such as blood glucose, total cholesterol, triglycerides, LDL-cholesterol, VLDL-cholesterol and HDL-cholesterol were also studied. The results of the experimental groups showed elevation of all above mentioned parameters in ethanol and methotrexate treated groups, where as significant reduction in liver markers enzymes with decrease in liver weight were observed in groups which are fed with fish oil along ethanol (Group 5) and MTX (Group 6), suggesting the possibility on the prevention of eventual increase in the release of lipoproteins from the liver even after prolonged intoxication with the development of liver dysfunctions. Histopathological studies showed Steatohepatitis in ethanol (Group 3) and methotrexate (Group 4) intoxicated groups, where as in groups which are fed with fish oil along ethanol (Group 5) and MTX (Group 6) showed ameliorating effects. The liver damage caused by alcohol intoxication was easily reversed; however, it was not in the case of NAFLD in the present study.

Keywords: fatty liver, Ethanol, methotrexate, liver markers, Steatohepatitis, Ameliorating effects

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INTRODUCTION

Fatty liver is caused by the build-up of fats in the liver in the form of triglycerides. In many instances the effect of having fat in their liver over a long period may lead to inflammation, causing swelling and tenderness (hepatitis) and then to scar (fibrosis). There are four stages of fatty liver disease; Acute hepatitis (sudden inflammation-short term), chronic (inflammation- long term), Fibrosis (scar tissue) and Cirrhosis (Fibrosis spread throughout the liver) to hepatocellular carcinoma [1, 2]. Deficiency of essential fatty acids (EFA) in diet, leads to a cascade of anomaly of nutrients in mammals, such as poorer cognitive function [3] and hepatocellular cytoplasmic vacuolation, *i.e*. fatty liver, due to an inability to form the lipoproteins responsible for transporting lipids out of the liver [4].

Alcoholic Fatty Liver Disease (AFLD) is caused due to common consequence of prolonged alcohol consumption and forms one of the major causes of morbidity and mortality worldwide. It is now well accepted that the progression of AFLD is due to the multi-factorial process that involves a number of genetic, nutritional and environmental factors [5]. Alcohol consumption was shown to compromise fatty liver status, either by affecting the

biosynthesis, absorption, or catabolism of fatty acids [6, 7]. The involvement of oxidative injury in ethanol toxicity has emerged from a number of reports showing that alcohol-fed experimental animals as well as in patients with Alcoholic Liver Disease (ALD) have increased level of lipid peroxidation products in liver and blood [8]. In this context, recent evidences indicates that oxidative stress induced by ethanol is responsible for promoting hepatic iron accumulation by down-modulating the liver expression of hepcidin, a 25 amino acid peptide that regulates the absorption and distribution of iron inside the body[9, 10]. During periods of heavy drinking, alcoholics often lack proper diets or consume diets which are not compromised with nutrients, such as EFAs and vitamins.

Non Alcoholic fatty liver disease (NAFLD) is a condition characterized by the build-up of fat in the liver cells of people who do not drink alcohol excessively; NAFLD can be defined as fat accumulation in the liver that exceeds 5–10% by wet weight [11]. Excess liver fat is believed to be a manifestation of the metabolic syndrome [12]. The pathogenesis of Non Alcoholic Steatohepatitis (NASH) includes insulin resistance, increased inflammation, tumor necrosis factor (TNF)-α, interleukin (IL)-6 and increased oxidative stress [13]. Most experimental studies proved that fatty liver in rodents are also diet-inducible, like that of human diseases driven by diet. The process of the NAFLD is same as that of AFLD with minor variations in cross reactions due to different initiative of these diseases. In addition, patients with NAFLD are prone to higher prevalence of insulin resistance and cardiovascular disease [15, 16, 17].

Experimental studies on animal models and humans suggested that dietary factors can affect fatty infiltration and lipid peroxidation in various types of liver disease including AFLD and NAFLD [17, 18]. Various studies conducted by Kraegen [19]; Storlien [20]; Kraegen [21]; Storlien [22] and Oakes [23] concluded that dietary inclusion of unsaturated fatty acids in rats completely prevented liver and muscle insulin resistance in 3 weeks. Fish oil prevents insulin resistance, when substituted for one third of the amount of safflower oil, in a 60% high fat diet in rats, by preventing a decrease in PI3 kinase activity in muscles and decrease in the GLUT4 level induced by the high fat diet [24]. The mechanisms relating dietary ω-3 fatty acid substitution to the protection towards insulin resistance remains hypothetical to a greater extent. Diets which have rich in olive oil, fish oil and fiber appear to improve hepatic steatosis in animal models [25, 26, 27]. Out of 9 of 15 NASH patients undergone one year of intense dietary intervention, 9 showed profound histological improvements [28], where the diet composed of 40-45% of carbohydrates with an emphasis on complex carbohydrates with fibre; 35-40% fat with emphasis on mono and polyunsaturated fatty acids (fish oil); and 15- 20% protein.

It has been a major challenge to clinicians and allied researchers of how one can achieve good compliance in these fatty liver diseases. Since there are clues and evidences proving that the unsaturated fatty acids have the potential to stimulate positive liver metabolism (regeneration), which may play a positive role against the fatty liver disease, both in alcoholic and non alcoholic. In this backdrop, an attempt has been made in the present study to unravel the role of fish oil which was extracted from *Sardinella fimbriata* against fatty liver diseases in rat experimental models and regenerative property of the liver in the incidence of fish oil.

MATERIALS AND METHODS

The crude fish body oil was extracted from *Sardinella fimbriata* by adopting the conventional method (Direct steaming). The crude fish oil composed of total monounsaturated fatty acids 25.25 % w/w of oil, total polyunsaturated fatty acids 17.617 % w/w of oil, total non essential amino acids 13.2µg/g and essential amino acids 8.452µg/g, respectively and was used for further pharmacological applications on fatty liver disease. All analytical reagents and enzyme test kits required for the present investigation were purchased from Hi-media and Fisher Scientific (Mumbai, India).

Animal models

The present study was approved by the Institutional Animal Ethics Committee (IAEC) for animal experiments of Annamalai University. Eight weeks old Wistar male albino rats weighing at 200±10 g were procured from Central Animal House of Annamalai University. The rats were acclimatized to laboratory conditions and fed with commercial pellet rat chow (Hindustan Lever Limited, India) and given water *ad libitum*. Ethanol and Methotrexate (MTX) was administered to animals to induce AFLD and NAFLD respectively.

Experimental design

The rats were divided into six groups, each group comprising of six rats. Group 1 received normal diet (Normal), Group 2 received 50ml/kg of fish oil alone for 4 weeks (Positive control) Group 3a received 50% v/v Alcohol, 50ml/kg for 4 weeks (Negative control-1a), Group 3b received 50% v/v Alcohol, 50ml/kg for 4 weeks and analyzed after 6th week (Negative control-1b), Group 4a received MTX 200 mg/kg for 4 weeks (Negative control-2a), Group 4b received MTX 200 mg/kg for 4 weeks and analyzed after 6th week (Negative control-2b), Group 5 received 50% v/v Alcohol + 50ml/kg of fish oil for 4 weeks, Group 6 received MTX 200 mg/kg + 50ml/kg of fish oil for 4 weeks. Fish oil, alcohol and Methotrexate (MTX) were administered orally by feeding tubes (enteral feeding).

At the end of the experiment period, the blood was collected from the retro orbital plexus for the estimation of alanine amino transferase (ALT) and aspartate amino transferase (AST) by Transaminases Colorimetric End Point Method, alkaline phosphatase (ALP) by BioAssay Systems QuantiChrom^{TM} Alkaline Phosphatase Assay Kit, direct bilirubin (DB) and total bilirubin (TB) by Life Chem^{TM} Kit [29] and gamma- glutamyl transpeptidase (GGT) by Mindray Gamma Glutamyl transferase Kit based on Orlowski method as described by [30].

Parameters evaluated such as blood glucose (BL) described by [31], total cholesterol (TC) described by [32], triglycerides (TG) described by [33], Low density lipoprotein cholesterol (LDL-C) described by [34], Very low density lipoprotein cholesterol (VLDL-C) using ELISA kit of Bio Assay Systems (USA) and High density lipoprotein cholesterol (HDL-C) described by [35] in blood serum were tested. The animal was euthanized with anaesthetic ether and the liver was excised, weighed and immediately used for histo-chemical studies. Histopathology studies were carried out [36]. Formalin-fixed portions of organ (Liver) were prepared for histological studies by standard procedures from dehydration through paraffin infiltration in an automatic tissue processor. After paraffin embedding, all sections were cut at 6µm thickness and routinely stained in hematoxylin-eosin dye. Selected frozen sections were made to ensure that the vacuolated appearance of the paraffin sections was due to the presence of lipid droplets. Histopathological observation was recorded using Photomicrograph system (Nickon Eclipse-E-200).

RESULTS

Experimental rats intoxicated with ethanol and methotrexate showed significant elevated levels of the serum as Alanine amino Transferase (ALT), Aspartate amino Transferase (AST), Alkaline Phosphatase (ALP), Direct Bilirubin (DB), Total Bilirubin (TB) and Gamma- Glutamyl Transpeptidase (GGT) as compared to animals in normal, positive control and fish oil treated groups. The treatment of fish oil ameliorated alcohol and methotrexate induced toxic changes in ALT, AST, ALP, TB, DB and GGT levels were shown in figure 7 to 12. Similarly, lipid profile too showed elevated levels in the ethanol and methotrexate intoxicated groups as compared to the other groups. Administration of ethanol and methotrexate showed a significant increase in liver weight, compared to the normal and fish oil treated groups. A significant reversal was observed in all the parameters and in the weight of liver following the treatment of fish oil (50ml/Kg) for 4 weeks.

Lipid Profile

The results of blood glucose are shown in Fig. 1. The levels of blood glucose were 82±8, 78±4, 91±7, 88±6, 87±7, 86±5, 83±4 and 81±3mg/dl for group 1, 2, 3a, 3b, 4a, 4b, 5 and 6 respectively. The results of Total cholesterol are shown in Fig. 2. The levels of total cholesterol were 73±5, 62±4, 83±6, 79±6, 86±5, mg/dl for group 1, 2, 3a, 3b, 4a, 4b, 5 and 6 respectively. The results of HDL cholesterol are shown in Fig. 3. The levels of HDL cholesterol was 29±5, 36±3, 22±5, 25±7, 19±4, 24±8, 29±4 & 31±9 mg/dl for group 1, 2, 3a, 3b, 4a, 4b, 5 and 6 respectively. The results of LDL cholesterol are shown in Fig. 4. The levels of LDL cholesterol was 35±5, 31±3, 46±8, 42±3, 49±6, 40±7, 32±4 & 30±5 mg/dl for group 1, 2, 3a, 3b, 4a, 4b, 5 and 6 respectively. The results of VLDL cholesterol are shown in Fig. 5. The VLDL cholesterol levels were 47±6, 40±5, 76±2, 71±7, 65±6, 59±6, 52±4 & 45±5 mg/dl for group 1, 2, 3a, 3b, 4a, 4b, 5 and 6 respectively. The results of Triglycerides are shown in Fig. 6. The triglycerides was 47±6, 40±5, 76±2, 71±7, 65±6, 59±6, 52±4 & 45±5 mg/dl for group 1, 2, 3a, 3b, 4a, 4b, 5 and 6 respectively.

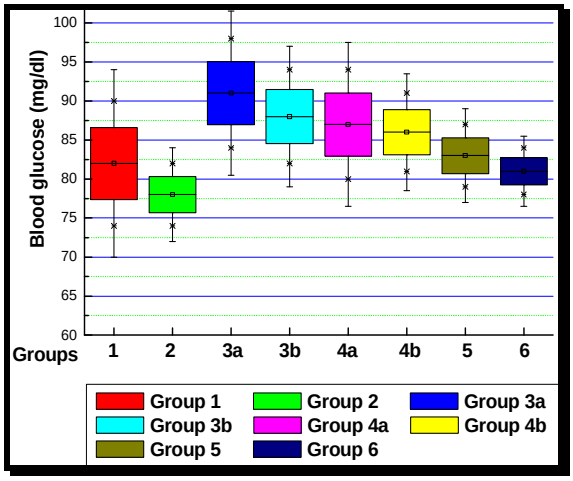


Figure 1. Variation in Blood Glucose levels between the groups

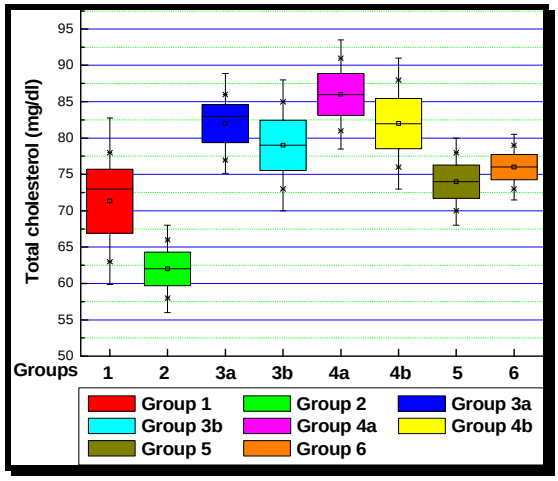


Figure 2. Variation in Total Cholesterol levels between groups

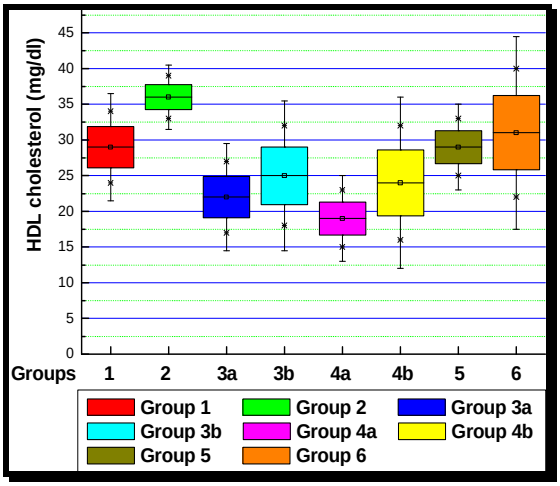


Figure 3. Variation in HDL cholesterol levels between the groups

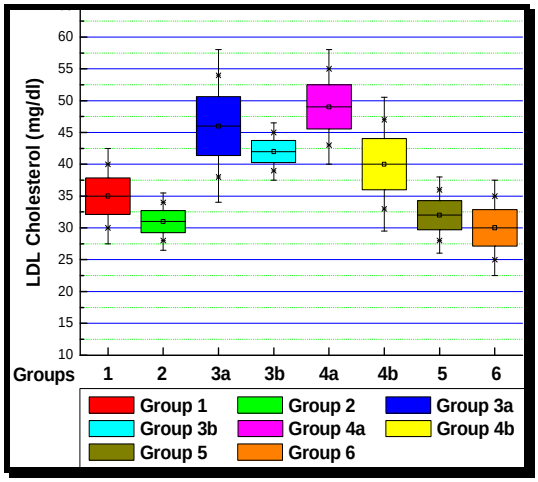


Figure 4. Variation in LDL cholesterol levels between the groups

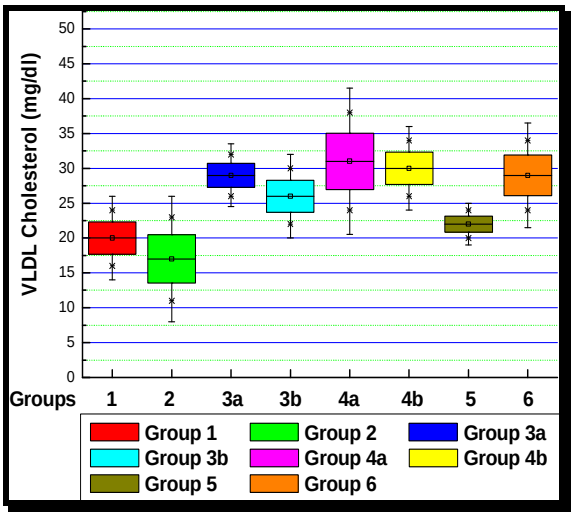


Figure 5. Variation in VLDL cholesterol levels between groups

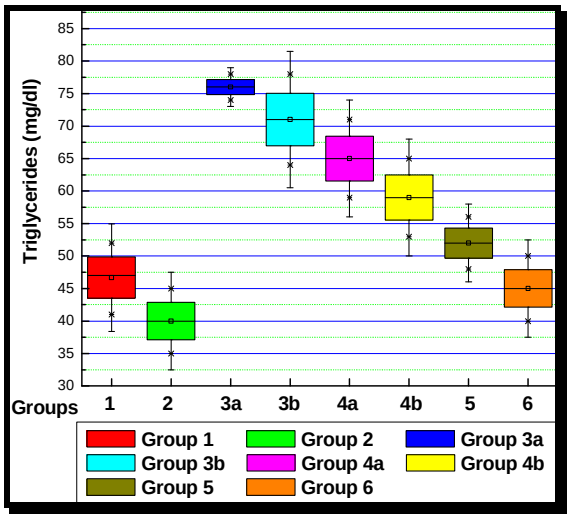


Figure 6. Variation in Triglyceride levels between the groups

Liver function tests

The results of ALT were shown in Fig. 7. The levels of ALT were 54±3.85, 55±2.15, 90±6.07, 88±7.32, 86±5.41, 85±3.11, 67±4.22 and 63±3.67 IU/l for group 1, 2, 3a, 3b, 4a, 4b, 5 and 6 respectively. The present study on chronic alcohol and methotrexate intoxication showed significant increase in all liver function tests, showing the indices of liver injury. The results of AST were shown in Fig. 8. The levels of AST were 113±5.87, 116±1.03, 176±8.09, 167±6.34, 148±9.96, 145±8.65, 140±3.38 and 131±5.18 IU/l for group 1, 2, 3a, 3b, 4a, 4b, 5 and 6 respectively. The results of ALP were shown in Fig. 9. The levels of ALP were 259±13.34, 246±16.45, 497±23.1, 468±19.87, 461±21.76, 453±18.11, 380±14.2 and 341±15.7 IU/l for group 1, 2, 3a, 3b, 4a, 4b, 5 and 6 respectively. The results of DB were shown in Fig. 10. The levels of DB were 0.17±0.02, 0.15±0.021, 0.41±0.04, 0.38±0.03, 0.45±0.06, 0.41±0.04, 0.29±0.01 and 0.30±0.04 IU/l for group 1, 2, 3a, 3b, 4a, 4b, 5 and 6 respectively. The results of TB were shown in Fig. 11. The levels of TB were 0.69±0.08, 0.63±0.05, 1.06±0.07, 1.01±0.04, 1.12±0.07, 1.06±0.09, 0.9±0.05 and 0.9±0.1 IU/l for group 1, 2, 3a, 3b, 4a, 4b, 5 and 6 respectively. The results of GGT were shown in Fig. 12. The levels of GGT were 32±3.3, 30±5.6, 49±9.1, 45±7.6, 51±6.3, 48±5.9, 39±4.6 and 35±6.2 IU/l for group 1, 2, 3a, 3b, 4a, 4b, 5 and 6 respectively.

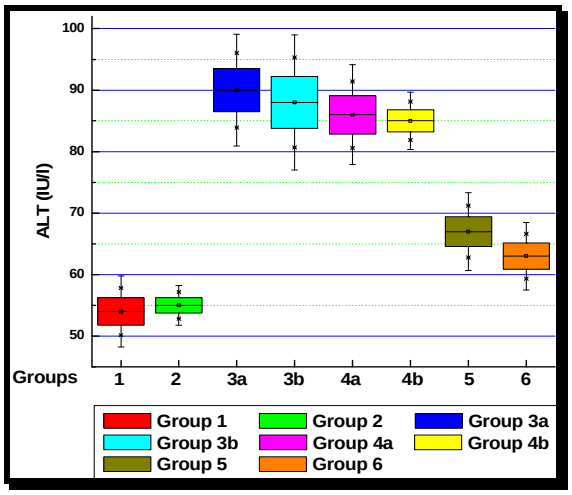


Figure 7. Variation in ALT levels between the groups

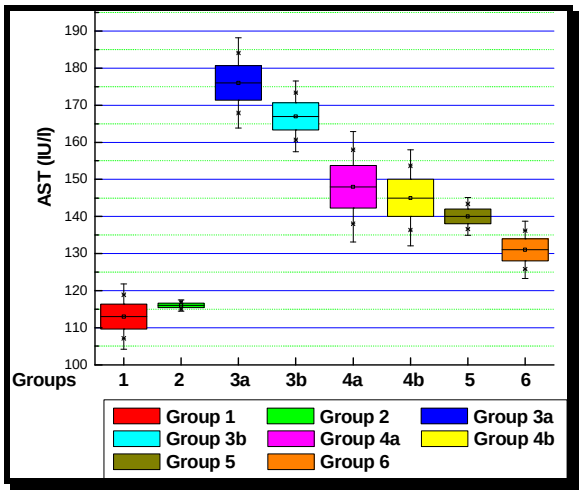


Figure 8. Variation in AST levels between the groups

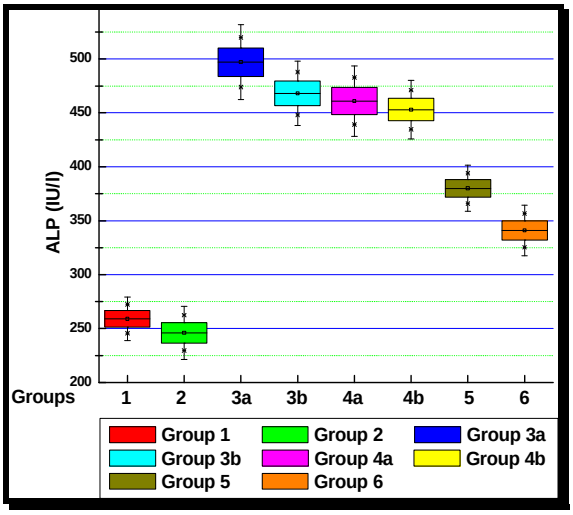


Figure 9. Variation in ALP levels between the groups

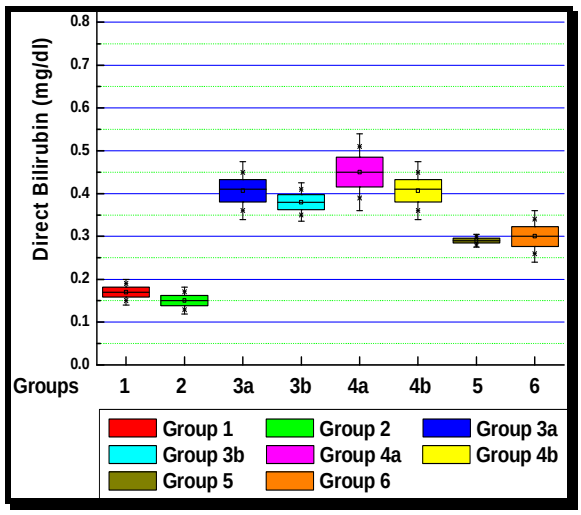


Figure 10. Variation in Direct Bilirubin levels between the groups

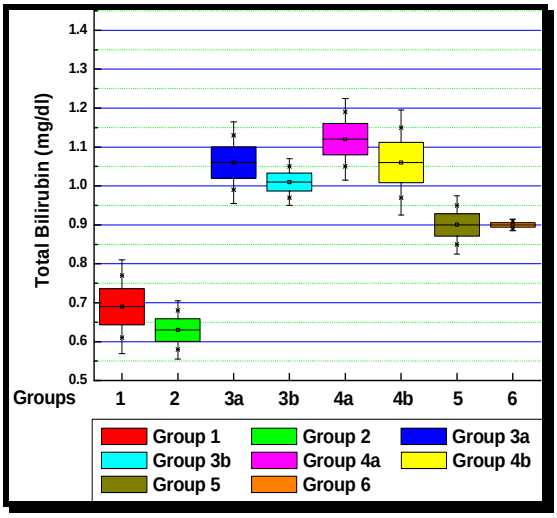


Figure 11. Variation in Total Bilirubin levels between the groups

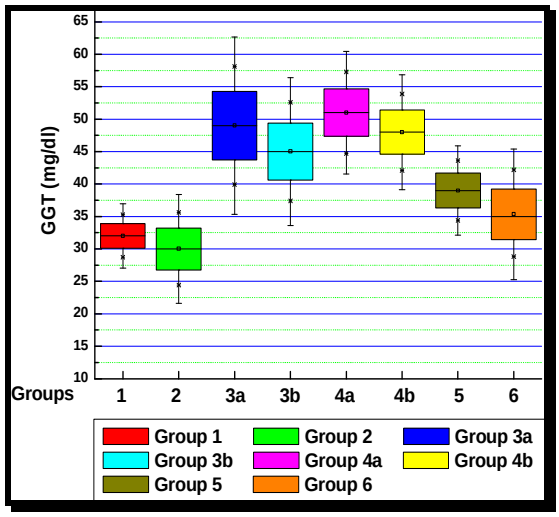


Figure 12. Variation in GGT levels between the groups

Food intake, Body weight and Weight of liver

The mean weight of the liver prior to histochemical studies was recorded, which showed increase in mass when compared to the normal group (4.2±0.09g and 4.1±0.08g versus 3.61±0.2 g). The group supplemented with fish oil recorded the least mean value of liver weight as compared to the intoxicated groups (3.53±0.2 vs. 4.2±0.09g respectively). Food intake (g/day for each rat) decreased in the ALD and NAFLD induced groups as compared to the normal and fish oil alone treated groups (5.6±0.5 and 5.2±0.7 vs. 10.6±1.1 and 10.1±0.9 g/day respectively). However the groups intoxicated with alcohol & methotrexate and treated with fish oil showed significant increase in food intake as compared to the intoxicated groups. Rats supplemented with alcohol + fish oil and methotrexate + fish oil recorded higher food intake values than other intoxicated groups (8.0±0.5 and 7.6±0.34 g/day). The data is summarized in table 1.

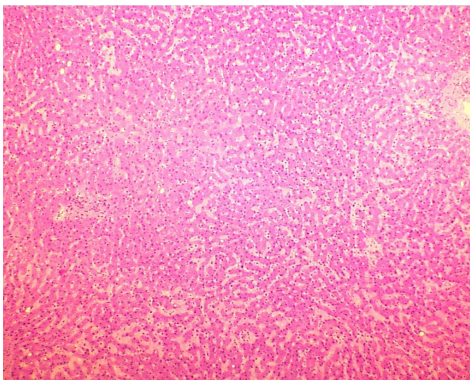
Table 1. Depicts the details of Body weight and Weight of liver in experimental rats

Parameters	Group I	Group II	Group III		Group IV		Group V	Group VI
			a	b	a	b		
Average Body wt. of rats (Initial) (g)	100±10	100±10	100±10	100±10	100±10	100±10	100±10	100±10
Average Body wt. of rats (Final) (g)	110±15	111±12	123±12.5	130±10.5	121±9.0	130±7.0	116±5.0	118±8.5
Average food intake (g/each day)	10.6±1.1	10.1±0.9	5.6±0.5	6.2±0.23	5.2±0.7	6.9±0.9	8.0±0.5	7.6±0.34
Wt. of Liver (g/100g)	3.61±0.2	3.53±0.2	4.2±0.09	4.1±0.05	4.1±0.08	4.0± 0.11	3.8±0.06	3.7±0. 1

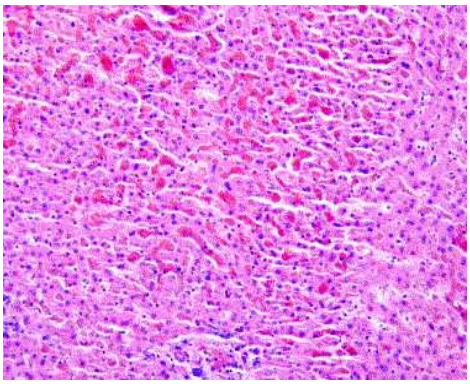
Histochemical studies

The liver sections of normal and positive control (fish oil alone) animals showed normal structure and architecture. Group 1 showed normal hepatic cells and intact hepatocytes. Group 2 showed structural and hepatic architecture intactness as well as glycogen deposition in the hepatocytes. Group 3 showed macrovesicular and microvesicular steatosis, spotty necrosis and mild infiltration of inflammatory cells, accumulation of fats and depletion of glycogen stores. Group 4 showed ballooning of hepatocytes, focal vacuolated fatty change, vacuolar degeneration and necrosis. Group 5 showed slight steatosis (regeneration of cells), but no inflammation or necrosis roughly coming back to normal liver histology. Group 6 showing complete absence of necrosis and slight inflammation, however steatosis was still present but tended to decrease compared with Group 4.

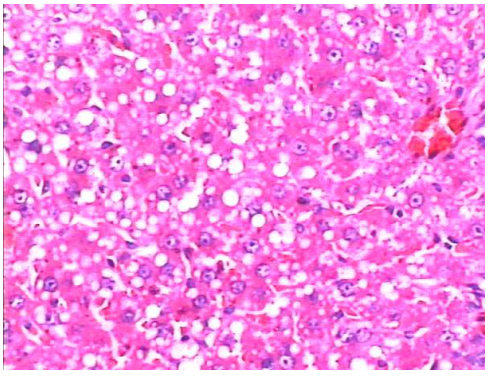
Histopathology of liver (Magnification at 200X)



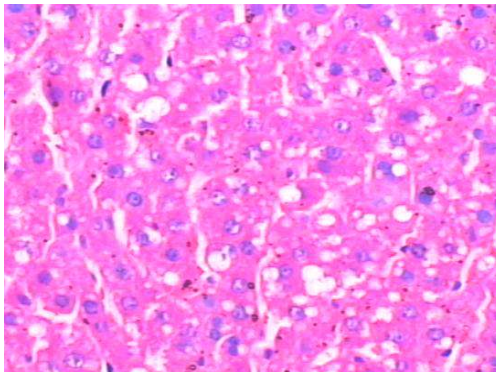
Slide 1. Group 1 showing normal hepatic cells.



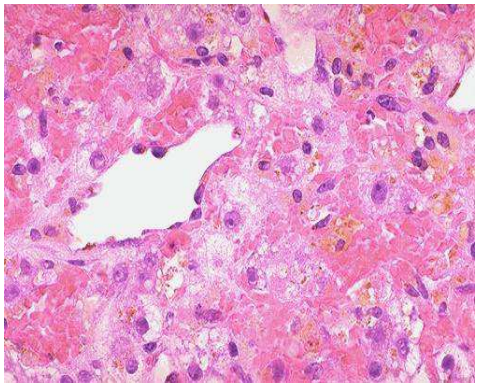
Slide 2. Group 2 (Positive control) showing structural and hepatic architecture intactness as well as glycogen deposition.



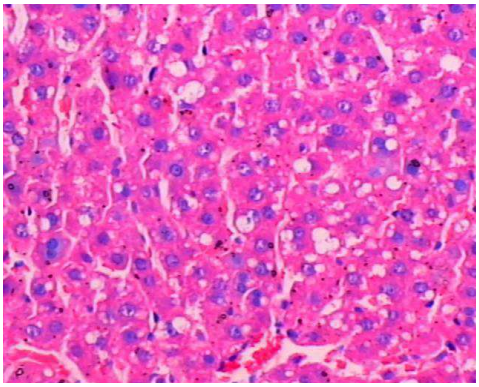
Slide 3. Group 3 (Negative control 1) showing macrovesicular and microvesicular, spotty necrosis and mild infiltration of inflammatory cells, accumulation of fats and depletion of glycogen stores.



Slide 4. Group 4 (Negative control 2) showing allooning of epatocytes, focal vacuolated fatty change, vacuolar degeneration and necrosis.



Slide 5. Group 5 (ALD rats + with fish oil) showing slight steatosis (regeneration of cells), but no inflammation or necrosis roughly coming back to normal histology.



Slide 6. Group 6 (NALD rats + with fish oil) showing complete absence of necrosis and slight inflammation, however steatosis was still present but tended to decrease.

DISCUSSION

In recent times, fish oil rich in unsaturated fatty acids has attracted attention due to its ability of reduction of accumulation of fats in the liver [37]. [38] and [39] suggested that polyunsaturated fatty acids rich fish oil has the potential to reduce Alcoholic FLD (AFLD) and Non Alcoholic FLD (NAFLD), prevent insulin resistance associated with type II diabetes and obesity, up regulates immune reacted genes, etc. The present study is a fact finding mission to unravel the importance of fish oil and its consequence on fatty liver diseases. In the present study, ethanol and methotrexate along with feed were used to induce AFLD and NAFLD in experimental rats.

Ethanol or any drug mediated liver damage progresses through the course of fatty liver, hepatitis, fibrosis and cirrhosis [40]. The severity and prognosis of ethanol-mediated liver diseases mainly depend on the amount, frequency and duration of alcohol intake, coupled with other factors such as the presence of inflammation, diets, nutritional status and genetic makeup of the host. The increase in cell size could be the basis for ballooning of the hepatocytes. Present observations in ethanol intoxicated animals were in concurrence with the earlier findings of [41 and 42].

The liver weight increased in the intoxicated groups compared to normal and positive control groups. The group 5 and 6 which were intoxicated and supplemented with fish oil normalized the liver weight, while it never decreased in the other groups 3 and 4 (intoxicated alone) which was supported by the works of [43]. The serum cholesterol levels were also in elevated levels in the intoxicated groups 3 and 4 (83±6 and 86±5 mg/dl, respectively), where as in fish oil supplemented groups 5 and 6 (74±4 and 76±3 mg/dl, respectively), the serum cholesterol levels lies in between the positive (62±4 mg/dl) and negative control groups. Chronic intoxications showed significant increase in serum transaminases, ALP and Bilirubin levels, which are the indices of liver injury. The elevated levels are primarily due to the leakage of cellular enzymes into the blood stream, leading to their increase in serum [44]. In the present study, AST of intoxicated groups was almost 3 times greater than ALT, which is in agreement with the results of [45, 46, 47, 48 and 49]. It could be noticed that the rats of both negative control groups showed significant elevation in the levels of AST and ALT, compared to those of positive control and normal group. In this aspect, [50] reported that a drug induced hepatotoxicity paved way to significant elevation of parameters such as ALT, AST, ALP, TB and DB.

Methotrexate is hepatotoxic, induces scare in the liver, followed by the occurrence of fibrosis/cirrhosis occurs; pave way for NAFLD [51], which is more potent in obese conditions [52]. The fish oil supplemented groups (5 and 6) showed positive results against the ethanol and methotrexate intoxication, providing an evidence for their efficacy against the fatty liver disease. The following mechanism was likely to be implicated: the decrease in serum triglyceride content (insulin sensitivity is related to serum triglycerides) due to fish oil supplementation [53, 54]. When fish oil is substituted as diet, which are enriched in saturated or polyunsaturated fatty acids, liver insulin resistance does not occur. ω-3 Long Chain PUFA (ω-3 LC-PUFA) decreases the activity and the expression of glucose-6-phosphatase, the last enzyme responsible for liver glucose output and ω-3 PUFA stimulate fatty acid oxidation in the liver via the activation of peroxisome proliferator activated receptor α (PPAR-α) [55, 56]. Such an inhibiting effect of ω-3 Long Chain Poly Unsaturated Fatty Acids (LC-PUFA) on 6-ATPase activity could overcome it's over activity and help to restore normal exhibition of hepatic glucose production. [54 and 57] opined that substitution of fish oil in the diet reduces liver triglyceride content. ω-3 LC-PUFA exert their effects by coordinately suppressing lipogenesis and upregulating fatty acid oxidation.

Immune reaction-related genes, antioxidant genes (several glutathione transferases, uncoupling protein 2, and Mn-superoxide dismutase) and lipid catabolism-related genes were upregulated in rats fed with fish oil, whereas cholesterol and fatty acid synthesis-related genes, 17-alpha hydroxylase and sulfotransferase genes (related to production of endogenous PPARα ligands and Reactive Oxygen Species (ROS)) were down regulated markedly when supplemented with fish oil. These results indicate that dietary intake of fish oil down regulate the endogenous PPARα activation system and increase antioxidant gene expressions to protect against ROS, if in excess. Several long term feeding studies proved that, fish oil fatty acids have been shown to divert fatty acids from esterification with glycerol to form triglycerides through beta-oxidation [58-61]. Detailed *in vitro* studies are needed to clearly elucidate the molecular mechanisms by which fish oil fatty acids inhibit triglyceride secretion. [62] endorsed our results, in which they mentioned that monounsaturated fatty acids are responsible for the decrease of triglycerides in the liver of rats with NAFLD, but do not provide the greatest antioxidant activity.

PUFA may restore the expression of the PPAR-α, whose expression is down regulated by ethanol [63]. Alternatively, PUFAs facilitate the uptake of fatty acids by directly activating the carnitine-palmitoyl transferase-1 for efficient fat oxidation [64]. Though not measured in this study, our results demonstrates that PUFA and MUFA rich fish oil blocked the ethanol mediated acetyl-CoA carboxylase (ACC) activation and thus protect against oxidative inactivation of 3- ketoacyl-CoA thiolase **(**involves in fat oxidation). Based on these results, our study provides a new mechanism of regulating fatty liver by dietary manipulation through inhibition of CYP2E1, sources of ROS. Our current results are reliable with protective roles of PUFA against alcoholic liver fibrosis or cirrhosis in non-human primates [65 and 66]. Therefore, the results of the present study and from other investigators emphasize the Importance of the fatty acids in preventing alcohol and methotrexate induced liver steatosis. In conclusion, our results strongly indicate the beneficial effects of PUFAs on the incidence of fatty liver in animal models, which might be due to prevention against increased ROS following alcohol and methotrexate exposure.

Histopathological evaluation in the liver of intoxicated groups showed the presence of an alimentary load of triglycerides, which is in corroboration with studies done by [67, 68 and 69]. These observations suggested that the results of the present study which exhibited cellular disorder, lipid accumulation, vacuolar degeneration, necrosis and prominent vacuolated Ito cells in the hepatocytes of both ethanol and methotrexate intoxicated groups confirms the Steatohepatitis stages. It is clearly apparent in our study that the effect of ethanol and methotrexate on the liver concurrently increased with intestinal absorption of triglyceride. [70] described ultra structural changes in liver cells of rats, which are acutely intoxicated with ethanol which were apparently not absorbing fat from the intestine. They described mitochondrial swelling and the gradual accumulation of “hollow lipid spherules” which gradually became “solid lipid droplets” during the development of hepatic steatosis. Histopathological studies showed steatohepatitis in both ethanol and methotrexate intoxicated groups, where as in groups which are fed with fish oil along ethanol and MTX showed ameliorating effects. These findings suggest that the fish oil prevents the eventual increase in the release of lipoproteins from the liver even after prolonged intoxication with the development of liver dysfunctions. The results of our findings were similar with that of [71], were the liver damage caused by alcohol intoxication was easily reversed; however, it was not reversible in the case of NAFLD in the present study, which still remains unknown. This situation warrants the need of rational and more in-depth studies pertain to this aspect.

CONCLUSION

The present investigation reveals that the fish oil extracted under Direct from the tissues of *Sardinella fimbriata* showed better results both in quantity and quality wise. The crude fish body oil showed positive results in the reduction of the fatty liver disease, thereby exhibiting hepatoprotective property against both alcoholic and non alcoholic fatty liver diseases. However, more clinical trials are required in molecular levels so to confirm the hepatoprotective property of the fish oil extracted from these low valued fishes. Hence the study concludes with the recommendation for the consumption of fish body oil obtained from *Sardinella fimbriata* which are very vital for human consumption.

REFERENCES

[1] M. Thursz. Fatty liver and Nash. British liver trust: Fighting liver disease (FLD), **2007** 1-36.
[2] E.S. Zafrani. *Virchows Arch.*, **2004**, 444: 3-12.
[3] U. N. Das. *Biotechnol. J.*, **2006**, 1: 420–439.
[4] E.J.A. Schaefer, H. Lichtenstein, S.L. Fava, J.R. McNamara, M.M. Schaefer, H. Rasmussen. *JAMA.*, **1995**, 274: 1450–1455.
[5] C.P. Day. *Liver Int.*, **2006**, 26: 1021–1028.
[6] R.J. Pawlosky, N. Jr. Salem. *Am. J. Clin. Nutr.*, **1995**,61: 1284–1289.
[7] N.Jr. Salem, M. Reyzer, J. Karanian. *Lipids.*, **1996**, 31: 153–156.
[8] E. Albano. Alcohol, oxidative stress and free radical damage. *Proc. Nutr. Soc.*, **2006**, 65: 278–290.
[9] D.D.H. Findik, D. Shafer, E. Klein, N. Timchenko, H. Kulaksiz, D. Clemens. *J. Biol. Chem*., **2006**, 281, 22974–22992.
[10] T. Ganz, E. Nemeth. *Am. J. Physiol.*, **2006**, 290: 199–203.
[11] S.H. Caldwell, D.M. Crespo, H.S. Kang, A.M. Al-Osaimi. *Gastroenterology*., **2004**,127 (5): 97-103.
[12] G. Marchesini, M. Babini. *Minerva Cardioangiol.*, **2006**, 54: 229-239.
[13] C. Postic, J. Girard. *J. Clin. Invest.*, **2008**, 118: 829-838.
[14] E. Park, A. Giacca. *Future Lipidol.*, **2007**, 2: 503-512.
[15] G. Targher, L. Bertolini, R. Padovani, S. Rodella, R. Tessari, L. Zenari. *Diabetes Care.*, **2007**, 30: 1212–1218.

[16] G. L. Vega, M. Chandalia, L.S. Szczepaniak, S.M. Grundy. *Hepatology.*, **2007**, 46: 716–722.
[17] M.I. Fernandez, M.I. Torres, A. Gil, A. Ríos. *Scand. J. Gastroenterol.*, **1997**, 32: 350-356.
[18] E. Mezey. Dietary fat and alcoholic liver disease. *Hepatology.*, **1998**, 28: 901–905.
[19] E.W. Kraegen, P.W. Clark, A.B. Jenkins, E.A. Daley, D.J. Chisholm, L.H. Storlien. *Diabetologia.*, **1986**, 29: 192–198.
[20] L.H. Storlien, E.W. Kraegen, D.J. Chisholm, G.L. Ford, D.G. Bruce, W.S. Pascoe. *Science.*, **1987**, 237: 885–888.
[21] E.W. Kraegen, P.W. Clark, A.B. Jenkins, E.A. Daley, D.J. hisholm L.H. Storlien. *Diabetes.*, **1991**, 40: 1397–1403.
[22] L.H. Storlien, A.B. Jenkins, D.J. Chisholm, W.S. Pascoe, S. Khouri, E.W. Kraegen. *Diabetes*, **1991**, 40: 280–289.
[23] N.D. Oakes, G.J. Cooney, S. Camilleri, D.J. Chisholm, E.W. Kraegen. *Diabetes*, **1997**, 46: 1768–1774.
[24] M. Taouis, C. Dagou, C. Ster, G. Durand, M. Pinault, J. Delarue. *Am. J. Physiol. Endocrinol. Metab.*, **2002**, 282: 664–671.
[25] L.S. Jacoby, C. Smythe, H. Phetteplace, A. Tabares. *JPEN J Parenter Enteral Nutr.*, **1992**, 16: 353-8.
[26] R. Hernandez, E.M. Lara, A. Canuelo. *World J. Gastroenterol.*, **2005**, 11: 7480-5.
[27] H.S. Lai, , W.H. Lin, P.R. Chen, H.C. Wu, P.H. Lee, W.J. Chen. *JPEN. J. Parenter. Enteral. Nutr.*, **2005**, 29: 401- 407.
[28] M.A. Huang, J.K. Greenson, C. Chao, L. Anderson, D. Peterson, J. Jacobson, *Am. J. Gastroenterol.*, **2005**,100: 1072 – 81.
[29] L. Jendrassik, P. Grof. *Biochem.*, **1938**, 297:81.
[30] G. Szasz. Methods of Enzymatic Analysis Ed. Bergmeyer, H. U. **1974**, 2-715.
[31] P. Trinder, *J. clin. Path.*, **1969**, 22: 158-161.
[32] D.S. Young, L.C. Pestaner, V. Gibberman. *Gun. Chem.*, **1975**, 21:1D.
[33] Fossati, L. Prencipe. *Clin. Chem.*, **1982**, 28(1): 2077–2080.
[34] J.R. Crouse, J.S. Parks, H.M. Schey, F.R. Kahl. *J. Lipid Res.*, **1985**, 25: 566-574.
[35] T. Gordon, *Am. J. Med*., **1977**, 62: 707-714.
[36] A. Awasthi, A. Das, R. Srinivasan, K. Joshi. *Histopathology*, **2004**, 45: 260-7.
[37] C.A. Lang, R.A. Davis. *Journal of Lipid Research*, **1990**, 31: 2079 – 2086.
[38] M. Takahashi, N.T. Kasaoka, T. Nakatani, M. Ishii, S. Tsutsumi, H. Aburatani, O. Ezaki. *Am. J. Physiol. Gastrointest. Liver Physiol.*, **2002**, 282: G338–G348.
[39] J. Delarue, C. Lefoll, C. Corporeau, D. Lucas. *Reprod. Nutr. Dev.*, **2004**, 44: 289–299.
[40] W. Lands, R.J. Pawlosky, N. Salem Jr. Alcoholism, antioxidant status, and essential fatty acids. In: Papas, AM., editor. Antioxidant Status, Diet, Nutrition, and Health. *Boca Raton, FL: CRC Press*, **1999**, 299-314.
[41] E. Mezey, *The Am. J. Clin. Nutrition*, **1980**, 33: 2709.
[42] G. Yuan, Z. Gong, X. Zhou, P. Zhang, X. Sun, Xi Li. *Int. J. Mol. Sci*., **2006**, 7: 204.
[43] R.S.A. Ismail, A.A.A.E. Megeid, A.R.A. Moemin. *German Medical Science*, **2009**, 7: 1612-3174.
[44] K.M. Soliman, M.A. Hamed, S.A. Ali. *Medical J. Islamic World Academy of Sci.*, **2006**, 16 (2): 77.
[45] O. Sanchez, M. Viladrich, I. Ramírez, M. Soley. *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, **2007**, 293(5): 1908-16.
[46] A.N.A.Wabel, H.M. Mousa, O.H. Omer, A.M.A. Salam. *Int. J. food agriculture environ.*, **2007**, 5(3- 4): 169-72.
[47] N. Cengiz, H. Ozbek, A. Him. *Pharmacology online.*, **2008**, 3: 870-874.
[48] A.A.R. Moemin, E.M. Mahamoud, E.M. Ghalab, M.K. Abdel-Rahman. *Agricultura. Sci. J.*, **2008**, 6: 30-7.
[49] T.H. Young, H.S. Tang, Y.C. Chao, H.S. Lee, C.H. Hsiong, L.H. Pao, O.Y. Hu. *Lab. Anim.*, **2008**, 42 (4): 495-504.
[50] M.A. Mansour, *Life Sci.*, **2000**, 66 (26): 2583-91.
[51] H. Zachariae, *J. Am. Acad. Dermatol.*, **2000**, 42: 531-534.
[52] J. Almeyda, H. Baker, G.M. Levene, D. Barnardo J.W. Landells. Methotrexate, Alcohol, and Liver Damage. *British Medical Journal*: Winter Meeting of the British Association of Dermatology, **1971**, 167.
[53] B.M. Jucker, G.W. Cline, N. Barucci, G.I. Shulman. *Diabetes.*, **1999**,48: 134–140.
[54] P. Simoncikova, S. Wein, D. Gasperikova, J. Ukropec, M. Certik, I. Klimes, E. Sebokova. *Endocr. Regul.*, **2002**, 36: 143–149.
[55] N. Daniele, J.C. Bordet, G. Mithieux. *J. Nutr.*, **1997**, 127: 2289–2292.
[56] F. Rajas, A. Gautier, I. Bady, S. Montano, G. Mithieux. *J. Biol. Chem.*, **2002**, 277: 15736–15744.
[57] S. Neschen, I. Moore, W. Regittnig, C.L. Yu, Y. Wang, M. Pypaert, K.F. Petersen, G.I. Shulman. *Am. J. Physiol. Endocrinol. Metab.*, **2002**, 282: E395–E401.

[58] S.H. Wong, P.J. Nestel, R.F. Trimble, G.B. Storer, R.J. Illman. D.L. Topping. *Biochim. Biofihys. Acta.*, **1984**, 792: 103-109.
[59] S. Bergseth, E.N. Christiansen, J. Bremer. *Lipids*, **1986**, 21: 508-514.
[60] E. Christensen, T.A. Hagve, B.O. Christophersen, **1986**. *Biochim.. Biophys. Acta.*, 879: 313-321.
[61] R.K. Yamazaki, T. Sehen, G.B. Schade. *Biochim. Biophys. Acta.*, **1987**, 920: 62-67.
[62] O. Hussein, M. Grosovski, E. Lasri, S. Svalb, U. Ravid, N. Assy. *World J. Gastroenterol.*, **2007**, 13(3): 361-368.
[63] M. Fischer, M. You, M. Matsumoto, D.W. Crabb. *J. Biol. Chem.*, **2003**, 278: 27997–28004.
[64] M.E. Surette, J. Whelan, K.S. Broughton, J.E. Kinsella. *Biochim. Biophys. Acta.*, **1992**, 1126: 199–205.
[65] C.S. Lieber, M.A. Leo, S.I. Aleynik, M.K. Aleynik, L.M. De Carli. *Alcohol Clin. Exp. Res.*, **1997**, 21: 375–379.
[66] R.J. Pawlosky, B.M. Flynn, N.JR. Salem. *Hepatology*, **1997**, 26 (6): 1386-1392.
[67] S. Mallov, J.L. Bloch. *Am. J. Physiol.*, **1956**, 184: 29.
[68] N.R. Di Luzio. *Am. J. Physiol.*, **1958**, 194: 453.
[69] C.S. Lieber, L.M. DeCarli, R. Schmid. *Biochem Biophys. Res. Commun.*, **1959**, 1: 303.
[70] A. Takada, E.A. Porta, W.S. Hartroft. *Am. J. Pathol.*, **1967**, 51: 929-957.
[71] T. Ide. *Diabetes*, **2005**, 54: 412-423.