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Assessment of hepatoproductive effect of spiny Eel fish Oil against alcohol induced liver marker enzymes and genotoxity in Albino Rats

Ragadevi M[1], Chitra E[1], Jayasurya K[1], Sakthivel M[1], Uthayakumar V[1*], Chandirasekar R[1], Sreedevi PR[2], Ramasubramanian V[3], and Sivasankar V[1]

[1]Department of Zoology, Sri Vasavi College, Erode, Tamilnadu, India | [2]Department of Zoology, Mar Athanasius College, Kothamangalam, Kerala, India | [3]Unit of Aquatic Biotechnology and live feed culture, Department of Zoology, Bharathiar University, Coimbatore, Tamilnadu.
Corresponding author, email: uthaya.kumar7777@gmail.com

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ABSTRACT

In the present study hepatoprotective activity of fish oil against alcohol induced hepatic damage in albino rats was evaluated. Hepatic injury was induced by administering 3 mL of 40% alcohol orally. The levels of liver marker enzymes such as ALT, AST, ACP, ALP, LDH, SGPT, SGOT and GGT significantly increased ($P<0.05$) in alcohol induced hepatic injury group when compare to control. But, when the rats are administered with the combination of Eel fish and Cod liver oil mixed standard diet, the levels of liver marker enzymes showed a better reduction than in rats administered with standard diet (control) and Eel fish oil standard diet. The level of DNA damage was assessed by Comet assay and the Mean Tail Length and Mean Tail Movement was found to be significantly lower ($P<0.05$) in rats administered with alcohol plus Eel and Cod liver oil mixed standard diet in comparison with control (standard diet) and Eel fish oil mixed standard diet groups. The number of micronucleated hepatocytes was significantly reduced in rats administered with Eel and Cod liver oil mixed standard diet (0.3616±0.04633) than in standard diet (1.4426±0.30871). On the whole, our study concludes that, oral administration of Eel and Cod liver oil reversed the alcohol induced hepatic injury and thus it can be used as a hepatoprotective agent.

Key words: Eel fish oil, Liver enzymes, Comet assay, Alcohol, Hepatoproductive.

INTRODUCTION

Liver diseases are mainly caused by toxic chemicals, excessive consumption of alcohol, liver infections and autoimmune disorders. Most of the hepatotoxic chemicals damage liver cells mainly by inducing lipid

peroxidation and other oxidative damage. Alcohol induced liver injury (ALI) and disease (ALD) is the major health problems affecting a broad patient population of different gender, race, and social backgrounds (Barrio *et al.,* 2004). In spite of tremendous strides in modern medicine, there are hardly any drugs that stimulate liver function, offer protection to the liver from damage or help regeneration employed in traditional system of medicine for liver affections of hepatic cells (Clinard and Ouazrir, 2002). However, there is no satisfactory therapy for alcoholic liver disease at present.

Long term alcohol consumption prolongs the inflammatory process leading to excessive production of free radicals, which can destroy healthy liver tissue (Nanji, 1994). Determination of the activity of hepatic enzymes released into the blood by the damaged liver is one of the most useful tools in the study of hepatotoxicity. In animals and man, xenobiotic metabolizing enzyme systems are present in most if not all tissues, with the highest concentration found in liver (Ennulat *et al.,* 2010). Expression of these enzymes is influenced by a range of factors including diet, sex, age, environmental exposures, and most importantly, endobiotics and xenobiotic, including drugs and chemicals. Although drug-metabolizing enzyme (DME) systems in the liver, can be rapidly and reversibly up regulated in response to endogenous or exogenous stimuli, a process known as enzyme induction. Increased alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and gamma glutamyl transferase (GGT) activities in liver parenchyma have been described in association with drug-induced CYP450 induction in the rat, the dog, and the human (Xu *et al*., 2005). The serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) are synthesized in the liver, heart and skeletal muscles. Elevation of these enzymes indicates liver, heart, muscle or brain injury (Calbreth, 1992).

Fish fatty acid s is considered as a strong antioxidant and its role as an anticancer agent has been extensively confirmed in most of the human malignancies (Sheikh *et al.,* 2010). Its role in enhancement cytotoxity of anticancer drugs to tumor cells and protection of normal cells was previously reported (Pardini, 2006). Furthermore, the anti inflammatory potential of long chain Omega-3 fatty acids in many chronic diseases has been suggested (Wall *et al.,* 2010). The role of Omega-3 fatty acids in inhibiting proliferation, inducing apoptosis and promoting differentiation in many cancers have been studied (Sun *et al.,* 2009) and relevant finding indicate that fish oil act synergistically with certain chemotherapeutic agents (Wendel and Hellar, 2009). The present study aimed to find the hepatoprotective efficacy of Eel fish and Cod liver oil mixed standard diet in Wistar rats by analyzing liver marker enzymes and the markers of genotoxic effects.

MATERIALS AND METHODS

Study Approval

The study was approved by the Institutional Animals Ethics Committee (722/02/a /CPCSEA-dt 04.12.2006) at Bharathiar University, Coimbatore, Tamil Nadu, India. The Wister strain rats were maintained as per the recommendation of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines and regulations.

Experimental Protocol

Animals were divided into five Experimental groups. **Group I-** (+) Control (standard diet), **Group-II:** (-) Control (alcohol+diet), Group**-III:** Cod liver Oil 5ml/kg/ 3times/day (alcohol+diet). **Group-IV:** 40% Alcohol and Standard Diet mixed with Spiny Eel fish Oil (5ml/kg). **Group-V**: 40% Alcohol and Standard Diet mixed with Spiny Eel fish Oil and Cod Liver Oil.

Liver marker enzymes activity

Experimental animals were sacrificed by cervical dislocation and a part of the liver was washed with ice cold tris-buffered saline, blotted dry and 10% homogenate was prepared using tris buffered saline (pH 7.4). The liver homogenate was centrifuged at 2000 rpm for 10 minutes and the supernatant was used for the experiment. Enzymes like AST, ALT, LDH, GOT and GPT were determined by the method of Reitman and Frankels, 1957. ALP and ACP activity was assayed by the method of Kings Armstrong, 1934. Gamma glutamyl transferase enzyme activity was analysed by the method of Rosalki *et al*., 1970.

Comet assay

The alkaline Comet assay was carried out according to the method described by Singh *et al.,* 1988. At the end of treatment, hepatic cells from liver was removed from rats of experimental groups and control group and thus collected cells washed thrice in Phosphate Buffered Solution (PBS**)** and applied on to a microscope slides with agarose in PBS. The slides were then incubated for 20

min in ice-cold electrophoresis solution, followed by electrophoresis. Measuring the lengths of DNA migration (Comet tail) in these cells was carried out directly by fixing an ocular micrometer in one of eyepieces of the microscope. About 60-100 comets per point were scored.

Micronucleus in Peripheral lymphocytes (Fenech and Morley, 1986)

Hepatic cells were collected from liver at the end of treatment by sacrificing the rats of experimental groups and control groups. Immediately after, live cells were added to 5 ml RPMI-1640 medium with 25% foetal bovine serum, 1% L-Glutamine and 2% phytohaemagg-lutinin. After 72 hour of incubation, cells were harvested by centrifugation. Before dropping cell suspension over the slide, slides were wiped with a small amount of cold fixative and the cell suspension dropped and dried over a hot plate maintained at 40°C. After that, cells were stained with Giemsa and gently washed with distilled water to remove the excess stain. Then, the slides were scanned under a high power microscope to score micronucleated lymphocytes.

Statistical analysis

All the statistical analysis was performed using SPSS 16.0 for windows. One-way ANOVA was used to analyze and compare the results of liver enzymes, DNA tail length and movement and number of micronucleated hepatocytes between different groups of rats. $P<0.05$ was considered as significant.

RESULTS

Liver Marker Enzymes

During the short-term (30 days treatments), the levels of liver marker enzymes such as ALT, AST, ACP and ALP were found to be significantly lower ($P<0.05$) in group V (Alcohol+ Eel fish and Cod liver oil mixed standard diet), group IV (Alcohol+ Eel fish oil mixed standard diet), group III (Alcohol+ Cod liver oil mixed standard diet) in comparison with Group II (Alcohol+standard diet). During the long term (90 days treatment), ALT, AST, ACP and ALP levels were found to be significantly lower ($P<0.05$) in group V, IV, III in comparison with group II (Alcohol+standard diet). When the duration of trea-tment was compared, long term treatment was found to be effective than short term treatment (Table 1).

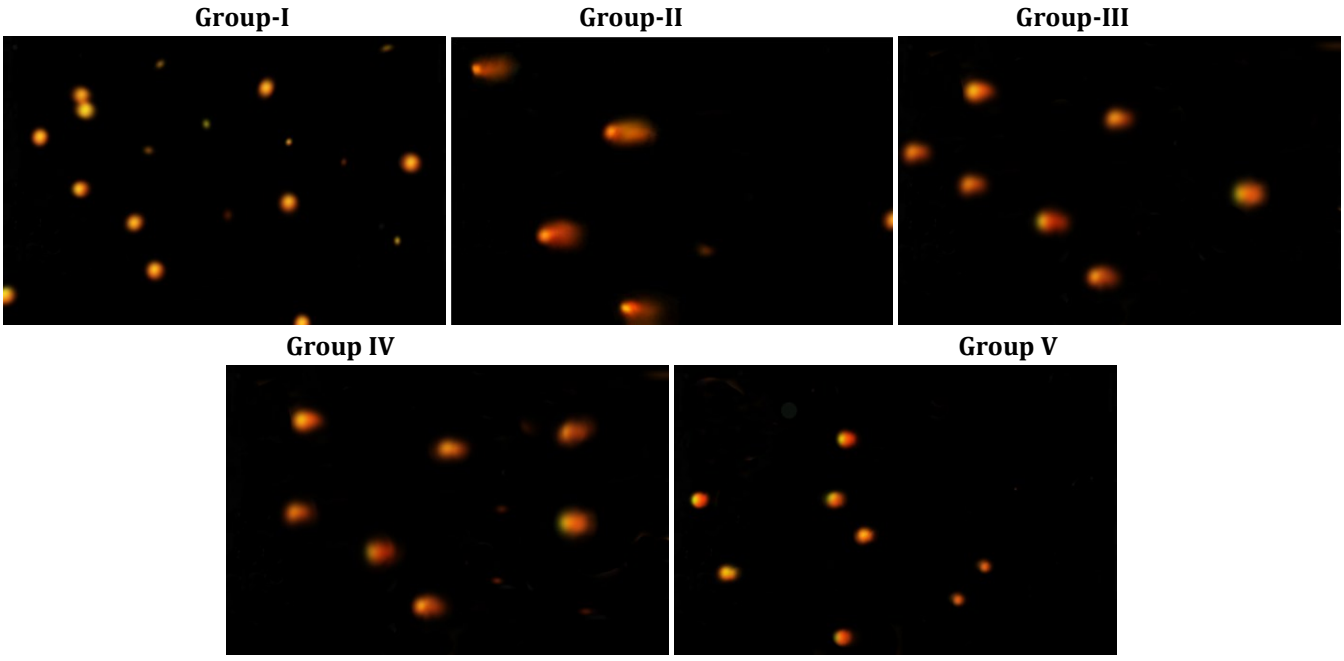

Figure 1 : Photograph of Comet assay in the Control and Experimental Groups rats

Group I – Standard diet; **Group II** – Standard diet+3mL of 40% alcohol; **Group III** - Standard diet+3mL of 40% alcohol+5mL/kg Cod liver oil; **Group IV** - Standard diet+3mL of 40% alcohol+5mL/kg Eel oil; **Group V** - Standard diet+3mL of 40% alcohol+5mL/kg combination of Eel fish oil and Cod liver oil.

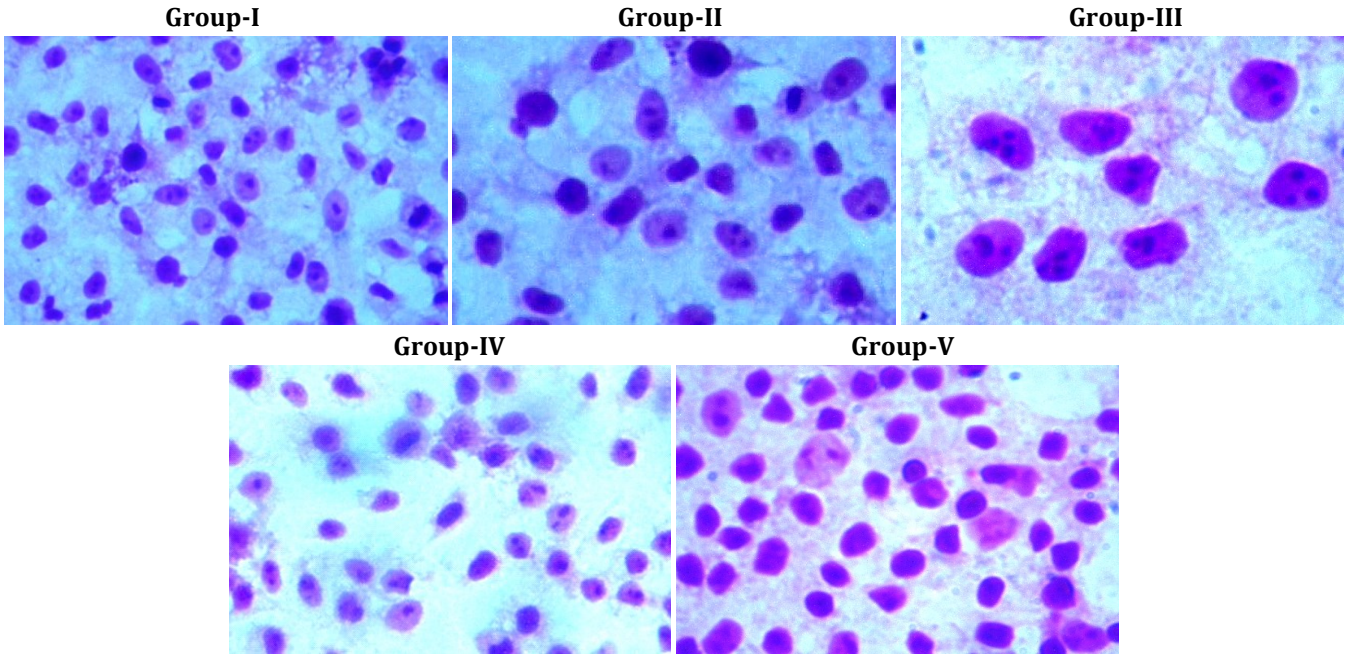

Figure 2: Photograph of Micronuclei in the Control and Experimental Groups rats

Group I – Standard diet; **Group II** – Standard diet+3mL of 40% alcohol; **Group III** - Standard diet+3mL of 40% alcohol+5mL/kg Cod liver oil; **Group IV** - Standard diet+3mL of 40% alcohol+5mL/kg Eel oil; **Group V** - Standard diet+3mL of 40% alcohol+5mL/kg combination of Eel fish oil and Cod liver oil.

Table1: Levels of Alanine transaminase (ALT), Aspartate transaminase (AST), Acid Phosphatase (ACP), Alkaline Phosphatase (ALP) in Liver and Serum of rats

Days	Groups	ALT Serum: Units/ml Liver: micro moles of Pyruvate liberated /min/mg/protein		AST Serum: Units/ml Liver: micro moles of pyruvate liberated /min/mg/protein		ACP Units: micro moles of phenol liberated /min/mg/protein		ALP Units: micro moles of phenol liberated /min/mg/protein	
		Liver	Serum	Liver	Serum	Liver	Serum	Liver	Serum
30 days	I	0.48 ±0.031^{f}	0.71 ±0.010^{g}	0.42 ±0.062^{g}	0.65 ±0.015^{d}	4.34 ±0.026^{i}	5.64 ±0.360^{h}	2.47 ±0.067^{h}	4.23 ±0.502^{g}
	II	0.76 ±0.020^{c}	1.95 ±0.012^{b}	0.98 ±0.038^{b}	1.35 ±0.045^{b}	8.12 ±0.079^{b}	9.86 ±0.045^{b}	7.87 ±0.017^{b}	11.11 ±0.952^{b}
	III	0.67 ±0.020^{d}	1.08 ±0.012^{d,e}	0.62 ±0.081^{c,d}	0.77 ±0.014^{d}	6.72 ±0.020^{e}	7.69 ±0.008^{e}	4.69 ±0.073^{e,f}	7.30 ±0.603^{c}
	IV	0.67 ±0.017^{d}	1.19 ±0.012^{c,d}	0.59 ±0.074^{d,e}	0.73 ±0.046^{d}	6.48±0.086^{f}	7.53 ±0.014^{f}	4.98 ±0.188^{f}	6.90 ±0.080^{c,d}
	V	0.61 ±0.014^{e}	1.02 ±0.120^{e}	0.48 ±0.045^{e,f}	0.69 ±0.014^{d}	6.43 ±0.053^{f}	7.59 ±0.040^{f}	4.36 ±0.065^{f}	7.21 ±0.686^{c}
90 days	I	0.76 ±0.034^{c}	0.87 ±0.320^{f}	0.67 ±0.016^{c,d}	0.71 ±0.010^{d}	5.43 ±0.028^{h}	6.61 ±0.120^{g}	3.20 ±0.524^{g}	6.62 ±0.063^{d}
	II	1.84 ±0.036^{a}	2.91 ±0.140^{a}	2.90 ±0.316^{a}	4.76 ±0.160^{a}	13.55 ±0.070^{a}	12.89 ±0.08^{a}	9.40 ±0.593^{a}	15.19 ±0.035^{a}
	III	0.87±0.022^{b}	1.87 ±0.075^{b}	0.73 ±0.033^{c}	1.23 ±0.012^{b,c}	7.08 ±0.054^{d}	8.94 ±0.029^{c}	4.98 ±0.283^{d,e}	5.98 ±0.091^{e}
	IV	0.91 ±0.054^{b}	1.98 ±0.236^{b}	0.87 ±0.010^{b}	1.19 ±0.000^{b,c}	7.61 ±0.044^{c}	8.76 ±0.030^{d}	5.50 ±0.432^{c,d}	5.88 ±0.091^{e}
	V	0.79 ±0.058^{c}	1.23 ±0.102^{c}	0.98 ±0.014^{b}	1.15 ±0.506^{c}	6.31 ±0.021^{g}	7.66 ±0.075^{e}	5.61 ±0.911^{c}	5.29 ±0.022^{f}

Mean± SD (n=5)

Mean values within the same row sharing the same superscript are not Significant different (P>0.05)

Table 2: Levels of LDH, SGPT, SGOT, GGT in liver and serum of rats

Days	Groups	ALT Serum: Units/ml Liver: micro moles of Pyruvate liberated /min/mg/protein		AST Serum: Units/ml Liver: micro moles of pyruvate liberated /min/mg/protein		ACP Units: micro moles of phenol liberated /min/mg/protein		ALP Units: micro moles of phenol liberated /min/mg/protein	
		Liver	Serum	Liver	Serum	Liver	Serum	Liver	Serum
30 days	I	36.32 ±0.816^{e}	18.76 ±2.440^{e}	65.33 ±0.693^{f}	11.21 ±1.474^{h}	106.25 ±1.500^{g}	57.50 ±2.64^{f}	9.03 ±0.304^{g}	5.26 ±0.315^{d,e}
	II	67.32 ±4.242^{b}	29.76 ±0.778^{b}	68.70 ±2.097^{d}	23.07 ±1.00^{c}	230.99 ±1.818^{b}	76.50 ±1.290^{c}	17.04 ±1.79^{c}	8.34 ±0.509^{b}
	III	42.76 ±3.559^{c}	20.13 ±4.082^{d}	63.72 ±0.920^{g}	12.25 ±0.79^{h}	100.23 ±0.962^{i}	67.50 ±0.577^{e,d}	13.04 ±0.744^{e}	4.08 ±0.681^{f}
	IV	43.98 ±1.632^{c}	18.54 ±3.162^{e}	68.96 ±0.206^{d}	17.27 ±0.426^{g}	108.71 ±1.251^{f}	66.0 ±0.816^{f}	11.71 ±0.335^{f}	4.94 ±0.302^{e}
	V	40.94 ±6.531^{c}	17.61 ±2.82^{e}	62.44 ±0.417^{h}	18.93 ±1.021^{f}	103.49 ±0.571^{h}	58.50 ±1.29^{f}	13.76 ±0.102^{e}	5.31 ±0.252^{d,e}
90 days	I	37.54 ±2.449^{d,e}	24.65 ±5.656^{c}	67.40 ±0.469^{e}	11.77 ±0.272^{h}	121.99 ±1.000^{e}	57.75 ±1.258^{f}	10.86 ±0.84^{f}	7.02 ±0.405^{c}
	II	87.49 ±2.943^{a}	34.88 ±3.265^{a}	178.66 ±0.833^{a}	43.52 ±0.994^{a}	353.06 ±4.375^{a}	126.25 ±1.89^{a}	25.83 ±0.872^{a}	9.24 ±0.602^{a}
	III	43.08 ±3.559^{c}	23.26 ±1.290^{c,d}	72.91 ±0.060^{b}	21.05 ±0.659^{e}	164.12 ±1.36^{e}	69.75 ±3.685^{d}	18.31 ±0.842^{b}	5.66 ±0.021^{d}
	IV	44.65 ±2.828^{c}	25.87 ±1.414^{c}	70.85 ±0.645^{c}	22.0 ±0.816^{d}	167.10 ±0.754^{d}	68.0 ±2.44^{d,e}	15.96 ±0.176^{d}	6.79 ±0.017^{c}
	V	42.78 ±2.160^{c}	22.91 ±1.632^{c,d}	60.46 ±0.646^{i}	26.67 ±0.483^{b}	214.60 ±3.049^{c}	92.0 ±0.816^{b}	18.93 ±0.087^{b}	5.66 ±0.008^{d}

Mean± SD (n=5)

Mean values within the same row sharing the same superscript are not Significant different (P>0.05)

Table 3: Effect of different diet on alcohol induced liver damage

Experimental Groups	Dose (ml/kg)	No. of rats	No. of Mortality	Exposure Periods (Days)	No. of Cells Analyzed	% of DNA Damage	Tail Length (µm)	Mean Tail Movement (%)
Positive Control (+ve)	Standard Diet	6	-	90	100	0^{e}	0^{d}	0^{c}
Negative Control (- ve)	Standard Diet +3ml of 40% Alcohol Thrice a Day Orally	6	2	90	100	40.71 ±3.979^{a} *(0.39716)	63.42 ±14.536^{a} *(01.45126)	22.55 ±8.061^{a} *(0.70544)
Ex1 (Cod Liver Oil)	Standard Diet +3ml of 40% Alcohol+ Cod Liver Oil (5ml/ kg) Thrice a Day Orally Administrated	6	-	90	100	12.26 ±1.306^{b} *(0.13356)	12.94 ±2.734^{c} *(0.27190)	2.07 ±2.384^{b} *(0.25512)
Ex2 (Spiny Eel Oil)	Standard Diet +3ml of 40% Alcohol+ Spiny Eel Oil(5ml/kg) Thrice a Day Orally Administrated	6	-	90	100	4.42 ±0.776^{d} *(0.08011)	11.38 ±2.356^{c} *(0.23753)	0.508 ±0.180^{c} *(0.01812)
Ex3 (Combined Fish oil-Cod + Eel)	Standard Diet +3ml of 40% Alcohol+ Combined Oil (Cod and Eel oil (5ml/kg) Thrice a Day Orally Administrated	6	-	90	100	10.95 ±1.191^{c} *(0.11973)	19.39 ±2.527^{b} *(0.25496)	2.11 ±0.320^{b} *(0.03299)

Mean± SD (n=5)
SE (n=5)

Mean values within the same row sharing the same superscript are not Significant different (P>0.05)

Table 4: Effect of different diet on the number of micronucleated hepatocytes in alcohol induced liver damage

Experimental Groups	Dose (ml/kg)	No. of rats	No. of Mortality	Exposure Periods (Days)	No of Cells Analyzed	MNHEP%/2000 mean Standard Deviation
Positive Control (+ve)	Standard Diet	6	-	90	2000	0.0437 ±0.02208 *(0.00049)
Negative Control (- ve)	Standard Diet +3ml of 40% Alcohol Thrice a Day Orally Administrated	6	2	90	2000	1.4426 ±0.30871 *(0 .00690)
Ex1 (Cod Liver Oil)	Standard Diet +3ml of 40% Alcohol+ Cod Liver Oil(5ml/kg) Thrice a Day Orally Administrated	6	-	90	2000	0.4612 ±0.40060 *(0.00896)
Ex2 (Spiny Eel Oil)	Standard Diet +3ml of 40% Alcohol+ Spiny Eel Oil(5ml/kg) Thrice a Day Orally Administrated	6	-	90	2000	0.2493 ±0.25600 *(0.00572)
Ex3 (Combined Fish oil-Cod + Eel)	Standard Diet +3ml of 40% Alcohol+ Combined Oil (Cod and Eel oil (5ml/kg) Thrice a Day Orally Administrated	6	-	90	2000	0.3616 ±0.04633 *(0.00104)

Mean± SD (n=5)
SE (n=5)
Mean values within the same row sharing the same superscript are not Significant different (P>0.05)

DISCUSSION

In the present study, fish oils decreased the levels of AST and ALT towards the respective control values that were an indication of stabilization of plasma membrane as well as repair of hepatic tissue damage caused by alcohol. A higher level of AST and ALT in the circulation indicates disintegration of cell membrane of liver. The damage provoked by free radicals to macromolecule plays an essential role in the pathophysiological process of atherosclerosis, inflammation, carcinogenesis, aging, drug reaction and toxicity. Alcohol-induced hepatic tissue damage is mediated by acetaldehyde and reactive oxygen species (Zima *et al.,* 2001).

In the present study Cod liver and Eel oil at a dose of 5mL/kg caused a significant decrease in the levels of SGOT and SGPT towards the respective normal range and this is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damage caused by ethanol. Our results are in agreement with a previous study in which Omega 3 fatty acids reduced paracetamol and ethanol induced hepatic injury in rats (Meganathan *et al.,* 2011).

Alcohol drinkers accumulate acetaldehyde in the body and consequently suffer from its genotoxic damage (Singh and Khan, 2010). Alcoholics have been found to have a higher frequency of chromosomal aberrations, sister chromatid exchanges and micronuclei in their lymphocytes than non-alcoholics (Maffei *et al.,* 2000), which is in line with the results of our study, with some slight differences.

DNA damage was detected in liver cells of rats following administration of alcohol. The rats administered with Eel fish and Cod liver oil mixed standard diet showed reduced DNA damage than rats administered with standard diet. A possible reason for the observed reduction in DNA damage in rats administered with fish oil mixed diet may be due to its anti-carcinogenic or anti-oxidant properties. Several investigations documented its beneficiary effect against DNA damage. The fish oil contains various biologically important molecules with anti-cancer and anti-oxidant properties. Omega poly unsaturated fatty acids (PUFAs) present in the eel oil inhibit the arachidonic acid pathway. Eicosapentanic acid (EPA) and Docosahexanic acid (DHA) which are abundant in fish oil, suppress colon carcinogenesis in experimental animals (Takahashi *et al.,* 1997). Conjugated linoleic acids are reported to show anti-carcinogenic properties (Narisawa *et al.,* 1991).

On the whole, our study demonstrated that, fish oil mixed diet can reverse the genotoxic effects of ethanol in ethanol induced hepatic injury. Hence, it is suggested

that, fish oil may be an effective dietary supplement in the management of alcohol induced liver damage. However, detailed studies are required to establish the toxicity and protective effect of this fish oil on ethanol-induced liver disorders in humans before it can be recommended for clinical trials.

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Conflicts of interest: The authors stated that no conflicts of interest.

REFERENCES

Barrio E *et al*. (2004) Liver disease in heavy drinkers with and without alcohol withdrawal syndrome. Alcohol. *Clinical Experimental Research*, 28:131-136.

Calbreth DF (1992) Clinical Chemistry, A fundamental approach, HBF International Edition; 5th Edn.12: 154-169.

Clinard F and Ouazrir K (2002) Incidence of drug induced hepatic injuries in French population based study. *Hepatology,* 36: 451- 455.

Ennulat D, Magid Slav M, Rehm S and Tatsuoka K (2010) Diagnostic performance of traditional hepatobiliary markers of drug-induced liver injury in the rat. *Toxicological Sciences*, 10: 138-144.

Fenech M and Morley AA (1986) Cytokinesis-block micronucleus method in human lymphocytes: effect of in vivo aging and low dose X irradiation. *Mutation Research*, 161: 193-198.

King EJ and Armstrong AR (1934) Convenient method for determining serum and bile Phosphatase activity. *Canad. Med. ASS. Journals*, 31:376.

Maffei F (2000) Increased cytogenetic damage detected by FISH analysis on micronuclei in peripheral lymphocytes from alcoholics. *Mutagenesis,* 15(6): 517–523.

Meganathan M *et al*. (2011) Evaluation of Hepatoprotective Effect of Omega 3-Fatty Acid against Paracetamol Induced Liver Injury in Albino Rats*. Global Journal of Pharmacology* 5 (1): 50-53.

Nanji (1994). Alcoholic liver (Disease). Proceedings of the society for experimental biology and Medicine, 205(3): 243-247.

Narisawa T *et al*. (1991) Inhibitory effect of Perilla oil rich in the n-3 poly unsaturated fatty acid α-linolenic acid on carcinogenesis in rats. *Jpn. J. Cancer Research*, 82: 1089-1096.

Pardini RS (2006) Nutritional intervention with omega-3 fatty acids enhances tumor response to anti-neoplastic agents. *Chemico-Biol. Interact* 162: 89-105.

Reitman S and Frankel S (1957) A Colorimetric method for the determination of Serum Glutamic Oxaloacetate and Glutamic Pyruvic Transominases. *American Journal Clinical Pathology* 28: 56-61.

Rosalki SB, Rau D, Lehmann D and Prentice M (1970) Determination of serum Gamma-Glutamyl Transpeptidase activity and its clinical applications. *Annals of Clinical Biochemistry*, 7: 143-147.

Shaikh IA, Brown I, Wahle KW and Heys SD (2010) Enhancing cytotoxic therapies for breast and prostate cancers with polyunsaturated fatty acids. Nutr Canc, 62(3): 284-296.

Singh NP and Khan A (1995) Acetaldehyde: genotoxicity and cytotoxicity in human lymphocytes. *Mutation Research*. 337(1): 9 -17.

Singh NP, Mccoy MT, Tice RR and Schneider EL (1988) A simple technique for quantitation of low levels of dna damage in individual cells. *Experimental Cell Research*, 175(1): 184-191.

Sun W, Chen G, Ou X, Yang Y, Luo C and Zhang Y (2009) Inhibition of COX-2 and activation of peroxisome proliferator activated receptor γ synergistically inhibits proliferation and induces apoptosis of human pancreatic carcinoma cells. *Canc Lett*, 275(2): 247-255.

Takahashi M *et al*. (1997) Suppression of azoxymethane induced rat colan carcinoma development by a fish oil component Docosahexanic acid (DHA). *Carcinogenesis*, 18: 1337-1342.

Wall R, Ross RP, Fitzgerald GF and Stanton C (2010) Fatty acids from fish: the anti-inflammatory potential of long chain omega-3 fatty acids. *Nutr Rev*, 68(5): 280-289.

Wendel M and Heller AR (2009) Anticancer action of omega-3 fatty acids-current state and future perspectives. *Anticancer Agent Med Chem*, 9(4): 457.

Xu C, Li CY and Kong AN (2005) Induction of phase I, II and III drug metabolism/transport by xenobiotics. *Arch Pharm Reseash*, 28: 249-258.

Zima T *et al*. (2001) Oxidative stress, metabolism of ethanol and alcohol related diseases. *Journal of Biomedical Science*, 8: 59-70.