

Protective Effects of Fish Oil Omega-3 Supplement on Liver-related Biochemical Factors Changes Induced by Thioacetamide in Male Rats

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Exposure to thioacetamide causes hepatotoxicity and hepatocellular carcinoma in human, while fish oil Omega-3 supplement has anti-inflammatory effects. In this study, the protective effect of Fish oil Omega-3 supplement against liver-related biochemical factors changes induced by thioacetamide in rat is investigated. 42 male rats were divided into 6 groups of seven. The control group, The sham 1 group receiving 0.4ml/kg olive oil as the solvent of Fish oil Omega-3 supplement, the sham2 group inter peritoneally receiving a dose of 150 mg/kg thioacetamide at the end of the experiment, the experimental groups 1, 2 and 3 orally receiving a daily dose of 100, 200 and 300 mg/kg Fish oil Omega-3 supplements respectively for 3 month followed by an inter peritoneal dose of 150 mg/kg thioacetamide at the end of the experiment. The serum levels of GGT, LDH, albumin and total protein were measured. Following hematoxylin-eosin staining, liver tissue samples were pathologically studied. The mean level of Albumin showed a significant reduction in experimental groups 1, 2 and 3 receiving thioacetamide. Also, the mean concentration of GGT in the experimental groups 1 and 2 compared to the group receiving thioacetamide decreased significantly; whereas, the mean levels of LDH and total protein showed no significant changes in the experimental groups 1, 2, and 3 ($p < 0.05$). The results of this study indicate that Fish oil Omega-3 supplement has a protective effect on liver-related biochemical factors changes induced by thioacetamide in rat.

Key words: Fish Oil Omega-3 Supplement, Thioacetamide, Biochemical Factors, Male Rat.

Liver diseases are considered one of the most important causes of death in the world¹. Oxidative stresses are known as a mechanism involved in the onset and progress of hepatic damages². The increase in the levels of reactive oxygen and nitrogen species can cause hepatocellular injuries³.

Unsaturated fatty acids are named according to the location of double bond from the carbon of the terminal methyl called Omega carbon. The fatty acids, eicosapentaenoic acid (EPA) and acid and

docosahexaenoic acid (DHA) belong to the Omega-3 fatty acid category. These acids can't be synthesized by humans and should be included in diet; the main sources of Omega-3 are Fish oil, marine planktons and oceanic fish⁴. In a study, Wergedahl et al (2009) showed that combination of fish oil (FO) and fish-protein hydrolysate (FPH) reduce plasma cholesterol levels, which are related to their effects on the reduction of HDL cholesterol level, while the total hepatic cholesterol concentration increased compared to control mice and those receiving FPH and FO alone. The cholesterol reducing effects of combined FPH and FO is related to decreased secretion of low density

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lipoproteins (LDL) from the liver⁵. The studies of Kim *et al.* (2013) demonstrated that Omega-3 fatty acid has protective effects against insulin resistance induced by obesity and liver steatosis. Omega-3 hyperlipidemia induced by diet and fatty liver can be improved through induction of cytochrome CYP7A1 expression and the activity of cholesterol catabolism to bile acids⁶. In a study by Haast *et al.* (2015), a direct correlation was found between Omega-3 fatty acid intake and reduced age-related brain deterioration and damage⁷. Also, Siegel *et al.* (2012) showed that Omega-3 fatty acids have beneficial effects on cerebral-cardiovascular diseases⁸. Likewise, fish oil Omega-3 emulsion significantly reduces hepatic damage following liver transplantation⁹, and has protective effects against liver fibrosis and injuries as well as oxidative stress induced by carbon tetrachloride¹⁰.

Thioacetamide (TAA) is an organic compound containing Thiono-sulfur that is used as a fungicide, an organic solvent and a stabilizer of motor oil. In 1984, Hugh and Nelson first reported that TAA is a hepatotoxic agent. A single dose of this agent can produce lobular necrosis sentry in animals, and chronic induction of thioacetamide can lead to liver cirrhosis and carcinoma¹¹. The toxic effects of thioacetamide is due to its biological activity exerted through oxidase systems, particularly FAD mono oxygenases and CYP2E1¹². Hence, in conjunction with the measurement of hepatic enzymes, thioacetamide can be useful in pharmaceutical research to induce pathological condition, because many blood factors and enzymes are synthesized in hepatocytes and their measurement is a diagnostic criteria for liver function.

Due to high prevalence of liver disease and the high side effects of current chemotherapies, there is a growing need for a medicine (s) with minimum complications and high effectiveness. In this regard, the use of fish oil Omega-3 is most promising, since it has antioxidant and anti-inflammatory properties and shows low side effects. Thus, in this research we tried to examine the possible protective effects of this oil on changes in the levels of gamma glutamyl transferase (GGT), lactate dehydrogenase (LDH), albumin and total proteins induced by thioacetamide in pretreated rats.

MATERIALS AND METHODS

Laboratory animals

In This experimental study, 42 adult male Westar rats in a weight range of 200±10g and the age range of 2.5-3 months were used. Animals were randomly divided in 6 Groups of seven, and kept under standard conditions of 20-22 ° c and light cycle of 12 hours light and 12 hours dark. They had easy access to food and water, and all ethical considerations and animal rights were ensured.

Animal treatment : Animals were divided into 6 groups and were treated as follow: the Control which left untreated and subjected to no stresses; the sham 1 which daily received 0.4ml/kg olive oil as a solvent supplement of Fish oil omega-3; the sham 2 which received a single dose of 150 mg/kg thioacetamide; the experimental groups 1, 2 and 3 received a daily dose of 100, 200 and 300 mg/kg Fish oil Omega-3 supplements respectively followed by a single dose of 150 mg/kg thioacetamide. Fish oil omega-3 was administered orally for 3 months, and thioacetamide was injected interperitonally at the end of treating period.

48 hours after the last injection, animals were anesthetized with ether, and blood samples were taken from the heart. These samples were kept under laboratory conditions for 20 minutes; then, centrifuged at 5000 RPM for 15 minutes^{13,14}.

Serum concentrations of various parameters were measured by appropriate methods: LDH by buffer phosphate method and albumen by Bromocresol Green method, total protein by biuret reaction end point method¹⁵, and GGT by enzymatic method¹⁶.

Statistical analysis

The software program SPSS18 and statistical ANOVA test were used for data analysis. In order to study statistically significant differences among data, the Tukey HSD test was used and significant average difference was sat at $P \leq 0.05$. The plasma concentrations of GGT, LDH, total protein and albumin are presented as average \pm deviation mean \pm SE¹⁷.

RESULTS AND DISCUSSION

In addition, the mean serum concentration of bilirubin rose significantly in the sham 2 group relative to control and sham 1 groups

while it showed no significant changes in all experimental groups relative to control and sham 1 groups, but a significant decline was seen between all three experimental groups, and the group receiving thioacetamide ($p \leq 0.05$). Also, In regard to concentrations of total proteins, no significant changes were observed between group receiving thioacetamide, and control and sham 1 groups as well as between various experimental groups, and control, sham 1 and sham 2 groups (table 1).

In contrast, The mean levels of albumen increased significantly in Group receiving thioacetamide compared with control and sham 1 groups, whereas it showed no significant changes in all experimental groups relative to control and sham 1 groups, but a significant decline was seen between all three experimental groups, and the group receiving thioacetamide (table 1).

The mean concentrations of GGT and LDH indicated a significant increase in the sham 2 group compared with control and sham 1 groups. Nevertheless, the mean level of GGT did not show a significant change in all three experimental groups relative to the control and sham 1 groups while the mean level of LDH rose significantly in these

groups. Conversely, the average concentration of GGT in experimental groups 1 and 2 (receiving 100 and 200 mg/kg fish oil omega-3 supplement and thioacetamide respectively) showed a significant decrease relative to sham 2 group whereas no differences were observed in the mean concentration of LDH among all three experimental groups and sham 2 group ($p \leq 0.05$; table 1).

Letter a Represents significant differences between group receiving thioacetamide alone (sham 2), and control and sham 1 groups at the level of $P < 0.05$; letter b represents significant differences between sham 2 group, and various experimental groups (fish oil omega-3 supplement and thioacetamide) at the level of $P < 0.05$; and letter c represents significant differences among different experimental groups, and control and sham 1 groups at the level of $P < 0.05$.

Liver injury was also determined by biochemical parameters(plasma SGPT, SGOT, ALP,LDH levels).Many useful medicines such as acetaminophen, and some industrial and environmental toxins can cause severe liver damage through functional interference with reactive free radicals. One of these industrial toxins is

Table 1. The effects of different amounts of fish oil omega-3 supplement on various biochemical serum parameters in Male rats poisoned by thioacetamide

All group	Total protein (mg/dL)	Albumin (mg/dL)	GGT (U/L)	LDH (U/L)
Control	0.5±8.88	0.03 ^a ±4.30	0.26 ^a ±2	6.58 ^a ±554.28
Sham1	0.07±8.70	0.04 ^a ±4.30	0.22 ^a ±2.50	33.84 ^a ±599.66
Sham2(TAA)	0.08±8.56	0.00±4.70	0.28±4.50	0.57±849
100mg/kg omega3+TAA	0.05±8.58	0.02 ^b ±4.40	0.15 ^b ±2.17	18.17 ^c ±962.75
200mg/kg omega3+TAA	0.20±8.53	0.10 ^b ±4.32	0.18 ^b ±2.20	9.59 ^c ±955.25
300mg/kg omega3+TAA	0.24±8.63	0.10 ^b ±4.34	0.25±3.75	30.23 ^c ±1022

thioacetamide. This toxin induces hepatic centrilobular necrosis, liver cirrhosis and hepatocellular carcinoma (HCC) ¹⁸.

According to the results of this study, the values of albumin, bilirubin,LDH, GGT in groups treated with thioacetamide increased significantly compared to the control and sham1 groups. The mean serum albumin concentration in all experimental groups receiving fish oil omega-3 supplement and thioacetamide significantly decreased compared to the thioacetamide group. The mean serum GGT in the experimental

groups receiving 100,200 mg/kg of fish oil omega-3 supplement and thioacetamide significantly decreased($P < 0.05$). This means that the fish oil omega-3 supplement had protective effects on liver –related biochemical factors against damage caused by thioacetamide .

Similarly, Chen *et al.* (2012) showed that DHA has beneficial effects on cholestatic liver disease. The beneficial effects of DHA supplement are related to its strong anti-inflammatory and anti-oxidative effects as well as down-regulation of NF- κ B, signaling of the transforming growth factor-

beta and Smad protein through functional interference in the activity of extracellular signal regulating kinase (ERK)¹⁹. More recently, Sherif *et al.* (2015) found that cod liver oil can improve damage induced by sodium nitrite through several mechanisms, including blocking of cell death signs, fibrotic mediators and inflammatory cytokines induced by sodium nitrite²⁰. Other evidence showed that DHA supplement and fish oil EPA Omega-3 may be the preventive agents in the treatment of liver cirrhosis in mice²¹.

According to Li *et al.* (2014), consumption of diet containing fish oil can reduce systemic inflammation and liver damage induced by infection through up-regulation of the peroxisome proliferator-activated receptor gamma-mediated pathway (PPAR) in septic mice²². Also, Jangale *et al.* (2013) determined that fish oil and flax seed oil can alleviate inflammation in diabetic mice induced by streptozotocin-nicotinamide²³. Kim *et al.* (2013) showed that diet containing Omega-3 can attenuate Hepatic damage caused by ischemia and tissue Reperfusion via reduction of NF-Kb activity²⁴. At the same time, Popescu *et al.* (2013) indicated that Omega-3 fatty acid along with diet containing natural calorie and diet with natural lipid has protective effects on nonalcoholic fatty liver disease²⁵. Other studies have shown that fish oil diet prevents hepatocyte cancer in B6C3F1 mice²⁶. Similarly, Omega-3-rich fish oil improves liver damage caused by LPS through the inhibition of TLR4 signaling pathway and NOD²⁷.

De Meijer *et al.* (2009) showed that emulsion based on fish oil prevents parenteral nutrition-associated liver disease²⁸. Also, Khan *et al.* (2015) demonstrated that fish and flax seed oil can protect against apoptosis, tissue damage and hepatotoxicity induced by nitric oxide; it can reduce lipid peroxidation and improve body's antioxidant system²⁹. Other research showed that the cod liver oil improves hepatic damage induced by sodium nitrite via oxidative stress alleviation, and blocking of MCP-1 and mitochondrial functional response as well as reducing DNA fragmentation³⁰. It was also found that due to the presence of the antioxidant compounds, Omega-3, lipid emulsion based on fish oil prevents liver diseases associated with intestinal failure³¹, since omega-3 fatty acid

improves the hepatic inflammatory responses by suppressing inflammatory cytokine production in hepatocytes. In addition, EPA reduces levels of TNF-a and IL-6 in the hepatocytes³², and DHA improves hepatic injuries induced by valproate through reforming of oxidative stresses and inflammation without having any effect on plasma level of valproate³³. Moreover, it has been shown that addition of fish oil supplement to parental diet reforms the increased levels of hepatic enzymes resulted from hepatic mal-function related to parental nutrition³⁴. Studies have shown that 10% fish oil and 1 g% artichoke leaf can restore hepatocellular carcinoma in rats³⁵. Lee *et al.* (2008) determined that Omega-3 fatty acid can repair hepatocellular damage caused by obstruction of the bile ducts³⁶; it was also shown that fish oil along with allopurinol and verapamil improve hepatic injuries resulting from ischemia by a significant reduction in oxidative stress and hepatic enzymes³⁷. In a study by Mardones *et al.* (2012), it was demonstrated that combination of thyroid hormone and fish oil protocol prevents liver damage resulted from tissue injury and ischemia³⁸. Furthermore, Omega-3 fatty acid prevents acute liver defects and stimulates liver regeneration following 90% hepatectomy in rats³⁹. Chiang *et al.* (2009) showed that fish oil can stimulate anti cell proliferation effect of 1- α 25-dihydroxy vitamin D3 on hepatic cancer cells⁴⁰; EPA can also improve hepatic toxicity, oxidative stress and inflammation induced by valproate⁴¹.

In general the results of this study are in line with the results of other studies. It seems that the oral administration of fish oil omega-3 supplement has protective effect on thioacetamide induced liver-related biochemical factors changes by neutralizing free radicals, stimulating the activity of antioxidant enzymes, and reducing the production of inflammatory cytokinin. As no similar study on the protective effects of fish oil omega-3 supplement on liver-related biochemical factors changes could be found, it was not possible to do a comparative study in this respect. Anyway, more studies should be conducted to examine the hepatic antioxidant enzymes and molecular changes inducing apoptosis so that the effects of fish oil omega-3 supplement on healing liver toxicity can be determined with higher certainty.

CONCLUSION

In general, the results of present study showed that Fish oil Omega-3 supplement in rat model with hepatic mal-function can cause desirable improvements. Thus, if supported by more experiments, it is possible to add Fish oil Omega-3 supplement to the diet of patients with liver mal-function.

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