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Evaluation of Hepatoprotective Effect of Omega 3-Fatty Acid against Paracetamol Induced Liver Injury in Albino Rats

[1]M. Meganathan, [1]K. Madhana Gopal, [1]P. Sasikala, [1]J. Mohan, [1]N. Gowdhaman,
[2]K. Balamurugan, [3]P. Nirmala, [3]Sylvia Santhakumari and [3]Vanitha Samuel

[1]Arupadaivedu Medical College and Hospital, Kirmapakkam, Puducherry, India
[2]Department of Pharmacy, Annamalai University, Annamalai Nagar, Chidambaram, India
[3]Division of Pharmacology, RMMC and H, Annamalai University, Annamalai Nagar, Chidambaram, India

Abstract: In the present studies hepatoprotective activity of Omega-3-fatty acids against paracetamol induced hepatic damage in albino rats was evaluated. Hepatic injury was induced by administering 2g/Kg body wt. p.o of paracetamol. Omega-3-fatty acid at dose levels of 100 and 300 mg/Kg/day were administered for albino rats which reduced serum liver enzymes like Aspartate Transferase (AST), Alanine Transferase (ALT), Alkaline Phosphatase (ALP) and serum albumin. The result obtained were compared with silymarin (25 mg/Kg body wt.p.o), the standard drug. In conclusion omega-3-Fatty acids at (300 mg/Kg/day) showed significant $p<0.001$ hepatoprotective activity similar to that standard drug, silymarin.

Key words: Omega-3-Fatty acids · Hepatoprotective activity · Free radicals · Silymarin

INTRODUCTION

Liver is the most important organ, which plays a pivotal role in regulating various physiological processes in the body. It is involved in several vital functions, such as metabolism, secretion and storage. It has great capacity to detoxicate toxic substances and synthesize useful principles [1]. Therefore, damage to the liver inflicted by hepatotoxic agents is of grave consequences. Liver diseases are mainly caused by toxic chemicals, excessive consumption of alcohol, infections and autoimmune disorders. Most of the hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation and other oxidative damages. Paracetamol hepatotoxicity is caused by the reaction metabolite N-acetyl-p-benzo quinoneimine (NAPQI) [2], which causes oxidative stress and glutathione (GSH) depletion. It is a well-known antipyretic and analgesic agent, which produces hepatic necrosis at higher doses [3]. Paracetamol toxicity is due to the formation of toxic metabolites when a part of its metabolized by cytochrome P-450. Introduction of cytochrome or depletion of hepatic glutathione is a prerequisite for paracetamol-induced hepatotoxicity [4-6]. 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 of hepatic cell [7]. There are however, number of drugs employed in traditional system of medicine for liver affections [8].

Through pioneering epidemiological studies in the early 1970s, Dyerberg and Bang [9] proposed the hypothesis that long chain highly unsaturated omega-3-fatty acids occurring in the oil of fishes and other marine animals which the Eskimos consumed produced beneficial effect. Fish and marine life are rich sources of a special class of polyunsaturated fatty acids known as omega-3-fatty acids [10]. Our ancestors consumed food containing a lot more omega-3 fatty acids than we do today. Scientific evidence revealed that a diet rich in long chain omega-3 fatty acids help in the development of healthy brain, heart and immune system. It has a role in joint movement, balanced mood, a sense of well being, strength, stamina and helps to maintain cholesterol levels within the normal range. omega-3-fatty acids contains about 60% of long-chain omega-3 fatty acids DHA and EPA as combined. The most widely available source of EPA and DHA is cold water oily fish such as *salmon, herring, mackerel, anchovies and sardines* [11]. The oil from these fish has a profile of around seven times as much omega-3 as omega-6. Therefore, the present investigation has been

Corresponding Author: M.Meganathan, Arupadaivedu Medical College and Hospital, Kirmampakkam, Puducherry, India, Tel: +91-9443252577, E-mail: dr.mega1969@yahoo.co.in.

designed to study the possible mechanism of omega-3 fatty acids on the biochemical parameter against paracetamol induced hepatic damage in albino rats.

MATERIALS AND METHODS

Animals: The institutional animal ethical committee (Register No.160/1999/CPCSEA), Annamalai University, Annamalai Nagar, India had approved the experimental design (Proposal No.436, dated 21.03.2007). A total of 30 *albino wistar* male rats of 140-160g was used for the study. Animals were housed in well ventilated room (temperature 23 ± 2°C, humidity 65-70% and 12h light/dark cycle) at Central Animal House, Rajah Muthiah Medical College, Annamalai University. Animals were fed with standard pellet diet and water *ad libitum*. All studies were conducted in accordance with Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) norms and the National Institute of Health guidelines "Guide for the Care and use of Laboratory Animals".

Assessment of Hepatotoxicity Activity: Briefly the animals were divided into five groups, six animals in each group. Group I served as the normal control (untreated rats). Group II served as paracetamol control, animals were administered distilled water for 14 days. Group III and IV were administered omega 3-fatty acid of 100 and 300 mg/kg/day for 14 days. Group V was treated with silymarin (25mg/kg body wt, p.o) for 14 days. Paracetamol at a dose of 2g/kg body wt, p.o was administered on the 15th day to all animals in groups II, III, IV and V. The food was withdrawn on preceding night of the experiment. After 48 hrs administration of paracetamol dosing the blood samples were collected in centrifuge tubes by retro orbital puncture under mild katamine anaesthesia (30mg/kg body wt, i.p route) for biochemical estimation.

Determination of Liver Enzymes: The activities of serum aspartate tranferase (AST), serum alanine tranferase (ALT), serum alkaline phosphatase(ALP)and serum bilirubin were assayed by the reporting methods [12,13].

Statistical Analysis: The serum biochemical and tissue enzyme parameters were determined for both test and control. Results were expressed as mean ± SEM; differences in mean values were estimated by the use of ANOVA followed by Dunnet's post hoc test. The minimum level of significance was setup at $P<0.05$.

RESULTS

The activities of serum AST, ALT and ALP were markedly elevated in paracetamol treated animals compared to normal, indicating liver damage. Administration of omega 3-fatty acids at dose 100mg/Kg remarkably prevented paracetamol induced elevation of serum enzymes. omega 3-fatty acids at dose 300mg/Kg has shown pronounced activity.

DISCUSSION

Drug induced hepatotoxicity, with paracetamol as an inducing agent is well documented. This well-known antipyretic and analgesic agent, is safe in therapeutic doses, but can produce fatal hepatic necrosis at toxic doses in humans, rats and mice. Paracetamol is metabolized in the liver via three pathways 1) glucuronidation, 2) sulfation (both account for 95% of metabolism) 3) via the cytochrome P450 enzyme system (5%) [14]. In this pathway, paracetamol is converted to a toxic metabolite, NAPQI. Glutathione (a tripeptide) then binds to this toxic metabolite forming a non-toxic compound. Hepatotoxicity occurs when there is a rapid depletion of glutathione leading to the accumulation of

Table 1: Serum Biochemical Parameters (Effect of Omega-3-fatty acids on serum AST, ALT and ALP, Total Bilirubin and Direct bilirubin in rats)

Groups	AST (U/L)	ALT (U/L)	ALP (U/L)	Total Bilirubin (mg/100ml)	Direct Bilirubin (mg/100ml)
Group I (Normal control)	54.30±0.315	25.21±0.496	96.10±0.194	0.88±0.008	0.25±0.004
Group II (Paracetamol control)	140.02±1.278***	62.11±2.287***	392.13±4.211***	2.16±0.0225***	0.69±0.023***
Group III (Test -I ; Paracetamol +100 mg/kg/day of omega-3-Fatty acids received rats)	65.43±1.304***/a	27.36±0.045***/a	141.26±6.353***/b	0.95±0.01**/a	0.36±0.005**/a
Group IV (Test -II ; Paracetamol +300 mg/kg/day of omega-3-Fatty acids received rats)	59.6±1.352***/a	20.81±0.344***/a	128.45±5.697***/a	0.90±0.008***/a	0.33±0.032***/a
Group V(Reference; Paracetamol + Silymarin treated rats)	52.14±1.254	24.73±0.547	93.42±4.354	0.87±0.346	0.21±0.048

Values are mean ±S.E.M. of six animals in each group. Comparisons: a. Group II compared with group I; b. Group III compared with group II and V; c. Group IV compared with group II and V; *** = P<0.001 highly significant, **/b= P<0.01 moderately significant, * /a=P<0.05 significant`

the toxic metabolite in the liver [15]. This toxicity occurs because of its reactive metabolite, N-acetyl-P-benzoquinoneimine (NAPQI). NAPQI exerts its toxicity primarily via its oxidative effect on cellular proteins.

Sulfhydryl compounds are among the most important endogenous antioxidants. Glutathione (GSH) is the main intracellular non protein sulfhydryl compound which plays an important role in the maintenance of cellular proteins and lipids in their functional states. NAPQI binds to GSH, forming a conjugate which results in conversion of GSH to an oxidized form of glutathione. When GSH levels are lowered, the toxic effects of oxidative insult are exacerbated, resulting in increased membrane and cellular damage. At this point, other protein and non-protein Sulfhydryl groups present in the cell provide an important alternate protection [16,17].

Omega-3-Fatty acids are comprised of the essential fatty acids, Eicosapentaenoic acid (EPA, C20:5 n-3) and Docosahexaenoic acid (DHA, C22:6n-3). Both EPA and DHA fall into an even larger category of polyunsaturated fatty acids (PUFAs). As reviewed earlier, the liver predisposes to oxidative stress presumably by amplifying the capacity of free radical chain reaction.An obvious sign of hepatic injury is the leakage of cellular enzymes into the plasma due to the disturbance caused in the transport functions of hepatocytes. When liver cell membrane is damaged, a variety of enzymes located normally in cytosol is released into the blood, thereby causing increased enzyme levels in the serum such as AST, ALT, ALP and TB. The abnormaly high levels of serum ALT, AST, ALP and TB observed in our study are the consequence of paracetamol induced liver dysfunction which denotes damage to the hepatic cells.

Analysis of the rat serum liver enzymes at the dose of 300 mg/kg/day of Omega-3-Fatty acids revealed a statistically significant lowering of enzymes (i.e.) AST, ALT, with a less marked effect in the levels of ALP and TB. These effects were comparable to the enzyme levels in the Silymarin treated group, which is suggestive of protective action in the liver.With regard to the free radical scavenging enzymes, the increase in the levels of SOD, GSH and CAT is presumably because of its anti-oxidant action, which helps to attenuate oxidative stress. The hepatoprotective effect of omega-3-Fatty acid was further confirmed(as in our previous work) by histopathological examination of the liver.

Possible mechanisms that may be responsible for the protection of paracetamol induced liver damage by omega-3-Fatty acids includes membrane stabilizing action on the hepatocytes. This present study has been shown that omega-3-fattyacids protect the liver against paracetamol induced liver injury. Although the exact mechanism of cytoprotection by omega-3-fattyacids remains unresolved, yet data suggests that this substance could serve as an antioxidant/cofactor that makes the hepatocytes less susceptible to the damaging action of noxious agents. The parameters such as AST,ALT,ALP and TB in blood have been found to be of great importance in the assessment of liver damage.

In conclusion, from this study it was concluded that omega-3-fattyacids, at a dose of 300mg/kg/day protects the liver against paracetamol induced hepatotoxicity in *albino wistar* rats. The probable mechanisms postulated are its membrane stabilizing and antioxidant effects as well as its conversion to lipid protective mediators. Hence omega-3-fattyacids pretreatment with other known hepatotoxic drugs like sodium Valproate would go a long way in reversing or ameliorating the severity of liver injury. As the toxicity with this drug is low, such an innocuous agent like omega-3-fatty acids would be useful to counter the drug induced liver toxicity. However, further studies in humans are yet to be done to substantiate its clinical effectiveness.

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