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Evaluation of antioxidant effect of Omega 3-fatty acid against paracetamol induced liver injury in albino rats

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ABSTRACT

In the present studies hepatoprotective activity of omega -3-fatty acids against paracetamol induced hepatic damage in albino rats was observed. Hepatic injury was induced by administering 2g/Kg body wt. p.o. of paracetamol, omega-3-fatty acid at dose levels of 100 mg/Kg/day and 300 mg/Kg/day were administered for albino rats altered and proved to be hepatoprotective and antioxidant action by increasing the levels of Superoxide Dismutase (SOD), Glutathione (GSH), Catalase (CAT). Wet weight of the liver and histopathological studies further confirmed the hepatoprotective and antioxidant activity of omega-3-Fatty acids when compared with paracetamol treated control groups. 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 and antioxidant activity similar to that standard drug, silymarin.

Keywords: omega-3-Fatty acids, hepatoprotective activity, free radicals, silymarin.

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INTRODUCTION

Liver is the vital organ of metabolism and excretion. About 20,000 deaths found every year due to liver disorders. Hepatocellular carcinoma is one of the ten most common tumors in the world with over 2, 50,000 new cases each year [1]. 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 [2]. There are however, members of drugs employed in traditional system of medicine for liver affections [3]. Many formulations containing herbal extracts are sold in the Indian market for liver disorders. But management of liver disorders by a simple and precise herbal drug is still an intriguing problem.

Paracetamol hepatotoxicity is caused by the reaction metabolite N-acetyl-p-benzo quinoneimine (NAPQI), which causes oxidative stress and glutathione (GSH) depletion. It is a well-known antipyretic and analgesic agent, which produces hepatic necrosis at higher doses [4]. Paracetamol toxicity is due to the formation of toxic metabolites when a part of it's metabolized by cytochrome P-450. Introduction of cytochrome [5] or depletion of hepatic glutathione is a prerequisite for paracetamol-induced hepatotoxicity [6, 7].

Through pioneering epidemiological studies in the early 1970s, Dyerberg and Bang 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 [8]. Our ancestors consumed food containing a lot more omega-3 fatty acids than we do today. Scientific evidence reveals that a diet rich in long chain omega-3 fatty acids helps in the development of healthy brain, heart, and immune system. It has a role in joint movement, balanced mood, a sense of well eing, 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. 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 designed to study the hepatoprotective action of omega-3 fatty acids 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 were used for the study. Animals were housed in well ventilated room (temperature 23 $\pm$ 2^{o}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 antioxidant activity

Liver was excised washed thoroughly in 0.86% ice cold saline to remove the red blood cell. They were then gently blotted between the folds of filter paper and weighed in an analytical balance (wet weight analysis). Then it was suspended in 10 %(w/v) in ice cold buffer 0.05M then phosphate buffer cut into small pieces and homogenized using a polytron homogenizer at 200C. The homogenate was centrifuged at 3000 rpm for 20 min to remove the cell debris, unbroken cells, nuclei erythrocytes and mitochondria. The supernatant was used for the estimation of enzymic and non enzymic anti-oxidants like reduced glutathione (GSH), superoxide dismutase (SOD), Catalase (CAT) activities as described by the method [9-12].

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 AND DISCUSSION

Administration of omega 3-fatty acids at dose 100mg/Kg remarkably prevented paracetamol induced of serum enzymes. Omega 3-fatty acids at dose 300mg/Kg has shown pronounced activity.

Table 1 - Tissue Enzyme Parameters (Effect of Omega -3- fatty acids on Tissue enzymes GSH, SOD& CAT in rats)

Groups	GSH (µmol)	SOD (nmol)	CAT (µmol)
Group I **(Normal control)**	11.01±0.11	5.89±0.21	9.4±0.12
Group II **(Paracetamol control)**	5.31±0.12***	1.02±0.12***	2.9±0.44***
Group III **(Test –I ;** Paracetamol +100 mg/kg/day of **omega** -3-Fatty acids received rats**)**	6.96±0.17**/b	3.64±0.1**/b	6.78±0.01***/b
Group IV **(Test –II ;** Paracetamol +300 mg/kg/day of **omega** -3-Fatty acids received rats)	7.9±0.52***/a	4.34±0.25***/a	7.98±0.55***/a

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Group V **(Reference;** Paracetamol + Silymarin treated rats**)**	10.02±0.14	5.2±0.15	8.92±0.24

Reduced glutathione (GSH)	µ moles oxidised /min /mg of protein.
Superoxide dismutase (SOD)	n mol /mg of protein.
Catalase (CAT)	µ moles of H_2O_2 decomposed /min/ mg of protein.

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 & V; c. Group IV compared with group II & V; *** = P<0.001 highly significant; **/b= P<0.01 moderately significant, * /a=P<0.05 significant.

Table 2 - Wet Weight of the Liver

Groups	Wet weight of the liver (gms)
Group I (Normal control)	3.77±0.070
Group II (Paracetamol control)	6.43±0.022***
Group III (Test –I ; Paracetamol +100 mg of omega -3-Fatty acids received rats)	5.76±0.035**/b
Group IV (Test –II ; Paracetamol +300 mg of omega -3-Fatty acids received rats)	4.02±0.052***/a
Group V (Reference; Paracetamol + Silymarin treated rats)	4.32±0.024

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 & V; c. Group IV compared with group II & V *** = P<0.001 highly significant, **/b= P<0.01 moderately significant, * /a=P<0.05 significant.

As given in table no (1) reduced activities of enzymic and non-enzymic antioxidants and enhanced activity of lipid peroxidation were seen in the paracetamol –treated group, whereas standard Silymarin and the drug treated groups showed significant (p<0.001) rise in antioxidant levels with reduction in lipid peroxidation level when compared with the paracetamol–treated control group. From the tables and figures it is clear that Omega 3-fatty acids showed dose dependent hepatoprotective activity. However Omega 3-fatty acids at dose 300mg/kg **group IV** exhibited relatively higher protective action than dose of 100mg/kg **(group III)**. The weight of the liver at doses of 100 mg/kg/day **(group III)** showed a moderately lower mean weight when compared with **group II**. The decrease in liver weight was also evident, but to a more profound degree in **group IV** at doses of 300 mg/kg/day. (Table no 2).

The Histopathological studies of liver showed intense centrilobular necrosis, derangement and disarray of hepatocytes and periportal inflammatory infiltrates in paracetamol treated group (**group II)**. The drug treated groups at dose 100mg/Kg (**group III)** has

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showed lesser degeneration and sinusoidal dialation and minimal inflammation, whereas in (**group IV)** at dose 300mg/Kg has showed very minimal change in inflammation and no evidence of necrosis. The silymarin treated **group V** showed almost reveals normal histopathological picture. [Fig: A-E].

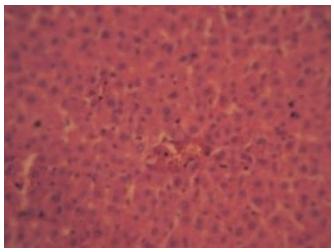

Fig A: Group – I normal control (H &E × 400) Normal cellular architecture

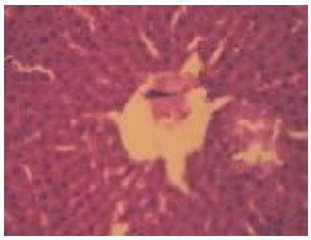

Figure: B- Group II (Paracetamol control) H &E × 400 Intense centrilobular necrosis, Derangement and disarray of hepatocytes and Periportal inflammatory infiltrates seen

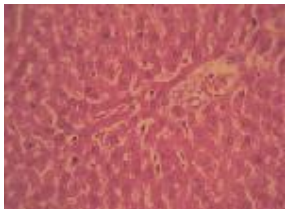

Figure:C -Group III (Test -I) H &E × 400 Lesser degeneration, sinusoidal dialation, minimal infiltration was observed near portal triad.

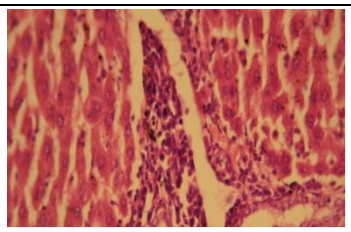

Figure: D - Group IV (Test - II) H&E × 400 No evidence of necrosis, very minimal change in the arrangement of hepatocytes and sinusoids, central vein were apparently normal

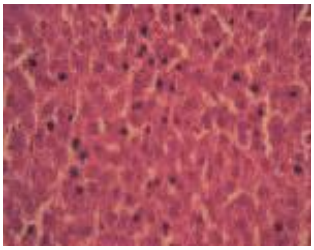

Figure: E - Group V (Reference) H&E × 400
The sheets of hepatocytes were positioned in typical radiation pattern and appears normal

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) or 3)via the cytochrome P450 enzyme system (5%) [13]. 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 the toxic metabolite in the liver

[14]. 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 [15,16]. 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 [15,16]. 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). Compared to saturated fats, PUFAs are more readily used for energy when ingested. Increasing the degree of unsaturation at a given carbon chain length increases the relative mobility of stored fat, making PUFAs more bioavailable.

In 1963, it was discovered that omega-6-AA is converted by the body into pro-inflammatory agents called prostaglandins. Later the other eicosanoids like Thromboxane, Prostacyclins and Leukotrienes were discovered. These eicosanoids have improtant biological function but with short half life, as they are metabolised by enzymes. However if the rate of synthesis exceeds the rate of metabolism, the excess eicosanoids may have deleterious effects. Researchers found that Omega-3-fatty acids are also converted to eicosanoids but at a much slower rate. When both omega-3 and omega-6 are present, they will"compete" to be transformed, so the amount of omega-3 present is directly related to a decrease is eicosanoid production. This competition was recognised as the mechanism for antiplatelet action and vasodilatation. Also its role in blunting the inflammatory response is due to its effect of leukotrienes.

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. The parameters such as GSH,SOD,CAT enzymes in liver tissue have been found to be of great importance in the assessment of liver damage.Analysis of the rat liver enzymes at the dose of 300 mg/kg/day of Omega-3-Fatty acids revealed a statistically significant increase in the levels of SOD, GSH& 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 by histopathological examination of the liver.

Liver sections of the rats of the control group **(Group 1)**, showed normal cellular architecture with distinct hepatic cells, sinusoidal spaces and central vein **(Figure A).** In rats treated with paracetamol **(Group II)**, there is intense centrilobular necrosis,derangement and disarray of hepatocytes with periportal inflammatory infiltrates **(Figure-B).** The histopathological profile of the rats treated with 100 mg/kg/day of omega -3- fatty acids and paracetamol **(Group III),** liver showed minimal infiltration, lesser degeneration and sinusoidal

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dialation indicating moderate hepatoprotective activity **(Figure-C),** Liver sections of the rats treated with 300 mg/kg/day of omega -3- fatty acids with paracetamol **(Group IV)** showed no evidence of necrosis with very minimum change in the arrangement of hepatocytes indicating the significatint hepatoprotective activity **(Figure-D)**. Rats treated with Silymarin along with paracetamol **(Group V)** showed normal hepatocytes, sinusoids, central vein which also appear apparently normal, indicating the significant hepatoprotective activity **(Figure-E)**.

CONCLUSION

Several publications have documented the evidence that agents which trap free radicals or supplement depleted agents like glutathione help to protect against paracetamol hepatotoxicity. Possible mechanisms that may be responsible for the protection of paracetamol induced liver damage by Omega-3-Fatty acids include the following-

[a] Free radical scavenging by antioxidant enzymes SOD and CAT. An increase in the levels of these enzymes intercepts the radicals involved in paracetamol metabolism.
[b] Supplementing glutathione stores which blunts liver injury.
[c] Its membrane stabilizing action on the hepatocytes.

This present study has been shown that Omega-3-fattyacids protect the liver against paracetamol induced liver injury.

Recently a novel series of DHA derived lipid mediators with potent protective action have been identified. *Invitro*, supplementation of hepatocytes with DHA significantly reduced hydrogen peroxide induced DNA damage, as evaluated by "Comet assays" and oxidative stress. [17,18] *Invivo* , dietary supplementation of rat with DHA and DHA & EPA combination ameliorated CCl_4 induced necro inflammatory damage[17]. The two DHA derived lipid mediators detected by HPLC Gas chromatography/Mass spectrometry analysis are 17S- hydroxy DHA and protectin D1 [19]. Taken together ,these findings identify a potential role for DHA derived products, specifically 17S – hydroxy DHA and protectin D1, in mediating the protective effects of dietary DHA and EPA (Omega-3-fattyacids) in liver toxicity.PD-1 is a potent regulator of inflammation, the formation of which results in significant anti-inflammatory action. Our results, which demonstrate abrogation of liver injury by Omega-3-fatty acids dietary supplementation, are consistent with earlier observation suggesting that the amount and type of dietary essential Fatty acids may modulate progression of liver damage.

Recent report also suggest an immuno modulatory role for Omega-3-fattyacids based on the fact that it reduces the release of TNF–α [20,21]. 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.

ISSN: 0975-8585

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