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RESEARCH ARTICLE

ROLE OF OMEGA-3 FATTY ACID IN HEPATOPROTECTION AGAINST CARBON TETRA CHLORIDE INDUCED LIVER INJURY IN ALBINO RABBITS

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ABSTRACT

In the present study hepatoprotective activity of Omega-3-fatty acid against carbon tetrachloride (CCl_4) induced hepatic damage in albino rabbits was evaluated. Hepatic injury was induced by administering 0.05ml/Kg body wt. intraperitoneally of CCl_4. Omega-3-fatty acid at dose levels of 600 mg/Kg/day were administered to albino rabbits that showed protection from hepatic injury. Omega 3-fatty acid reduced the elevated serum liver enzymes like Aspartate Transferase (SGOT), Alanine Transferase (SGPT), Alkaline Phosphatase (ALP) and serum bilirubin. The result obtained were compared with silymarin (100 mg/Kg body wt. p.o), the standard drug. In conclusion omega-3-Fatty acid (600 mg/Kg/day) were found to hvae very highly significant ($p<0.001$) hepatoprotective activity comparable to silymarin.

Key words: Omega-3-Fatty acids, Carbon tetra chloride, Hepatoprotective activity, Silymarin

INTRODUCTION:

Liver is the key organ regulating homeostasis in the body. It is involved with almost all the biochemical pathways related to growth, fight against disease, nutrient supply, energy production and reproduction. Liver is an important target for toxicity produced by drugs, xenobiotics and oxidative stress.[1] Therefore, damage to the liver inflicted by hepatotoxic agents is of grave consequence. Most of the hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation and other oxidative damages.[2] CCl_4 is used as hepatotoxic agent in animal research work to study the hepatoprotective action of plants and other compounds. [3, 4] This model of CCl_4-induced liver injury has been widely used in new drug development for liver diseases.[5] Liver damage induced by carbon tetrachloride (CCl_4) involves biotransformation of free radical derivatives, increased lipid peroxidation and excessive cell death in liver tissue.[6, 7] In spite of tremendous strides in modern medicine, there are hardly any drugs that simulate liver function, offer protection to the liver from damage or help regeneration of hepatic cells.[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 being, strength, stamina, and helps to maintain cholesterol levels within the normal range.[9] Fish and marine life are rich sources of a special class of polyunsaturated fatty acids known as omega -3 fatty acids.[10] Omega-3-fatty acids contain 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 oily fish such as salmon, herring, mackerel, anchovies and sardines. The concept that nutrition may modify or ameliorate the toxicity of environmental chemicals is provocative and warrants further study, the implications for human health could be significant.[11]

So in the present study an attempt is planned to find out whether omega-3 fatty acid can prevent the hepatotoxicity induced by carbon tetra chloride in the laboratory animal like albino rabbits.

MATERIAL AND METHODS:

The study was conducted in the department of pharmacology and therapeutics in collaboration with department of pathology, G.S.V.M medical college

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Kanpur, after clearance from Institutional Animal Ethics committee. The animal care and handling was done as per the guidelines set by CPCSEA and the Indian National Science Academy, New Delhi, India. The study was conducted on 42 healthy albino rabbits of either sex weighing 1.5-2.0 kg. The animals were made available by the animal house of department of pharmacology. All the animals housed individually in clean cage and maintained under standard conditions (12 hrs light and dark cycle, at room temperature 25 ± 3º C and 50-60% humidity). All animals fed on gram diet and water ad libitum.

Omega 3 fatty acid capsule was purchased from an authorised chemist. Carbon tetrachloride was obtained from Ranbaxy Laboratories Limited.

Omega-3 fatty acids were administrated orally for 21 days from day zero to day 20. Carbon tetra chloride (CCl_4) was administrated intraperitoneally for 10 days along with Omega-3 fatty acid from day 11 to day 20. Dose calculation was done by using conversion factor developed according to Surface area [12].

Experimental Design for Hepatoprotective Activity:

The rabbits were divided randomly into four groups of six rabbits in each. The hepatoprotective activity of the Omega-3 fatty acid was tested using CCl_4 model.

Group I (normal control) received neither fatty acid nor CCl_4 that is they receive food and water only, **Group II** was given a intraperitoneal CCl_4 (0.05ml/kg) for 10 day (from 11^{th} to 20^{th} day) only. **Group III** was given omega 3 fatty acid (600 mg/kg/day) for 20 days from 1^{st} to 20^{th} days along with CCl_4 from 11^{th} to 20^{th} day. **Group IV** (Standard control) was given Sylimarin (100 mg/kg/day) for 20 days from 1^{st} to 20^{th} day and along with CCl_4 from 11^{th} to 20^{th} day.

Blood samples were collected on **day zero** before giving omega-3 fatty acid to see the control value of liver function tests (L.F.T), and **on day 11** to see the per se effect of omega-3 fatty acids on L.F.T and **on day 21** to see the protective effect of omega-3 fatty acid on L.F.T.

Biochemical Analysis

Blood sample was collected inside centrifugation tubes and allowed to clot for five to ten minutes at room temperature. The sample was later centrifuged to obtain sera. Sera samples were tested for Alanine aminotransferase (SGPT), Aspartate aminotransferase (SGOT), Alkaline Phosphate (ALP), Serum bilirubin and Total protein by standard methods [11].

Histopathological analysis

Liver was taken out after sacrificing the rabbit. Liver samples were immediately collected and fixed in 10% buffered formaldehyde solution for a period of at least 24 hours before histopathological study. Tissue was processed for the purpose of dehydration, clearing (dealcoholisation) impregnation and embedding. Tissue was kept in the solution of graded ethyl alcohol (75% of absolute) then chloroform then wax, paraffin wax blocks were made, containing the tissue and sectioned (five microns). These thin sections were stained with hematoxylin and eosin (H&E) and mounted on glass slides. Degrees of liver damage were assessed under a light microscope and images were captured with at original magnification of 10 × 10. Assessment of injury was done in term of steatosis, portal inflammation, and necrosis.

Statistical Analysis

Mean, standard error of means were calculated and results were analyzed by using one way analysis of variance (ANOVA) test and Post hoc analyses were employed using Turkey-Kramer multiple comparison test. For statistical analysis we used GraphPad InStat-3 and Microsoft Office Excel 2007 software. $p < 0.05$ was accepted as significant, $p < 0.01$ as highly significant and $p<0.001$ as very highly significant.

RESULTS:

1. Serum biochemical parameters

In the present study, activities of total bilirubin, Serum SGPT, SGOT and ALP from CCl_4 group had a sharp and significant increase as compared to a normal control group indicating successful induction of hepatotoxicity by CCl_4 (Table-1). Administration of omega-3 fatty acid at a dose of 600mg/kg/day had a beneficial effect on the levels of these biochemical markers in the treatment group showing significant shift in biochemical parameter when compared with CCl_4 group which was comparable to Silymarin group. (Table-1)

Table1: Effect of omega-3 fatty acid on liver biochemical indices in CCl_4-induced albino rabbit

Groups	S.Bilirubin (mg/dl)	SGPT (IU/L)	SGOT (IU/L)	ALP (IU/L)	Total protein (g/dl)
Group1(Normal control)	0.33 ± 0.16	30.16 ±0.49	30 ± 0.47	73.6 ± 1.51	6.55 ± 0.10
Group2 (CCl_4 group)	1.28±0.02###	139±1.85###	85.66±1.38###	142±2.13###	3.03±0.10###
Group3(CCl_4+omega3fatty acid)	0.66±0.03***	50.66±1.21***	50.66±1.14***	120.33±1.23***	5.18±0.07***
Group4(CCl_4+Silymarin)	0.65±0.03***	53.66±1.82***	54.16±1.44***	122.33±0.73***	5.36±0.14***

N=6, Values are expressed as Mean ± S.E.M., ***p<0.001 when compared with CCl_4 intoxicated group, ###p<0.001 When compared with normal control.

2. Liver Histology

The hepatoprotective effects of omega-3 fatty acid were also evident from the prominent variations in liver histology (Fig1-4). The normal control group (Fig.1) displayed normal liver histology while the CCl_4 treated group (Fig.2) showed swollen or apoptotic hepatocytes. This group also displayed a large area of fatty changes with extensive perivenular necrosis. Omega-3 fatty acid treated groups (Fig.3) exhibited protection of hepatic lobules, this group showed mild fatty changes and little bit necrosed area when compared with CCl_4 only treated group. The group treated with the standard drug silymarin (Fig 4) had mild congestion of central vein and mildly swollen hepatocytes.

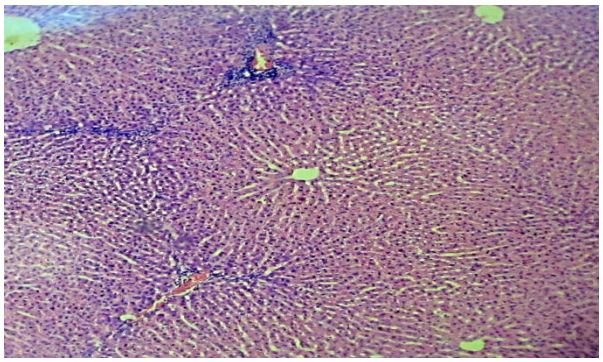

Figure 1: Normal Control Group

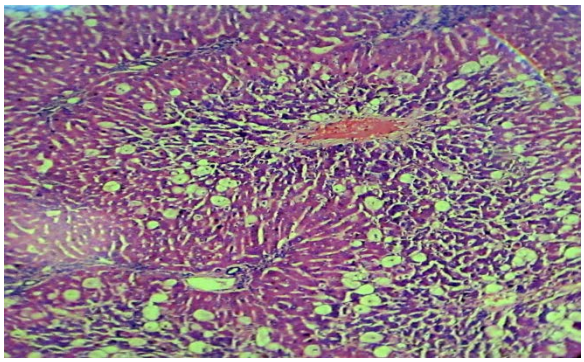

Figure 2: CCl_4 Group

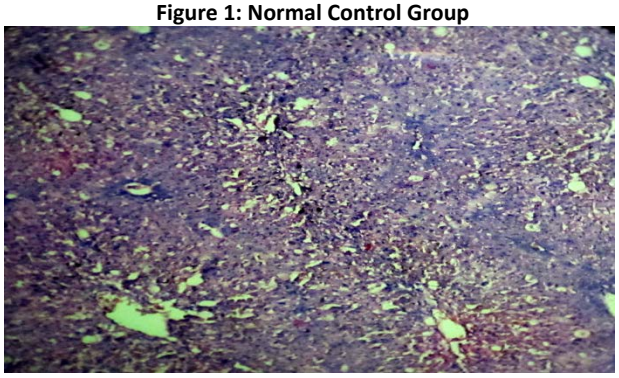

Figure 3: Omega-3 fatty acid +CCl_4 Group

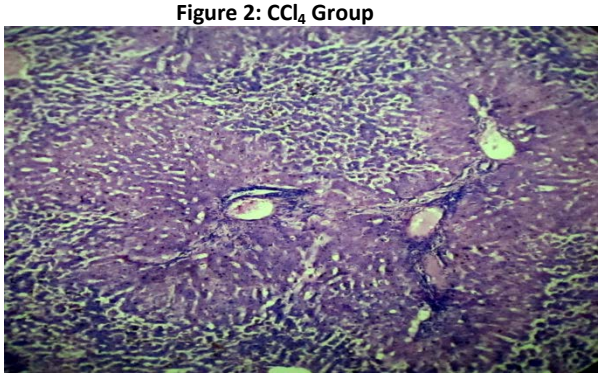

Figure 4: Sylimarin+ CCl_4 Group

DISCUSSION:

Hepatic injury through carbon tetrachloride (CCl_4) induced lipid peroxidation is well known and have been extensively used in the experimental models to understand the cellular mechanisms behind oxidative damage and further to evaluate the therapeutic potential of drugs and dietary antioxidants.[13]Serum bilirubin estimation helps in assessing the liver function.[14] Type of liver injury is determined by measuring the presence of hepatocellular enzymes in liver like SGOT, SGPT and ALP. The increased levels of these enzymes indicate mitochondrial damage and cell membrane damage.[15] Supplementation of Omega-3 fatty acid could reduce burden of number of disease(s) and have beneficial effects against a number of different pathologies. [16, 17] Fish oil and flax oil are the rich sources of omega 3-fatty acids and are consequently potent antioxidants. Dietary supplementation of fish oil is known to ameliorate hepatotoxicity due to cisplatin.[18] The fish (cod liver) oil pretreatment of rats has been shown to protect liver against toxicity due to acetaminophen.[19] In addition to fish oil as a source of omega 3-fatty acids, raw and baked flaxseed products have been shown to induce hypolipidemic, hypoglycemic and hypocholesterolemic effects which may be attributed mainly to seed oil which is rich in alpha linolenic acid. [20, 21, 22]

In our study we observed that omega-3 fatty acid shown significant shift in LFT parameters when compared with group administered CCl_4 only that indicates omega-3 fatty acid had shown signs of hepatoprotection. These findings were also well supported by histopathological assessment.

In addition to its metabolic functions, the liver is known to be involved in inflammation and immune responses.[23] Kupffer cell-derived cytokines, such as interleukin (IL)-1β, IL-6, TNF-α, and leukotrienes, promote infiltration and the antimicrobial activity of neutrophils.[24] TNF-α is known to be a central regulator that facilitates tissue repair by stimulating apoptosis and cell proliferation, it also exacerbates cell damage by initiating an

inflammatory process. [25] Omega-3 fatty acids have been shown by others to down regulate the TNF-α response to lipopolysaccharide insult, as would be seen in sepsis and this may have a direct role in hepatoprotection and that might contributing mechanism for hepatprotection in our study [26].

CONCLUSION:

By study it was concluded that omega-3-fatty acids, at a dose of 600 mg/kg/day protect the liver against CCl_4 induced hepatotoxicity in albino rabbits. The probable mechanisms postulated are its antiinflammatory and antioxidant effects. 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 and to get precise mechanism.

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