OMEGA-3 FATTY ACID PROTECTS AGAINST CHEMICAL INDUCED LIVER INJURY- AN EXPERIMENTAL STUDY

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ABSTRACT

INTRODUCTION: The mainstream detoxification of chemical agents is carried out in liver which is chief organ of body. Omega-3 is belonging to family of polyunsaturated fatty acids which has several beneficial effects on metabolism and inflammation.

OBJECTIVE: To assess to hepatoprotective effects of the Ω- 3-FA on carbon tetra chloride induced liver injury in laboratory animals at animal house.

METHODOLOGY: After ethical review committee approval this study was performed at the Department of Anatomy and Pathology, Bhitai Dental and Medical College and Animal house of Sindh Agriculture University Tando Jam. 80 Wistar albino rats were selected and randomly divided into 4 groups and drugs were given for allocated time. Blood sampling, biochemical and histopathological analysis were carried out.

RESULTS: The Ω- 3-FA and silymarin demonstrated protective effects against the CCl4 induced liver injury. Group C and D which were treated with the Ω- 3-FA and silymarin revealed significant amelioration of the liver function tests ($p<0.05$).

CONCLUSION: The rat models showed tremendous benefits of omega-3 on liver due to carbon tetrachloride induced damage.

KEY WORDS: Omega-3, Carbon tetrachloride, Hepatocellular injury

This article may be cited as: **Mawani H, Naz S, Irshad F, Ata MA. Omega-3 fatty acid protects against chemical induced liver injury- an experimental study. Northwest J Med Sci. 2018; 3(2):83-7**

INTRODUCTION

Liver is the largest gland of body and the key organ of regulating homeostasis in the body. Liver is major site of biochemical reactions related to detoxification, growth, gluconeogenesis, energy production and nutrient supply. It is major organ regulating the biochemical composition of blood within normal limits. It detoxifies the alcohol, toxins, drugs, xenobiotics and helps combat oxidative and peroxidant stress.[1] Therefore drug and toxin induced liver injury may produce grave consequences. Many of the toxic agents induce liver injury and hepatocyte necrosis through induction of oxidative and peroxidative free radicals.[2] The carbon tetrachloride (CCl4) is classical hepatotoxic agent used in laboratory animals for the research purpose, for example studying the hepatoprotective effects of herbal agents scientifically.[3,4] The CCl4 induced liver injury is widely used in animals models to investigate and invent newer drugs for the liver disease.[5] The CCl4 induced liver injury is produced by free radical formation which switches on the oxidation and peroxidation reactions resulting in hepatocellular injury.[6,7] Such CCl4 induced liver injury animal model is widely used in laboratory to evaluate hepatocellular protective and regenerative potential of herbal compounds for searching newer drug modalities.[8] The Omega-3 fatty acids (Ω- 3-FA) are long chain unsaturated fatty acids which help in blood clotting, keep brain, heart and immune system healthy. The Ω-3-FA play role in Eicosanoid synthesis, balanced mood, joint mobility, and help maintain a sense of well being. They antagonize the atherogenesis and improved blood lipids.9 The Ω- 3-FA is found in the fish and marine animals in abundant quantities.10 The DHA and EPA comprise approximately 60% of the Ω- 3 fatty acids. Cold oily fish such as salmon, sardines, anchovies, mackerel and herring are some of the rich sources of Ω- 3-FA. The concept that the Ω- 3-FA may modify and ameliorate the oxidative stress and peroxidative burden, the need for further studies is compelling. Further research on the Ω- 3-FA

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Date Received: January 02, 2018
Date Revised: February 07, 2018
Date Accepted: March 27, 2018

would be helpful for human health.[11] The present study was conducted to hepatoprotective effects of the Ω- 3-FA on carbon tetra chloride induced liver injury in laboratory animals at our animal house.

METHODOLOGY

The present study was conducted at the Department of Anatomy and Pathology, Bhitai Dental and Medical College and Animal house of Sindh Agriculture University Tando Jam. Ethical approval was taken from the institutional review committee. The study was conducted after clearance from Animal Ethics committee of the institute. Animals were catered according to the NIH guidelines. 80 Wistar albino rats were selected randomly according to inclusion and exclusion criteria. Male rats of 200- 300 grams were the inclusion criterion. Female rats and sick male rats were excluded. Animals were housed in stainless steel cages, with automatic nozzles. Free availability of chow diet and fresh water ensured ad libitum. Animals were kept 12/12 hour dark – light cycle at temperature 25 ± 5° C and 50-60% humidity. The Ω- 3-FA (Amarant Pharmaceuticals) was purchased from institute pharmacy. Intraperitoneal CCl4 was administered at dose of 0.05ml/kg, the Ω- 3-FA at dose of 600 mg/kg and Silymarin at dose of 100 mg/kg were administered daily for 3 weeks. Eighty rats were divided randomly into 4 groups of 20 rats in each. Animals were divided into groups as; Group A. Controls – received 0.9% NaCl as placebo, Experimental Groups- Group B. Intraperitoneal CCl4 (0.05ml/kg) daily for 3 weeks Group C. Intraperitoneal CCl4 (0.05ml/kg) + Ω- 3-FA (600 mg/kg/day) daily for 3 weeks, Group D. Intraperitoneal CCl4 (0.05ml/kg) + Silymarin (100 mg/kg/day) daily for 3 weeks.

Blood sampling: Blood samples were collected at the end of experiment period in all animal groups. Blood samples were collected by cardiac puncture.

Biochemical Analysis: Blood samples were allowed to colt for 5-10 minutes at 250C. Blood samples were centrifuged at 3000 rpm for 15 minutes. Sera were separated out. Sera were allowed to store at -20 0C if biochemical estimation was delayed. Sera were used for the biochemical estimation of serum bilirubin, serum creatinine, Prothrombin time and liver enzymes - Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline Phosphate (ALP), Y-glutamyl transferase Y-GT (U/L) and lactate dehydrogenase (LDH) by standard methods [11]. Serum superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase (CAT), reduced glutathione (GSSH) and serum malondialdehyde (MDA) were estimated by Elisa assay kit (Cayman and Fortress Diagnostics, USA).

Histopathological analysis: Abdominal dissection was performed after anesthesia with Ketamine and Xylazine. Liver was retracted, collected and fixed in 10% buffered formaldehyde solution in a plastic jar. Tissue was stored for at least 24 hours before tissue processing. Dehydration, dealcoholization, impregnation and embedding of tissue were the processing protocol. Tissue was processed in graded concentration of ethyl alcohol (75% of absolute), followed by chloroform and wax. Paraffin wax blocks were prepared. 3-5 µ thick tissue sections were stained Hematoxylin and eosin (H&E) and mounted on glass slides for microscopic examination.

Statistical Analysis: Data was analyzed on SPSS 22.0 (IBM, incorporation, USA). One way analysis of variance was used for numerical variable comparison among groups. Post Hoc Tukey-Cramer test was used for inter group comparisons. Results were presented as mean and standard deviation (SD). Confidence interval for data analysis was taken as 95% significance ($p \leq 0.05$).

RESULTS

The Ω- 3-FA and silymarin exhibited significant biochemical and tissue protective effects against the CCl4 induced liver injury. Table 1 shows the serum bilirubin, serum creatinine, Prothrombin time and ALT, AST, ALP, Y-GT and LDH. Group C and D which were treated with the Ω- 3-FA and silymarin revealed significant amelioration of the liver function tests ($p<0.05$). The Ω- 3-FA and silymarin treated rats showed a rise in the anti oxidant enzymes – the SOD, GPX, CAT and GSSH and a reduction in the lipid peroxidation marker- the MDA (table 2). Microphotographs 1-4 show the histopathological examination of four rat groups. The liver architecture and histology was found intact with minimal tissue injury in the Ω- 3-FA and silymarin treated rats compared to CCl4 treated rats which showed severe hepatocellular injury (figure 2). The CCl4 induced liver injury was found significantly decreased in the Ω- 3-FA and silymarin treated rats.

DISCUSSION

Carbon tetrachloride (CCl4) induced liver injury is well known in experimental studies. CCl4 induces free radical formation which set a vicious cycle to induce oxidative and peroxidative cell injury. CCl4 is extensively used in experimental animal models to investigate and understand the cellular mechanisms behind oxidative damage. Therapeutic potential of food and dietary agents is essential to evaluate for their positive effects against toxic agents.[12,13] Liver aminotransferase, serum bilirubin and Prothrombin time are biological markers of hepatocyte function.[14] Liver aminotransferases are markers of cytoplasmic, mitochondrial or cell membrane injury. They are also good indicator of toxic agent if ingested or present in the diet. The increases levels of liver aminotransferases indicate cell injury.[15] The present experimental animal model study evaluated the toxic effects of CCl4 on liver. Elevated liver aminotransferases indicate the hepatocellular injury in present study; these findings are in agreement with previous studies.[12-15] Alleviation of CCl4 induced liver injury by the omega-3 fatty acid (Ω- 3-FA) indicates towards the

TABLE 1: BIOCHEMICAL MARKERS OF HEPATOCELLULAR INJURY IN EXPERIMENTAL RATS (N=80)

	Group A	Group B	Group C	Group D	P-value
ALT (U/L)	32.8±6.1	73.3±14.8	53.1±8.6	54.1±8.9	0.0001
AST (U/L)	30.6±3.4	40.9±20.1	33.6±7.6	33.2±8.6	0.046
ALP (U/L)	70.1±16.6	133.7±34.4	97.5±43.4	81.2±16.9	0.0001
LDH (U/L)	119.2±26.5	171.9±29.1	140.6±35.1	128.6±26.0	0.0003
Y-GT (U/L)	32.5±4.6	78.9±5.2	61.5±18.6	64.8±21.0	0.0001
S. bilirubin (mg/dl)	0.59±0.13	2.63±0.76	1.77±1.01	1.33±0.34	0.0001
S. creatinine (mg/dl)	1.68±0.65	3.83±1.09	2.1±1.2	1.41±0.47	0.0001
PT (sec)	9.38±1.97	15.01±1.2	12.1±2.7	10.74±2.1	0.0001

TABLE 2: ANTI OXIDANT AND LIPID PEROXIDANT MARKERS IN EXPERIMENTAL RATS (N=80)

	Group A	Group B	Group C	Group D	P-value
Serum SOD (U/ml)	124.3±22.7	86.8±21.1	109.0±19.6	121.4±15.6	0.0001
Serum GPX (nM/ min/mL)	130.5±41.3	88.7±22.0	125.7±17.1	124.3±6.4	0.0001
Serum CAT (nM/ min/mL)	409.4±79.4	160.1±89.9	193.2±15.5	260.8±28.2	0.0001
Serum GSSH (µM)	3.88±0.35	1.82±0.61	2.6±0.91	2.85±0.91	0.0001
Serum MDA (µM)	2.44±1.1	6.78±2.5	4.69±1.4	3.51±2.0	0.0001

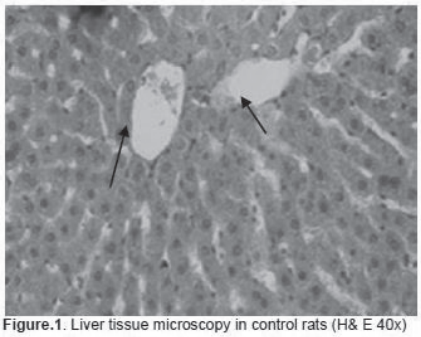

Figure.1. Liver tissue microscopy in control rats (H& E 40x)

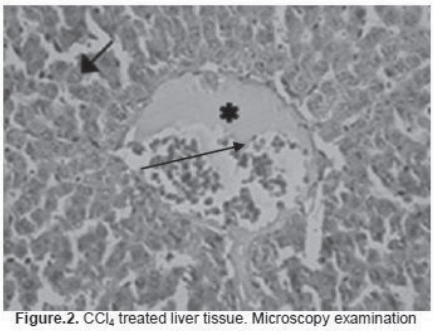

Figure.2. CCl_4 treated liver tissue. Microscopy examination reveals severe tissue injury (H& E 40x

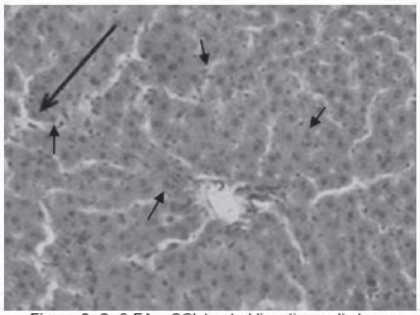

Figure.3. Ω- 3-FA + CCl_4 treated liver tissue. It shows hepatocyte cords with minimal tissue injury (H& E 40x)

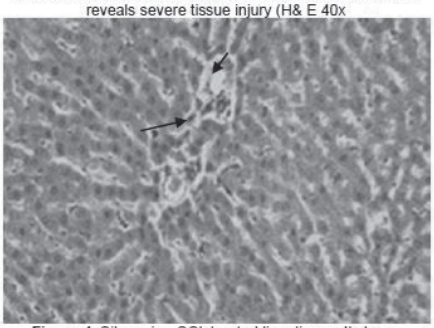

Figure 4. Silymarin+ CCl_4 treated liver tissue. It shows hepatocyte cords with decreased tissue injury (H& E 40x)

beneficial effects. Also improvement with observed in the anti oxidant enzyme activity i.e. SOD, GPX, CAT and GSSH and low MDA are observable findings. These findings are in agreement with previous studies.[16,17] Fish and vegetable oils are rich sources of Ω- 3-FA. Previous studies reported Ω- 3-FA protected cis-platin and acetaminophen induced liver injury in rat model.[18,19] Other studies have reported hypoglycemic, hypolipidemic and hypocholesterolemia potential of Ω- 3-FA.[20,21] In the present study, the Ω- 3-FA improved the hepatocellular markers of injury, and anti oxidant enzymes and a reduction in lipid peroxidation (MDA). Our findings corroborate with previous studies.[21-24]

The Ω- 3-FA is reported to decrease the inflammatory markers including the tumor necrosis factor-alpha

(TNF-α) in response to lipopolysaccharide insult in studies mimicking to as what has been observed in the systemic sepsis. This effect is reported exerted directly by the Ω- 3-FA might be contributing mechanism against toxin induced liver injury.[25] The histological examination of present study (figure 1-4) shows tissue protective effects of Ω- 3-FA in the treated animals. These findings are in agreement with Litreature reported similar results in the in the CCl4 treated rats.[26] The present study shows the Ω- 3-FA improved the hepatocellular markers of injury, anti oxidant enzymes, lipid peroxide marker (MDA) and tissue histology. All these findings show the effectiveness of Ω- 3-FA against prevention of CCl4 toxicity. The present study proposes the Ω- 3-FA exerts its hepatoprotective effects through these mechanisms. Previoulsy study reported the superoxide dismutase (SOD) and glutathione (GSH) were significantly low in mice treated with CCl4 and were increased after treatment with Ω- 3-FA.[27] The finding corroborates with the present study. Previoulsy study reported that the level of lipid peroxide marker - the malondialdehyde (MDA) was significantly highly in CCl4-treated rats in controls compared to those rats treated with Ω- 3-FA.[28] The findings of this study are also consistent to the present study as shown in Table-2. Similar findings have been reported by various previous studies.[29-31] Based on the evidence based findings, the present study proves the Ω- 3-FA has potential of protecting against the chemical and toxin induced injury.

CONCLUSION

The present study concludes that the omega-3-fatty acids (Ω- 3-FA) are effective against the carbon tetrachloride induced liver injury in rat model. The proposed mechanism of hepatoprotection is through free radical scavenging activity. The Ω- 3-FA may prove clinically useful to counter drug induced liver damage. Future studies in humans are recommended to substantiate its clinical effectiveness and to get precise mechanism of chemical mediated liver injury.

ACKNOWLEDGEMENT

We acknowledge the study participants who took time out for this study and shared their experience and views.

NOTES ON CONTRIBUTORS

The study was part of HM, SN, FI and MAA, all authors were involved in every part of Manuscript writing, analysis, Protocol developments, and data collection process.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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