Studies on the protective effect of fish oil against cisplatin induced hepatotoxicity

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Abstract

Cisplatin (CP) is considered as a major antineoplastic drug against a broad spectrum of malignancies. The tissue specific toxicity of cisplatin to the kidneys is well documented. However, at higher doses less common toxic effects such as hepatotoxicity may arise. Although cisplatin remains one of the most effective antineoplastic drugs used in chemotherapy, strategies to protect tissues against cisplatin toxicity are of clinical interest. Dietary fish oil (FO) enriched in ω-3 fatty acids is known to retard the progression of certain types of cancers, cardiovascular and tissue disorders. In view of this, the present study investigates the protective effect of FO on CP induced damage to liver. Rats were pre-fed normal diet and the diet rich in FO for 10 days and then a single dose of CP (6mg/kg body weight) was administered intraperitoneally while still on diet. Serum/urine parameters, enzymes of carbohydrate metabolism and oxidative stress were analyzed. CP caused perturbation of the antioxidant defense as is reflected by the decrease in the activities of catalase, superoxide dismutase and glutathione peroxidase. Further the activities of various enzymes involved in glycolysis, TCA cycle, gluconeogenesis and HMP shunt pathway were determined and were found to be differentially altered by CP treatment. However, these alterations were ameliorated in cisplatin treated rats fed on fish oil. Present results show that dietary supplementation of FO to CP treated rats ameliorated CP-induced hepatotoxic and other deleterious effects due to its intrinsic biochemical/antioxidant properties.

Keywords: Cisplatin; Hepatotoxicity; Fish oil; Carbohydrate metabolism; Oxidative stress.

Introduction

Cisplatin (*cis*-diamine-dichloroplatinum) is a prominent member of the effective broad-spectrum antitumor drugs. However, its clinical usage is restricted due to some adverse side effects, such as ototoxicity and nephrotoxicity (Born *et al.*, 2003; Iraz *et al.,* 2005; Yao *et al.,* 2007). However, at high doses, less common toxic effects, such as hepatotoxicity, may arise (Cvitkovic, 1998; Hanigan *et al.*, 2003; King *et al.,* 2001). Continued aggressive high-dose cisplatin chemotherapy necessitates the investigation of ways for prevention of the dose-limiting side effects that inhibit the cisplatin administration at tumoricidal doses. Until now a large number of studies have been focused on the ways for prevention of cisplatin side effects via supplementation of preventive agents simultaneously (Ali and Moundhri, 2006). Although the mechanism underlying the side effects induced by cisplatin are not understood clearly, it was considered to be attributed to the combination of multi-ways (Hong *et al.*, 2005; Ramesh and Ravees, 2002; Nowak, 2002; Townsend and Hanigan, 2002; Xiao *et al.,* 2003), such as the generation of reactive oxygen species (ROS), which could interfere with the antioxidant defense system and result in oxidative damage in different tissues (Pratibha *et al.,* 2006; Koc *et al.,* 2005; Mansour *et al.,* 2006; Iraz *et al.,* 2006). Indeed, some recent studies have suggested that oxidative stress plays an important role in cisplatin-induced liver damage (Lu and Cederbaum, 2006; Iraz *et al.*, 2006; Pratibha *et al.*, 2006; Mansour *et al.*, 2006).

The past three decades have been a period of rapid expansion in the scientific knowledge of ω-3 polyunsaturated fatty acids (PUFAs). A number of investigations have demonstrated that diet supplemented with fish oil (FO) enriched in ω-3 fatty acids has profound beneficial health effects against various pathologies (Simopoulos, 1991) including cardiovascular diseases, respiratory diseases, diabetes, depression, cancers, inflammatory and immune renal disorders (De Caterina *et al.,* 1994). Dietary ω-3 fatty acids have shown a significant protective effect against gastric ulcerations by inhibition of offensive mucosal factors and augmentation of defensive mucosal factors (Bhattacharya *et al.,* 2006) Reports showed that FO prevents gentamicin and cyclosporine-A-induced nephrotoxicity (Thakkar *et al.,* 2000; Abdel-Gayoum *et al.*, 1995; Priyamvada *et al.,* 2008). However, the

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biochemical mechanism or cellular response by which FO protects against CP induced damage to liver has not been examined.

        Considering great interest in expanding the clinical usefulness of CP and numerous health benefits of FO, the present work was undertaken to study the detailed mechanism of biochemical events/cellular response/mechanisms of CP induced hepatotoxicity and its protection by dietary fish oil. We hypothesized that fish oil would prevent CP-induced hepatotoxicity due to its intrinsic biochemical and antioxidant properties that would lead to improved metabolism and antioxidant defense mechanism of the liver. The results obtained indicate that dietary supplementation with fish oil markedly ameliorated CP induced adverse effects in liver. The activities of enzymes of carbohydrate metabolism and antioxidant defense mechanism were markedly enhanced by fish oil feeding to CP administered rats. Dietary supplementation with FO enriched in ω-3 fatty acids could prevent CP-induced suppression of metabolic, membrane and antioxidant enzymes. These results support a potential therapeutic use of CP + FO combination in combating cancer without hepatotoxic and other harmful side effects.

Materials and Methods

Chemicals and drugs
Fish oil (Menhaden, Sigma Chemical Co., USA), Cisplatin (Sigma Chemical Co., USA). All other chemicals used were of analytical grade and were purchased either from Sigma Chemical Co. (St Louis, MO, USA) or SRL, Mumbai, India.

Animals
Adult male Wistar rats (8 rats/group) weighing between 150-200 g were used in the study. The animal experiments were conducted according to the guidelines of Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Government of India. Animals were acclimatized to the animal facility for a week on standard rat chow and allowed water ad libitum under controlled conditions of 25±2°C temperature, 50±15% relative humidity and normal photoperiod (12 hour dark and light). Body weights of rats were recorded at the start and completion of the procedure.

Diet

A nutritionally adequate laboratory pellet diet was obtained from Aashirwaad Industries, Chandigarh (India). Pellets were crushed finely and mixed with 15% Fish oil was stored in airtight containers. Vitamin E as DL-α-tocopherol (270 mg/kg chow) was added to each of the modified rat chows in order to meet the increased metabolic requirement for antioxidants on a diet high in polyunsaturated fatty acids.

Experimental design
Four groups of rats entered the study after acclimatization. These groups were fed either on a normal diet (control and CP groups) or on diet containing 15% fish oil (CPF and FO groups). After 10 days rats in two groups (CP and CPF) was administered a single dose of CP intraperitoneally (6 mg/kg bwt). Animals in the control and FO group received an equivalent amount of normal saline. The rats were sacrificed 4 days after the injection under light ether anesthesia. Blood and urine samples were collected and liver was removed and processed for the preparation of homogenates as described below.

Serum/Urine chemistry
Serum/Urine parameters - Serum samples were deproteinated with 3% Trichloroacetic acid in a ratio of 1:3, left for 10 minutes and then centrifuged at 2000 X g for 10 minutes. The protein free supernatant was used to determine inorganic phosphate and creatinine. The precipitate was used to quantitate total phospholipids. Blood Urea Nitrogen (BUN) and cholesterol levels were determined directly in serum samples. Glucose was estimated by o-toluidene method using kit from Span Diagnostics, Mumbai, India. These parameters were determined by standard procedures as mentioned in a previous study (Khundmiri *et al.*, 2004).

Preparation of homogenates
After the completion of treatment schedule, liver was carefully separated from the treated and control animals, and homogenized in 0.1 M Tris-HCl buffer pH 7.5 by a glass-teflon homogenizer (Thomas PA, USA) by passing 5 pulses; at 4°C to make a 10% w/v homogenate. The homogenate was then subjected to high-speed Ultra-Turrex Kunkel homogenizer (Type T-25, Janke & Kunkel GMBH & Co. KG. Staufen) for 3 pulses of 30 s each with an interval of 30 s between each stroke. Homogenate was centrifuged at 2000 rpm at 4°C for 10 min in

Beckman J2-M1 (Beckman Instruments, Inc., Palo Alto, C.A., USA) high-speed refrigerated centrifuge to remove the cell debris. The supernatant was saved in aliquots and stored at -20°C for analyses of metabolic enzymes.

Assay of carbohydrate metabolism enzymes
The activities of the enzymes involving oxidation of NADH or reduction of NADP were determined spectrophotometrically on Cintra 5 fixed for 340 nm using 3 ml of assay in a 1-cm cuvette at room temperature (28-30 °C). The enzyme assays of Lactate dehydrogenase (LDH, E.C.1.1.1.27), malate dehydrogenase (MDH, E.C.1.1.1.37), malic enzyme (ME, E.C.1.1.1.40), glucose-6-phosphate dehydrogenase (G6PDH, E.C.1.1.1.49), glucose-6-phosphatase (G6Pase, E.C.3.1.3.3) and fructose-1, 6-bisphosphatase (FBPase, E.C.3.1.3.11) activities were studied as described by Khundmiri *et al*. Hexokinase was estimated by the method of Crane and Sols (1953) and the remaining glucose was measured by method of Nelson-Somogyi (Nelson, 1944).

Assay of membrane enzymes and lysosomal marker enzymes
The activities of membrane marker enzymes, alkaline phosphatase (ALP), leucine amino peptidase (LAP), γ- glutamyl transferase (GGTase) and lysosomal marker enzyme, acid phosphatase (ACPase) were determined as described by Farooq *et al.* (2004).

Assay of enzymes involved in free radical scavenging
Superoxide dismutase (SOD, E.C.1.15.1.1) was assayed by the method of Marklund (Marklund and Marklund, 1974). Catalase (CAT, E.C.1.11.1.6) and glutathione peroxidase (GSH-Px, E.C. 1.11.1.9) activities were assayed by the method of Giri *et al.* (1996).

Lipid peroxidation and total –SH group estimation
Total SH groups were determined by the method of Sedlak and Lindsay (1968) and lipid peroxidation (LPO) by the method of Ohkawa *et al.* (1979).

Statistical analysis
All data are expressed as Mean ± SEM for at least 4-5 different preparations. Statistical evaluation was conducted by one-way ANOVA. A probability level of p<0.05 was selected as indicating statistical significance. Most of the

changes between various groups were compared with control values for better understanding and clarity.

Results
The present work was undertaken to study detailed mechanism of CP-induced hepatotoxicity and other deleterious effects and its possible protection by feeding ω-3 fatty acids enriched diet to the rats. To address our hypothesis, the effect of CP alone and in combination with fish oil (FO) was determined on various enzymatic and non-enzymatic parameters of oxidative stress, and carbohydrate metabolism in rat liver.

Effect of dietary fish oil on CP induced alterations in serum and urinary parameters
Results summarized in Table 1 and 2 show the effect of CP alone and in combination with fish oil (FO) on blood and urine chemistry. CP treatment to control rats resulted in significant increase in serum creatinine (Scr) and blood urea nitrogen (BUN), but decrease in cholesterol, phospholipids (PL), inorganic phosphate and glucose compared to control rats. These changes were associated with profound phosphaturia, proteinuria and glucosuria accompanied by decreased creatinine clearance. FO diet alone caused significant increase in serum glucose, Pi and PL and decrease in BUN, urinary Pi and protein excretions. Scr, cholesterol and urinary glucose remain unchanged by FO diet accompanied by increased creatinine clearance.

Feeding of FO diet to CP administered (CPF) rats resulted in significant reversal of various CP elicited deleterious effects on serum and urine parameters. FO prevented CP induced increase of Scr, BUN and cholesterol and decrease of serum Pi and glucose. CP-induced phosphaturia, proteinuria and glucosuria were absent in CPF compared to CP rats.

Effect of fish oil (FO) on CP induced alterations on metabolic enzymes in liver
The effect of CP, FO diet and their combined treatment was determined on the activities of various enzymes of carbohydrate metabolism in liver. As shown in Table 3 and 4, CP treatment to control rats significantly increased the activity of lactate dehydrogenase (LDH) and hexokinase (HK) but decreased malate dehydrogenase (MDH), glucose-6-phosphatase (G6Pase) and

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fructose-1, 6-bisphosphatase (FBPase) activities. When CP treatment was extended to FO fed rats, CP-induced alterations in metabolic enzyme activities were not only prevented by FO diet, but G6Pase remained significantly higher in CPF and FO compared to control as well as CP rats in the liver.

Table 1: Effect of Fish oil (FO) on serum parameters with CP treatment.

Group	Creatinine (mg/dl)	BUN (mg/dl)	Cholesterol (mg/dl)	Phospholipid (mg/dl)	Phosphate (µmols/ml)	Glucose (mg/dl)
Control	0.999± 0.046	14.40 ± 0.669	129.2 ± 1.96	114.71 ± 0.86	2.3 ± 0.047	73.22 ± 4.07
CP	2.012 ± 0.139[*] (+101.40%)	34.24 ± 3.30[*] (+137.7%)	115.79 ± 1.29[*] (-10.379%)	80.68 ± 0.38[*†] (-29.66%)	1.92 ± 0.123[*] (-16.52%)	19.16 ± 0.59[*] (-73.83%)
CPF	1.23 ± 0.06[†] (+23%)	18.25 ± 0.890[*†] (+26.73%)	88.40 ± 1.33[*†] (-31.57%)	98.51 ± 3.58[*†] (-14.12%)	2 ± 0.063[*] (+13.04%)	75.74 ± 3.48[*†] (+3.44%)
FO	0.965 ± 0.046[†] (-3.40%)	11.79 ±0.430[*†] (-18.125%)	108.47 ± 2.63[*†] (-16.04%)	99.5 ± 1.97[*†] (-13.25%)	2.51±0.03 (-6.5%)	83.06 ± .32 (+13.435)

Results are Mean ± SEM for five different preparations.
* Significantly different from control; † significantly different from CP at $p < 0.05$ by one-way ANOVA.
Values in parentheses represent percent change from control.

Table 2: Effect of Fish oil (FO) on urine parameters of rats with CP treatment.

Group	Urine Flow Rate (UFR) (ml/day)	Creatinine clearance (ml/min/ 100 gm body wt.)	Phosphate (µmols/ml)	Protein (mg/mmols creatinine)	Glucose (mg/dl)
Control	18 ± 1.73	0.655 ± 0.002	1.16 ± 0.023	0.029 ± 0.003	13.98 ± 1.73
CP	24 ± 0.57* (+33.33%)	0.212 ± 0.057[*] (-67.63%)	1.65 ± 0.77[*†] (+84.78%)	0.103 ± 0.004[*] (+255.17%)	56.47 ± 5.51[*†] (+303.93%)
CPF	21 ± 2.88 (-20%)	0.355 ± 0[*] (-45.80%)	1.19 ± 0.028[†] (+2.58%)	0.064 ± 0.023 (+120.68%)	33.16 ± 1.69[*†] (+137.19%)
FO	20 ± 0.57 (-33.33%)	0.954 ± 0.0012[*†] (+45.64%)	0.644 ± 0[*†] (-44.84%)	0.020 ± 0[*†] (-31.03%)	4.66 ± 0.715[*†] (-66.66%)

Results (specific activity expressed as µmoles/mg protein/h) are Mean ± SEM for five different preparations.
* Significantly different from control; † significantly different from CP at $p < 0.05$ by one-way ANOVA.
Values in parentheses represent percent change from control.

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Table 3: Effect of Fish oil (FO) on activities of HK, LDH and MDH homogenate of liver.

Enzyme Groups	LDH (μmol/mg protein/h)	MDH (μmol/mg protein/h)	HK (μmol/mg protein/h)
Control	0.462 ± 0.009	0.66 ± 0.05	1.08±0.07
CP	0.551 ± 0.02[*] (+19.26%)	0.254 ± 0.043[*] (-61.51%)	1.27 ± 0.215 (+17.26%)
CPF	0.382 ± 0.368[*†] (-17.31%)	0.419 ± 0.215[*] (-36.51%)	1.19 ± 0.051 (+5.26%)
FO	0.392 ± 0.01[*] (-28.78%)	0.534 ± 0.130 (-19.09%)	0.98 ±0.036[†] (-14.31%)

Results (specific activity expressed as μmoles/mg protein/h) are Mean ± SEM for five different preparations.
* Significantly different from control, † significantly different from CP at $p < 0.05$ by one-way ANOVA.
Values in parentheses represent percent change from control.

Table 4: Effect of Fish oil (FO) on activities of G6Pase, FBPase, ME and G6PDH in homogenate of liver.

Enzyme Groups	G6Pase (μmol/mg protein/h)	FBPase (μmol/mg protein/h)	ME (μmol/mg protein/h)	G6PDH (μmol/mg protein/h)
Control	0.968±0.074	0.809±0.004	0.134±0.028	0.065±0.0045
CP	0.702 ± 0.023[*] (-27.47%)	0.643 ± 0.026[*] (-20.51%)	0.139±0.005 (+3.73%)	0.039 ± 0.009 (-40%)
CPF	0.892± 0.056[†] (-7.85%)	0.789 ± 0.075 (-2.47%)	0.072±0.007[†] (-46.26%)	0.028±0.0046[*] (-56.92%)
FO	1.109 ±0.023[†] (+14.56%)	0.807 ± 0.028[†] (-0.247%)	0.067±0.009[*] (-50%)	0.053 ± 0.03 (-18.46%)

Results (specific activity expressed as μmoles/mg protein/h) are Mean ± SEM for five different preparations.
* Significantly different from control, †significantly different from CP at $p < 0.05$ by one-way ANOVA.
Values in parentheses represent percent change from control.

Effect of dietary fish oil (FO) on CP induced alterations in membrane enzymes and marker enzyme of lysosomes

To assess the structural integrity of certain organelles e.g., plasma membrane and lysosomes, the effect of CP alone and in combination with FO diet was determined on membrane enzymes and lysosomal enzymes in the homogenate of liver. The activities of alkaline phosphatase (AlkPase), γ-glutamyl transpeptidase (GGTase) and leucine aminopeptidase (LAP) and acid phosphatase (ACPase) were determined under different experimental conditions in the homogenates of liver Table 5. CP treatment to control rats caused significant reduction in the specific activities of AlkPase (-38.02%), GGTase (-38.54%) and LAP (-50.74%) in liver homogenate. The prior feeding of FO diet with CP treatment prevented CP elicited decrease in membrane enzyme activities. As can be seen from the data, CP induced decrease in enzyme activities were similarly prevented by FO diet. However, the activity of acid phosphatase (ACPase) was increased (+51.02%) by CP in liver homogenate while FO diet was able to prevent the increase in ACPase activity (Table 5).

Effect of dietary fish oil (FO) on CP induced alterations in antioxidant defense parameters in liver

It is evident that reactive oxygen species generated by various toxicants are important mediators of cellular injury and pathogenesis of various diseases (Walker, 1999). Antioxidant status is a potential biomarker to determine the physiological state of the cell, tissue or organ. To ascertain the role of antioxidant system in CP-induced toxicity, the effect of CP was observed on oxidative stress parameters. CP enhanced lipid peroxidation (LPO) and significantly altered antioxidant enzymes albeit differently (Table 6). LPO measured in terms of malondialdehyde (MDA levels) significantly enhanced in the liver (+21.6%) whereas total-SH declined in the tissue (-15.88%). CP treatment caused decrease in superoxide dismutase (SOD,-55.1%), Glutathione peroxidase (GSH-Px, 78.08% %) and catalase (-24.33%) activities. In contrast, the effect of FO consumption increased the activities of antioxidant enzymes albeit to different extents. The results indicate marked protection by FO diet against CP induced oxidative damage to renal tissues.

Table 5: Effect of Fish oil (FO) on biomarker enzymes of membrane and lysosomes in homogenate of liver after CP treatment.

Enzyme Groups	ALP (μmol/mg protein/h)	GGTase (μmol/mg protein/h)	LAP (μmol/mg protein/h)	ACPase (μmol/mg protein/h)
Control	2.27 ± 0.066	1.79 ± 0.078	1.21 ± 0.123	2.45 ± 0.097
CP	1.32 ± 0.127[*] (-38.02%)	1.1± 0.0786[*] (-38.54%)	0.596 ± 0.013[*] (-50.74%)	3.7 ± 0.51[*] (+51.02%)
CPF	1.8 ± 0.153 (-20.70%)	1.45 ± 0.047[*†] (-18.99%)	0.802 ± 0.028[*†] (-33.71%)	3.27 ± 0.275[*] (+33.46%)
FO	2.023 ± 0.027 (-10.88%)	1.73 ± 0.074[†] (-3.35%)	1.69 ± 0.076[*†] (+39.66%)	2.46 ± 0.06[†] (+0.408%)

Results (specific activity expressed as μmoles/mg protein/h) are Mean ± SEM for five different preparations.
* Significantly different from control, † significantly different from CP at $p < 0.05$ by one-way ANOVA.
Values in parentheses represent percent change from control.

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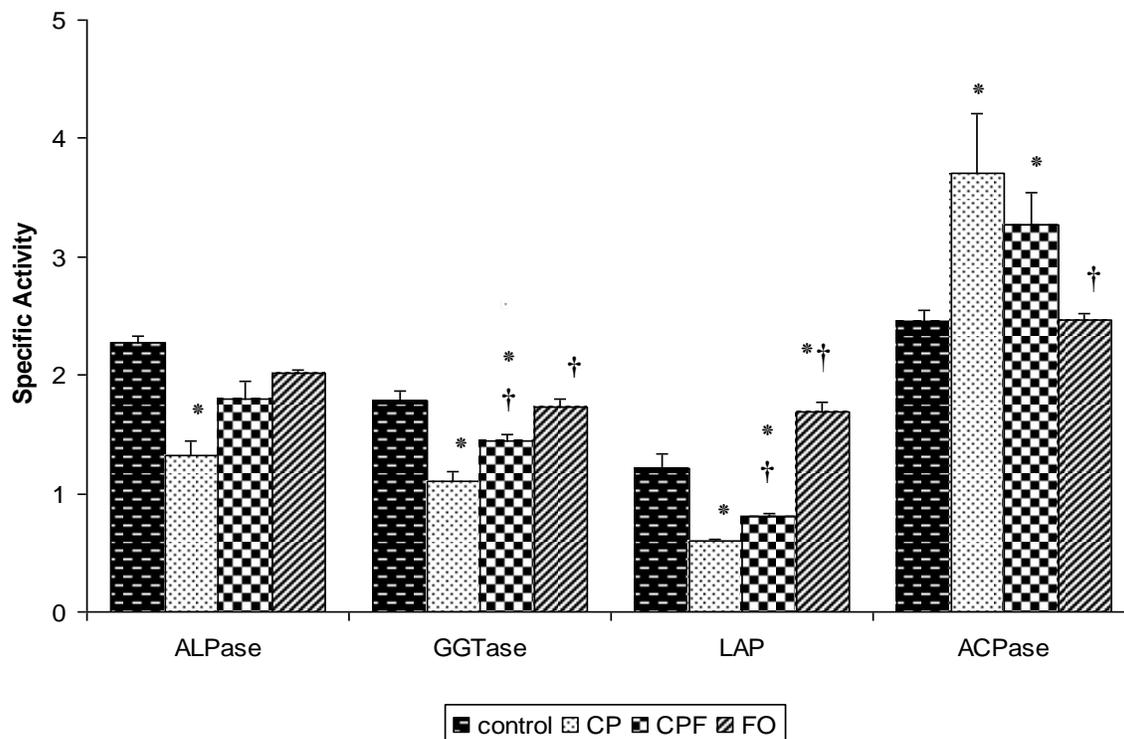
Figure 1: Effect of FO on the activities of ALPase, GGTase, LAP and ACPase in liver homogenate with CP treatment.
Results (μmols/mg protein/hour) are Mean ± SEM for five different preparations.
* Significantly different from control, † significantly different from CP at p<0.05 by one-way ANOVA.

Table 6: Effect of Fish oil (FO) on enzymatic and non-enzymatic antioxidant parameters in homogenates of liver with CP treatment.

Parameters / Groups	Lipid Peroxidation (nmols/gm tissue)	Total SH (μmols/gm tissue)	SOD (μmols /mg protein)	Catalase (μmols/mg protein/min)	GSH-Px (μmols/mg protein/min)
Control	165.89 ± 5.29	20.15 ± 0.811	15.13 ± 0.29	14.59 ± 0.531	0.0073 ± 0.001
CP	201.84 ± 5.79[*] (+21.6%)	16.95 ± 0.853 (-15.88%)	6.78 ± 0.40[*†] (-55.1%)	11.04 ± 0.57[*] (-24.33%)	0.0016 ± 0[*] (-78.08%)
CPF	167.69 ± 11.77[†] (+1.07%)	20.42 ± 0.05[†] (+1.33%)	11.50 ± 0.60[*†] (-23.99%)	13.05 ± 0.514 (-10.55%)	0.0038 ± 0[*†] (-47.94%)
FO	166.66 ± 3.27[†] (+0.464%)	22.3 ± 0.513[†] (+10.66%)	16.40 ±0.41[†] (+8.39%)	13.76 ± 1.11 (-5.68%)	0.0058 ± 0[*†] (-20.54%)

Results are Mean ± SEM for five different preparations.
* Significantly different from control, † significantly different from CP at p < 0.05 by one-way ANOVA.
Values in parentheses represent percent change from control.

Discussion

Cisplatin, a platinum co-ordinated complex, is a widely used antineoplastic agent for the treatment of metastatic tumors of the testis, metastatic ovarian tumors, lung cancer, advanced bladder cancer and many other solid tumors (Sweetman, 2002). The efficacy of cisplatin is limited, however, by its dose-limiting nephrotoxicity (Winston and Safirstein, 1985; Greggi Antunes *et al.*, 2000; Chirino *et al.*, 2004). Although cisplatin-induced nephrotoxicity has been very well documented in clinical oncology, hepatotoxicity has been rarely characterized, and is less studied. It is known that cisplatin is significantly taken up in human liver and that high doses of the drug produces hepatotoxicity (Hesketh *et al.*, 1990; Vermorken and Pinedo, 1982). The treatment of tumor cells with CP provokes several responses including membrane peroxidation, dysfunction of mitochondria, inhibition of protein synthesis and DNA damage (Cohen and Lippard, 2001; Sadowitz *et al.*, 2002). Formation of free radicals leading to oxidative stress has been shown to be one of the pathogenic mechanisms of these side effects (Jordan and Carmo-Fonseca, 2000).

Earlier studies have demonstrated that FO or its active component ω-3 fatty acids retard(s) progression of various forms of cancers, depression, arthritis, asthma, cardiovascular and renal disorders (Caterina *et al.*, 1994) and gastric ulcerations (Bhattacharya *et al.,* 2006). Dietary fish oil has also been shown to protect against acetaminophen (paracetamol)-induced hepatotoxicity (Speck and Lauterburgh, 1991), ethanol-induced gastric mucosal injury (Leung, 1992) in rats, a number of inflammatory diseases including lupus nephritis (Chandersekar and Fernandes, 1994), IgA nephropathy (Donadio, 2001) and murine AIDS (Xi and Chen, 2000). However, studies on possible beneficial effects of FO on drug or chemical-induced toxicity are very limited.

The present work was undertaken to study detailed mechanism of CP-induced hepatotoxic alterations and possible role of FO in preventing those deleterious changes in rat liver. Single CP injection caused marked alterations in serum and urine parameters, as is evident by decreased creatinine and urea clearance. The present results confirm the earlier findings (Kuhad *et al.,* 2006) and show that CP administration to control rats caused marked increase in serum creatinine and BUN, diagnostic indicators of nephrotoxicity, accompanied by the significant decrease in serum glucose, inorganic phosphate (Pi), phospholipids and serum cholesterol, accompanied by massive proteinuria, glucosuria and phosphaturia.

FO-diet given prior to and following CP administration prevented CP-induced alterations in various serum/urine parameters. CP elicited increase in levels of both serum creatinine and BUN were lowered when CP was administered to FO fed rats. Serum glucose, phospholipids were improved and cholesterol profoundly enhanced upon CP treatment to FO fed rats.

CP significantly decreased the activities of ALPase, GGTase and LAP in the liver homogenate. The decrease in membrane enzyme activities might have occurred due to loss of membrane enzyme and other components indicating adverse effects of CP on membrane integrity. The present results show that in contrast to CP treatment, dietary supplementation of FO to control rats caused significant increase in the activities of membrane enzymes in the liver homogenate. Prior to or along with CP treatment, FO supplementation prevented/retarded CP-induced decrease of membrane enzymes in the tissue. The activity of lysosomal enzyme, ACPase was significantly increased in liver homogenate by CP treatment. Alteration in ACPase activity demonstrates CP-induced loss of lysosomal function (Kuhlmann *et al.,* 1997; Courjault-Gautier *et al.,* 1995). However, the CP induced effect on lysosomal enzyme activity appeared to be ameliorated at least to some extent by dietary FO supplementation.

To assess the functional aspects, the activities of various metabolic enzymes were determined under different experimental conditions. The activities of various enzymes involved in glycolysis, TCA cycle, gluconeogenesis and HMP-shunt pathway were differentially altered by CP treatment and/or by FO consumption. CP caused significant increase in LDH and decrease in MDH in the tissue which was accompanied with a simultaneous increase in hexokinase activity in the liver tissue. Although the actual rates of glycolysis or TCA cycle were not determined, marked decrease in MDH activity appears to be due to CP-induced damage to mitochondria (Kuhlmann *et al.,* 1997; Zhang and Lindup, 1993). A marked increase in LDH and to some extent hexokinase activity with simultaneous decline in TCA cycle enzyme, MDH appears to be an adaptive cellular effect in energy metabolism from aerobic metabolism

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alternatively to anaerobic glycolysis due to CP induced mitochondrial dysfunction.

CP also altered the activities of enzymes of gluconeogenesis and HMP-shunt pathway. The activities of G6Pase, FBPase, G6PDH, were profoundly decreased albeit to different extent. However, the activity of NADP malic enzyme (ME) was increased in the tissue. The present data indicate that CP caused differential effects on different enzymes of carbohydrate metabolism. FO administration to CP-treated rats resulted in overall improvement of carbohydrate metabolism as evident by higher activities of MDH and gluconeogenic enzymes in CPF group of rats as compared to CP group. FO might have lowered number of damaged mitochondria or other affected macromolecules or may have increased number of normally active organelles or macromolecules.

Earlier reports reveal that heavy metals including CP (Fatima *et al.,* 2004; Baligha *et al.,* 1998; Banday *et al.*) exert their toxic effects by inducing the generation of reactive oxygen species (ROS). A major cellular defense against ROS is provided by SOD and catalase, which together convert superoxide radicals first to H_2O_2 and then to molecular oxygen and water. Other enzymes e.g. GSH-Px use thiol-reducing power of glutathione to reduce oxidized lipids and protein targets of ROS. However, oxidative stress can occur as a result of either increased ROS generation and/or decrease in antioxidant enzyme system. These antioxidant enzymes protect the cell against cytotoxic ROS. In agreement with the previous studies, present results show that CP enhanced lipid peroxidation (LPO), an indicator of tissue injury and deplete protein thiols (Sara *et al.,* 2009). CP administration to control rats caused severe damage to liver tissue most likely by ROS generation as apparent by perturbation in the antioxidant enzymes (SOD, Catalase and GPx-SH) and total-SH content that lead to increased lipid peroxidation. CP treated rats fed on FO rich diet caused significant increase of SOD, catalase and GSH-Px activities accompanied by lower LPO values in liver tissue. The protection against CP by FO can be attributed to its intrinsic biochemical and natural antioxidant properties. It appears that FO enriched in ω-3 fatty acids enhanced resistance to free radical attack generated by CP administration.

We conclude that while CP elicited deleterious hepatotoxic effects by causing severe damage to the plasma membrane, mitochondria and other organelles by suppressing antioxidant defense mechanism, however, these effects were ameliorated by dietary supplementation with FO. Present study thus supports the rationale that ω-3 fatty acid enriched FO may be effective dietary supplementation to maximize the clinical use of CP in the treatment of various malignancies without hepatotoxic and other side effects.

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