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Ameliorative Effect of Fish Oil on the Cisplatin Induced Hepatotoxicity and Nephrotoxicity in Rats

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ABSTRACT

Cisplatin is a widely used anticancer drug. It is documented that it elicits major side effects like nephrotoxicity and hepatotoxicity forcing the patients to limit its clinical use in long term treatment. The aim of this study was to investigate the possible protective role of fish oil on cisplatin-induced liver and kidney toxicity using biochemical and histopathological approaches. Fifty male rats were used, the first group (G **I)** was used as control received physiological saline; the second group (G **II)** received only fish oil by intraperitoneally administrated; animals in group **III:** received a single dose CP. Group **IV:** received a single dose CP following 10 days of fish oil pre treatment, and the last group (Group **V)** was treated with a single dose of CP before 5 days of fish oil post-treatment. Administration of cisplatin to rats induced a marked liver and renal failure, Liver damage was assessed by hepatic serum marker enzymes like sAST, sALT. Renal damage was observed by serum markers like creatinine and urea. The results showed that oral administration of fish oil significantly reduced the elevated serum markers in the liver and kidney that were induced by cisplatin in rats. Liver and renal histopathological changes were observed in the cisplatin group as compared to the control group. In contrast, these histopathological changes appeared nearly normal in the groups treated with fish oil pre- and post-injection. In conclusion, this study clearly indicated that cisplatin treatment markedly impaired liver and renal function and that treatment with fish oil might prevent this toxicity in rats.

Keywords: Cisplatin; Hepatotoxicity; Nephrotoxicity; Fish oil; Rat

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INTRODUCTION

Chemotherapy is one of the most valuable cancer treatments and many anticancer agents have been developed to achieve this over the years. Nevertheless, their serious side effects and consequent systemic toxicity has contributed to the limitations for cancer treatment [1]. Several investigators have observed that anticancer agents have been utilized for the chemotherapy of various cancers such as sarcoma small cell lung cancer, ovarian cancer, lymphomas germ cell tumors and CNS disability [2-3], but they have also been implicated in causing various kinds of toxicity after treatment in cancer patients measured as adverse effects [4].

Cisplatin (CP -diamminedichloroplatimum II) is a heavy metal complex that is one of the most effective anti-neoplastic drugs currently available. Cisplatin is frequently used to treat various malignancies, including cancer of the testis, metastatic ovarian tumors, lung cancer, advanced bladder cancer and many other types of solid tumors. Although high doses of cisplatin more effectively suppress cancer [5]. Cisplatin-induced an increase in lipid peroxidation in the kidney tissues. The drug causes generation of reactive oxygen metabolites (ROM) and inhibits the activity of antioxidant enzymes in renal tissue [6], which is responsible for the life-threatening side effects of CP therapy, including nephrotoxicity, hepatotoxicity and neurotoxicity [7-9]. It is known that cisplatin is significantly taken up in human liver and those high doses of the drug produces hepatotoxicity [10]. Generally liver toxicity of cisplatin is characterized by mild to moderate elevation of serum transaminases and less frequently, by a mild elevation of serum alkaline phophatase, lactate dehydrogenase (LDH), bilirubin and *c*-glutamyl transpeptidase levels [11].

Natural antioxidants as potential nutraceuticals have been studied to reduce severe side effects as well as enhance anticancer activities of antitumor drugs [12]. In animals, supplementation with antioxidants seems to protect against CP-induced side effects [13]. A number of investigations have demonstrated that diet supplemented with fish oil(FO) enriched in ώ-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 [14]. The marine-derived lipids, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), can differentially sensitize tissues to chemotherapy. These lipids sensitize cancer cells or tumors to anticancer drugs while preserving or even protecting non-tumor tissues. While the potential of DHA and EPA to improve chemotherapy has been reviewed [15]. Natural products (fish oils) or nώ3 PUFA-rich oils biosynthesized from micro-algae14 are considered to be the most effective way to supply DHA and EPA to tissues. These fatty acids are non-toxic nutrients [16]. Their human consumption has been granted GRAS (generally recognized as safe) status by the FDA. [17] DHA and EPA are incorporated into cell membrane phospholipids as structural components [18] and are ubiquitously distributed in tissues, although at different levels in various organs [19]. The present study was performed to investigate the possible role of fish oil in the prevention of CP-induced nephrotoxicity and hepatotoxicity in rats.

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MATERIAL AND METHODS

Chemicals:

Cisplatin was bought from Sigma–Aldrich Chemical Company, USA. Fish oil (Menhaden, Sigma Chemical Co., USA); all other chemicals used were of analytical grade and were purchased either from Sigma Chemical Co. (St Louis, MO, USA).

Animals:

The animal experiments were conducted according to the guidelines of Committee University of Alexandria, Egypt. All experiments were performed with adult albino male rats weighing approximately260 ±25 g. They were obtained from the animal house of Medical Research Institute, Alexandria University, Alexandria, Egypt. The animals were housed in stainless steel cages in a temperature-controlled room with a12-h light/dark cycle. They were fed standard laboratory food and provided with water *ad libitum*.

Experiment design:

The Animals were quarantined for 10 days before being randomized into five experimental groups of ten animals per group. Animals were i.p injected with single dose of Cisplatin dissolved in normal saline (7 mg/kg) at the beginning of the experiment [20]. Animals were fed with Menhaden oil (MO) by intragastric intubation at a dose of 1ml/toad, according to Sadek *et al*. [21].

All the animals were euthanized after 48 h of last treatment and various blood and hepatic biochemical parameters were performed:

Group I: (control) received a single dose i.p. injection of 1ml saline.
Group II**:** received only fish oil.
Group III**:** received a single dose CP.
Group IV**:** received a single dose CP following 10 days of fish oil pre-treatment.
Group V**:** received a single dose of CP before 5 days of fish oil post-treatment.

At the end of the experimental period of 12 weeks, animals in different groups were sacrificed by cervical decapitation. Blood samples were collected in two different tubes, i.e. one is heparinised, for plasma and another without heparin for serum collection. Serum and plasma were separated by centrifugation and used for various biochemical estimations.

Effect of the treatment on body and organs weights of the rats:

The body, liver and kidney weights of all rats were noted before and after the treatment to see effect of the treatment on the animals.

Preparation of samples:

The blood was centrifuged at 2500 rpm (1500g) for 10 min to collect their serum which was later stored in cold. All the samples were labeled properly and kept in -20 °C for further analysis.

Estimation of ASTand ALT as liver function markers:

The activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in the serum was assayed by [22].

Estimation of urea and creatinine as kidney function marker:

The level of urea and creatinine was estimated in the serum by the commercially available diagnostic kits, Urea [23], creatinine [24].

Histological studies:

Tissues (liver and kidney) were fixed in formalin, routinely processed and embedded in paraffin. Blocks were cut at 4 µm thick, paraffin sections were cut on glass slides and stained with hematoxylin and eosin (H&E), and examined by light microscope. Features of cell injury and necrotic changes were scored on a semi quantitative scale: [+, mild (less than 25% of the tissues were affected); ++, moderate (25–50% of the tissues were affected); +++, severe (more than 50% of the tissues were affected)].

Statistical analysis:

All the data have been expressed as in mean ± standard error of mean (SEM) for 5–6 different preparations in duplicate. Their statistical significance was evaluated by one-way ANOVA software. The probability of occurrence was selected at $p < 0.05$.

RESULTS

Effects of CP and fish oil treatment on body and organs weights:

At the end of the experimental period, animals of all groups (except *GroupIII*) showed no obvious symptoms or signs of toxicity and No mortality throughout the experiment. However, Cisplatin treated rats (*Group III*) showed varying degrees of clinical signs few minutes after dosing. The signs included huddling, conjunctivitis, mild tremor, piloerection diarrhea and dyspnea. Before starting of the treatment, weight of all the rats was in the range of 260 -263 g /rat but after the treatment, there was a significant decreased in final body weights in CP treated group when compared to the control group. But pre- and post-treatment with fish oil not significantly changed in body weight when compared to the weight before the treatment (Table 1). On the other hand, results showed that oral administration of Cisplatin (*Group III*),

significantly decreased the absolute and the relative liver and kidney weights compared with those of control group (po0.05). But no significant changes were observed between (*Group I*), (*Group II*), (*Group IV*) and (*Group V*) groups at the end of the experimental period (Table 1).

Table 1. Effect of cisplatin or its combination with fish oil on body weight, liver weight in rats.

Groups	Initial body weight(g)	Final Body weight(g)	Absolute liver weight(g)	Relative liver weight (g/100 gb.w)
Control	263±29.2	266± 32.4[a]	11.66 ± 1.66[a]	4.3 ± 0.10[a]
F O	262±30.4	268± 35.8[a]	11.66 ± 1.66[a]	4.9 ± 0.10[a]
CIS	262.32.6	219 ± 21.9b[b]	6.66 ± 0.66[b]	3.0 ± 0.15[b]
CP + Pre-treatment with FO	263 ±33.1	265± 27.9[ab]	11.66 ± 1.66[a]	4.3 ± 0.10[ab]
CP+ post-treatment with FO	263±33.5	264± 32.4[ab]	11.66 ± 1.66[a]	4.3 ± 0.10[ab]

Data represent mean ±SEM, n = 10. [a b] $p < 0.05$ vs. control.

Biochemical results:

The Cisplatin treatment markedly affected the liver specific enzymes. It was found that a significant increase in serum AST, ALT activities of rats given alone Cisplatin ($p < 0.05$). This result suggests that liver function markers are elevated in the serum due to release of the enzymes from damaged liver. Also, Cisplatin caused a marked reduction in renal functions, as characterized by significant increases in plasma creatinine and urea levels after administration of single dose of CP when compared to the control group ($p < 0.05$) (Table 2). Thus, these data indicate that a single intravenous injection of Cisplatin impairs both kidney and liver functions.

On the contrary, Treatment with fish oil markedly reversed cisplatin-induced increases in serum creatinine and urea levels. Fish oil was also found to be effective to reverse Cisplatin-induced changes in serum ALT, AST levels compared with the alone Cisplatin treated groups (Table 2).

Table 2. Effect of fish oil on the AST, ALT activities as well as in the creatinine and urea nitrogen levels changes induced by treatment of rats with Cisplatin

Treatment	Liver function tests		Kidney function tests	
	Ast(U/L)	ALT (U/L)	Creatinine (mg/dL)	Urea (mg/dL)
Control	36.5± 0.80	37.7±1.10	0.54±0.07	41±3.9
F O	37.3± 0.84	36.9±1.20	0.57±0.09	43±3.4
Cis	65.5± 3.40[a]	68.1± 8.01[a]	1.40±0.16[a]	107±5.l[a]
CP + Pre-treatment with FO	35.5± 0.89	37.9±1.10	0.58±0.10	44±3.0
CP + post-treatment with FO	39.52± 0.89 38.4±1.10		1.03±0.21	95±3.9

Each group contains 10 rats. Values are means±SD. Means with different letters (shown as superscripts a) within the same column indicate significant difference at $P < 0.05$.

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Histopathology:

Histopathology of rat liver:

Histopathological changes in all of the groups were scored as summarized in Table 3. In microscopic examinations of the samples, the central vein, hepatocyte cord, hepatocytes and portal areas were observed to be normal in the control and Fish oil groups (Fig. 1A&B). Cisplatin application constituted histopathological changes in the liver. Severe congestion was observed in the area surrounding the central veins .Wide vacuolar degeneration of hepatocytes, and lymphocyte and eosinophil infiltration were observed (Fig. 1C). Necrotic hepatocytes especially around the vena centralis (at the periphery of central vein) and occasional sinusoidal congestion were also determined (Fig. 1D). The histological appearance of the pre-treatment with fish oil group showed normal architecture (Fig. 1E). However, post-treatment with fish oil group was quite similar to that of the control group, and tissue damage and necrosis were of less extent in these groups than the Cisplatin group (Fig. 1F).

Table 3. Effect of pre and post-treatment with fish oil on the histopathological changes induced by treatment of rats with Cisplatin

Lesion	**Treatment**				
	Control	**fish oil**	**CP**	**CP+Pre-treatment with F O**	**CP+post-reatment with FO**
Liver					
Vacuolar degeneration	-	-	++	-	+
Centrilobular necrosis	-	-	+++	-	_
Inflammatory cell infiltration	-	-	++	_	+
Congestion	-	-	++	_	+
Kidney					
Tubular necrosis	-	-	+++	-	_
Tubular dilatation	-	-	++	-	+
Blood vessel congestion	-	-	++	+	+
Glomerular congestion	_	_	+	+	+

Tissue samples were collected for morphological analysis after 48 h of treatment of rats. (+) Mild, (++) Moderate; (+++) severe; (–) no pathological Change

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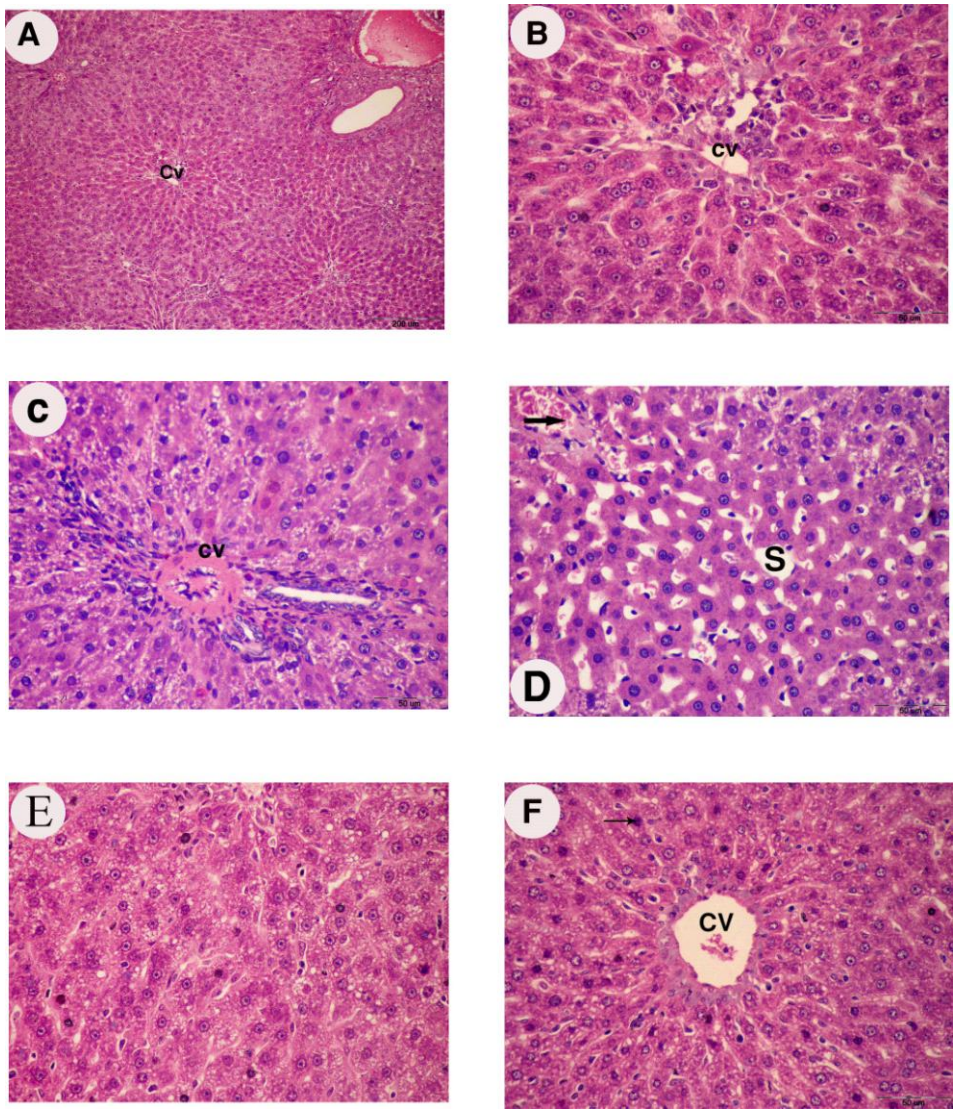

Figure 1. Photomicrographs of liver samples of the control and treated groups. Normal liver histological aspect from a control ((A), *Group II* (B), It is composed of hexagonal or pentagonal lobules with central veins (CV) and peripheral hepatic triads embedded in connective tissue with no leukocyte infiltration and necrosis. Hepatocytes are arranged in trabecules running radiantly from the central vein and are separated by sinusoids containing Kuppfer cells. Treated cisplatin groups (C&D), show disorganization and degeneration hepatocytes, diffuse cytoplasmic vacuolization, dilatation and vascular congestion in sinusoids, increased number of activated Kupffer cells and inflammatory cell infiltration. Pre and post -treatment with the fish oil, rats showing apparently normal architecture (E&F).

Histopathology of rat kidney:

The histological changes in kidneys were results are presented in Table 3. The kidneys of control and Fish oil group rats exhibited normal renal tissue, where normal glomeruli, tubular epithelium and interstitial tissue were observed (Fig. 2 A&B). The Cisplatin -exposed animals showed pathologic alterations such as congestion, hemorrhages, and tubular degeneration. Tubular degenerative changes included hyalinic degeneration, necrosis, and inflammation.

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Glomerular damage and tubular necrosis with invading inflammatory cells were also characteristic lesions (Figs. 2C&D). Despite the fact that the above mentioned changes were observed in all parts of the nephrons. Pre and post -treatment with the fish oil, the morphology revealed a normal appearance of glomeruli and tubuli was noted (Fig. 2E&F) when compared with the alone CP group.

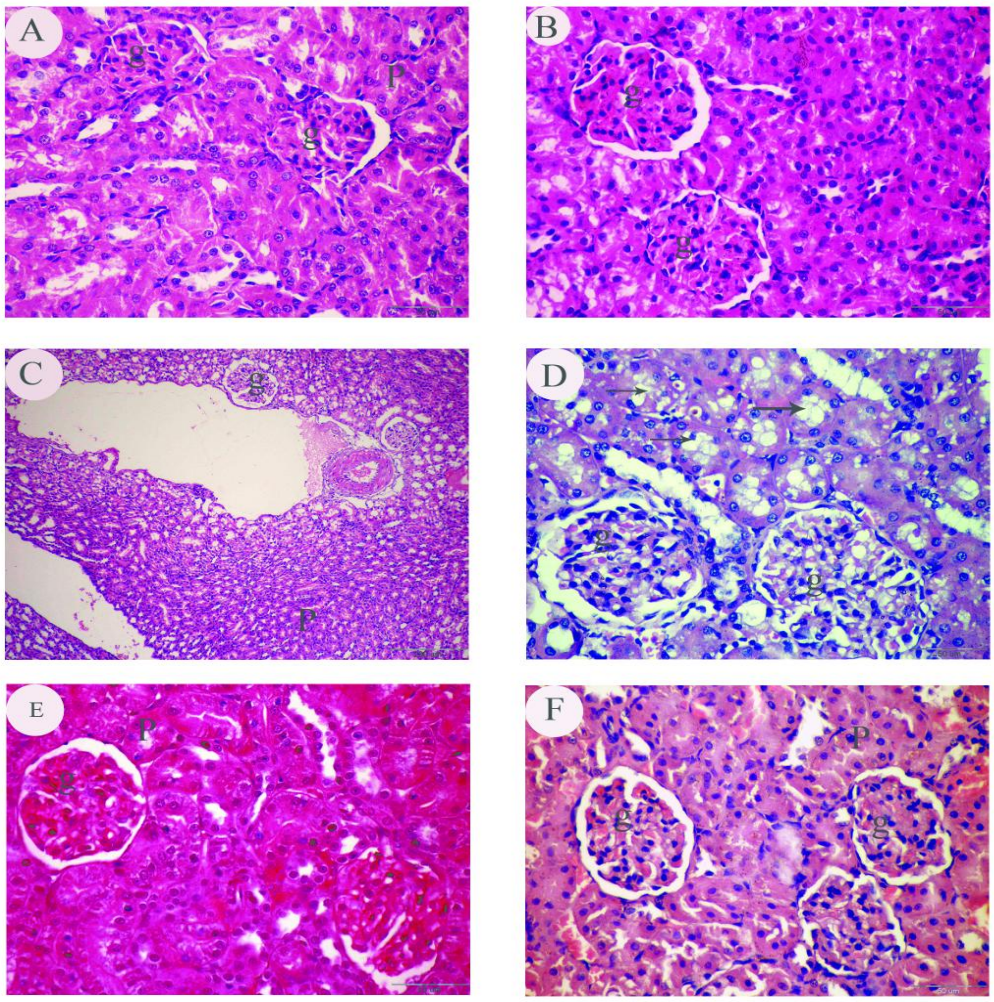

Fig. 2. Photomicrographs of kidney samples of the control and treated groups. (A&B) kidney section of control and fish oil rats showing normal glomeruli and normal tubules; (C&D) cisplatin group showing, severe glomerular congestion and degeneration, dilatation in Bowman's space, and degeneration in tubular cells (arrow) in kidney. Cortical renal tubules show various degenerative changes, light edema and focal tubular necrosis invaded by inflammatory cells; (E&F) Kidney of Pre and post -treatment with the fish oil, rats showing normal histology.

DISCUSSION

Anticancer agents used in chemotherapy causes several types of toxicity including neurotoxicity [25] and hepatotoxicity [26]. Cisplatin (CP) is one of the most widely used anticancer drugs for the treatment of various cancers and solid tumors [27].

Several investigators [6&28] showed that treatment with some of the most effective anti-cancer drugs provides a number of symptoms of direct toxicity. The present study evidenced that cisplatin treatment had a toxic effects on liver and kidney of rats. Administration of CP to rats resulted in decrease in the body weight of the animals. This weight loss observed in the CP -treated group may be because of reduced appetite and enhanced catabolic rate which are considered as the obvious side effects of chemotherapy. It could be also due to CP - induced dysfunction of the gastrointestinal system. The treatment affects the target organs – kidneys and liver as evidenced by their elevated functional markers in the serum of the CP - treated group. Other less frequent toxic effects, which is generally observed after administration of high doses of cisplatin, can also alter the clinical situation of patients [29]. The present investigation showed that rats fed fish oil (menhaden oil) with or without Cisplatin exhibited insignificant decrease in their body weight than control animals. Also, no clinical signs of toxicity were present in any of these groups. Similar to the present result, Mehra *et al*. [30] suggested that the Eicosapentanoic acid (EPA) is well tolerated and may stabilize weight in cachetic pancreatic cancer patients. Beck *et al.* [31] showed that maintenance of host body in animals bearing the MAC16 tumor and treated with EPA is associated with decreased protein degradation in skeletal muscle without an effect on protein synthesis.

It is documented that CP is accumulated in its target organs by covalently binding with their proteins [32]. This can affect their antioxidant enzymes which are the first line of defense against any oxidative insult to the cells. This may be the primary factor behind the alteration in the parameters observed in group *III*. In this study, Cisplatin -induced hepatotoxicity and nephrotoxicity were evidenced by biochemical measurements and histopathological changes that coincide with the observations of other investigators Parvez et al. [33]. This is a clear indication of nephrotoxicity and hepatotoxicity caused by CP as the markers are released by the damaged organs in the circulatory system. The data of our study also revealed that a single dose of cisplatin induced severe hepatic damage. Elevation of the serum levels of the hepatic enzymes; Deteriorations of liver function tests (serum ALT, AST) revealed hepatic dysfunction in this group. These alterations are consistent with previous data of the literature [34&35]. A marked recovery was observed in the markers combination treatment by CP with FO. Heller *et al.* [36] showed that, after major abdominal tumor surgery, fish oil supplementation improved liver and pancreas function, which might have been contributed to the faster recovery of patients. Hatzitolios *et al.* [37] reported that a decline in serum transaminase levels (AST & ALT) in patients with hypertriglyceridemia after treatment with omega-3 fatty acids. Studies on possible beneficial effects of FO on drug or chemical induced neph rotoxicity are very limited. Recently it has been documented that FO protects against gentamicin-induced nephrotoxicity [38].

It has been shown that administration of cisplatin to rat caused an elevation in plasma creatinine and urea levels. Several studies have reported that the alterations induced by cisplatin in the kidney functions were characterized by signs of injury, such as increase in products of creatinine and urea levels, in urine and plasma samples [39]. The present result was in agreement with that of Sueishi et al. [13] who demonstrated that an increase in creatinine in serum in proximal renal tubules on the fourth day after CP injection in rats. The present studies

were performed to test the hypothesis that FO consumption would ameliorate CP-induced nephrotoxic and other adverse effects thus maximizing the clinical use of CP in the treatment of various malignancies without any major side effects. The feeding of FO prior to and after CP treatment significantly lowered CP-elicited increased levels of serum creatinine. Also, Serum urea levels were improved upon CP treatment to FO consuming rats.

Moreover, cisplatin administration caused severe damage in the liver, as assessed microscopically. The liver morphology was characterized by severe degenerated hepatocytes and enlargement of sinusoids. Some reports suggest that cisplatin-induced hepatotoxicity may be dose-related [40&41], reaching an incidence of 86% in patients treated with a single dose of 100 mg/m2 cisplatin. Although hepatotoxicity has always been described of mild and of transient nature, at least when cisplatin is not administered at high doses, particular attention should be paid to the additional effects due to the co administration of other more hepatotoxic agents. Little is known about the mechanism of cisplatin-induced liver damage, although apoptotic lesions seem to characterize the damaged liver parenchyma.

In the present study, cisplatin injection produced severe degeneration in glomeruli and both proximal and distal tubuli compared to control group. In accordance with Jariyawat et al.[42] cisplatin treatment developed renal damage. In earlier studies, it is documented that CP is accumulated in its target organs by covalently binding with their proteins [32]. This can affect their antioxidant enzymes which are the first line of defense against any oxidative insult to the cells. In fact oxidative stress and involvement of oxidative reactive species in cisplatin toxicity have been shown in many studies and were suggested to play an important role in the pathogenesis [43]. Cisplatin (CP) causes several types of toxicity including neurotoxicity and hepatotoxicity mediated by mitochondrial dysfunction. Free radical generation or oxidative stress develops when there is an imbalance between pro-oxidants and antioxidants ratio, leading to the generation of ROS [26]. Studies have also shown that CP induces ROS production in liver mitochondria [5].

The nephrotoxicity of CP is well documented as the most important dose-limiting factor in cancer chemotherapy [44]. CP- induced cell injury and necrosis in the rat kidney are predominantly localized in the S3 segment of proximal tubules in the corticomedullary region [45]. However, CP-induced free radical production and LP in tubular cells have been suggested to be responsible for the oxidative renal damage [39]. In some animal studies CP was reported to enhance LP in renal tissue [46]. Also, renal GSH concentrations were found to be decreased after CP administration [13]. It was also observed that CP treatment increased creatinine levels [46]. A marked increase in creatinine in serum and histopathological changes including vacuolation, necrosis, and protein casts were observed in proximal renal tubules on the fourth day after CP injection in rats [13].

There are several possible sources of ROM production by the inflamed tissue. These include the epithelium, the microvascular endothelium or the inflammatory cells. Although, the mechanisms underlying the cisplatin-induced acute renal failure have not been fully understood, several investigators have shown that the ROS or free radicals are closely related

to the acute renal failure induced by cisplatin [13]. Cisplatin has been demonstrated to generate active oxygen species, such as superoxide anion and hydroxyl radical (Baliga et al., 1998) and to stimulate lipid peroxidation in the kidney tissue [47].

Thus, complementary therapies have been recently investigated for their potential to ameliorate these effects [48]. Antioxidants may also reduce certain types of toxicity associated with chemotherapy [49]. Antioxidants (vitamin E, etc.) are suggested to play a possible role in protecting against CP-induced nephrotoxicity mediated by oxidative stress [46]. Supplementation with well-known antioxidants, such as vitamin E and vitamin C administration, was reported to protect against CP-induced renal toxicity in animal studies [50&51] .

When histopathological analyses and other experimental results of the present study are evaluated together, fish oil seems to protect against CP-induced hepatotoxicity and nephrotoxicity. Our results confirm the other studies suggesting that antioxidants may have a protective effect against CP-induced nephrotoxicity [52]. A marked recovery was observed in the markers post combination treatment by CP with fish oil several possible mechanisms have been proposed to explain the pathological status of kidney and liver post- CP treatment [53]

As far as we know, this is the first study that fish oil in the prevention of CP-induced nephrotoxicity and hepatotoxicity. Our study provides encouraging results about the protective effect of fish oil against CP toxicity. The protective effects of fish oil may be mediated via their antioxidant effects. However, further studies are needed to elucidate the exact mechanism of this protection of fish oil.

The current study demonstrates that fish oil provides protection against cisplatin-induced acute nephrotoxicity also induced hepatotoxicity in rat.

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