SUMMARY

The kidney is a vital organ, which plays an essential role in health, disease and overall development and growth. The main function of kidney is to maintain total body fluid volume, its composition and acid base balance. A number of environmental variables including certain drugs influence these functions (Mahmood and Water, 1994; Begg and Barcay, 1995; Priyamvada et al., 2009). Platinum-based drugs are widely used anticancer agents with a broad range of antitumor activities (Alois et al., 2007). These platinum antitumor agents are unique, coordination complexes and the parent compound of this class cis-diammine-dichloroplatinum (II) (cisplatin, CP), contributes to the curative treatment of various cancers such as testicular teratoma, ovarian, head, neck, bladder, cervical and lung cancers. The compound is also highly toxic with a list of side effects including renal damage, severe nausea, vomiting, myelosuppression, ototoxicity and neurotoxicity (McKeage, 1995, Markman, 2003).

The optimal use of cisplatin as a chemotherapeutic drug has been limited by its nephrotoxicity. Following administration, CP is widely distributed into body fluids and tissues. The highest concentration can be seen in the kidney, liver and intestine although it has also been found in all other tissues (McEvoy, 1992). Induction of nephrotoxicity is assumed to be a rapid process involving reactions with proteins in renal tubules. Light and electron microscopy have shown that the CP-induced injury and necrosis in rat kidney are predominantly localized in the S3 segment of proximal tubules in the corticomedullary region (Townsend et al., 2003). CP toxicity in proximal tubular epithelial cells is morphologically characterized by tubular necrosis, loss of microvilli, alterations in the number and size of lysosomes and mitochondrial vacuolization accompanied by functional disturbances of various cell organelles (Kuhlmann et al., 1997). The mechanism by which cisplatin kills the proximal tubule cells in the kidney has been the focus of intense investigation for many years. In tumors and other dividing cells, cisplatin-DNA crosslinks are thought to be the cytotoxic lesion (Fink and Howell, 2000). Nonproliferating cells are less sensitive to the toxicity of DNA-damaging agents, yet the quiescent proximal tubules cells are selectively killed by cisplatin (Lieberthal et al., 1996). Experimental evidence has suggested that CP deteriorates renal function (Arany and Safirstein, 2003) and glomerular filtration rate (GFR) in a dose-dependent manner (Yao et al., 2007).
The mechanism underlying the side effects induced by cisplatin is not understood clearly, however it was considered to be attributed to the combination of multi-ways (Hong et al., 2005; Ramesh and Reeves, 2002; Nowmak, 2002; Townsend and Hanigen, 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 (Kuhlman et al., 1997; Matsushima et al., 1998; Kuhad et al., 2007; Khan et al., 2009) and reaction with thiols in protein and glutathione, which could cause cell dysfunction. Various studies have focused on the ways for prevention of cisplatin associated side effects via supplementation of preventive agents simultaneously. These include antioxidants, modulators of nitric oxide, diuretics, and cytoprotective and apoptotic agents (Ali and Al Moundhri, 2006). However, none of them have been found safe/suitable for clinical practice.

In past few years, much interest has been centered on the role of naturally occurring dietary substances for the control and management of various chronic diseases such as cancer and cardiovascular disorders (Connor, 2000; Hardman, 2002; Larsson et al., 2004; Williams et al., 2007; Kakar et al., 2008; Leaf, 2008). From ancient times the physicians and scholars in Asia have understood that food have both preventive and therapeutic value and are integral part of health. Omega-3 polyunsaturated fatty acids (ω-3 PUFA) enriched fish oil provides one such dietary source of biologically active components that has been shown to be co-preventative and co-therapeutic in a wide variety of ailments (Doughman et al., 2007).

A number of investigations have already demonstrated that diet supplemented with fish oil (FO) enriched in ω-3 PUFA has profound beneficial health effects against various pathologies including cancers, cardiovascular disorders, diabetes, depression, arthritis, asthma and inflammatory and immune disorders of the kidney and intestine (De Caterina et al., 1994; Tsujikawa et al., 2000; Barbosa et al., 2003; Bhattacharya et al., 2006). Recently, ω-3 PUFA from some plants/seeds e.g. flaxseed oil (FXO) showed many similar health benefits as demonstrated by ω-3 PUFA from FO (Freese and Mutanen 1997; Ide et al., 2000; Kim and Choi, 2001; Nannicini et al., 2006; Paschos et al., 2007). Alpha Linolenic acid (ALA, 18:3 n:3, ω-3 FA) from vegetable sources including grains and oils, offer an alternative source for those who are unable to regularly consume fish for religious or other reasons (Harper and Jacobson, 2001).
Dietary flaxseed oil affects the biochemistry of fatty acid metabolism and thus the balance of proinflammatory mediators and atherogenic lipids, holding a great promise for modulating inflammatory diseases (Chilton et al., 2008).

In view of numerous beneficial health effects of ω-3 PUFA "we hypothesized that: dietary FO and FXO enriched in ω-3 PUFA would be able to prevent/reduce single as well as multiple CP dose induced nephrotoxic and other adverse effects in the rat kidney."

To address this hypothesis the present work was undertaken to study the detailed biochemical events/cellular response/mechanism of CP induced nephrotoxic and other adverse effects in rat kidney. The effects of dietary ω-3 PUFA enriched FO and FXO were also determined to observe any protection provided by them against CP nephrotoxicity. The following parameters were determined:

(a) Certain biochemical parameters in serum/urine.

(b) The activities of BBM and lysosomal marker enzymes in renal cortical and medullary homogenates and BBM marker enzymes in isolated BBM preparations from renal cortex.

(c) The activities of certain enzymes of carbohydrate metabolism involved in glycolysis, TCA cycle, gluconeogenesis and HMP-shunt pathway in renal cortex and medulla.

(d) The enzymatic and non-enzymatic parameters of antioxidant defense system in renal cortex and medulla.

# THE RESULTS OBTAINED ARE SUMMARIZED AS FOLLOWS:

פעל-IA: EFFECT OF DIETARY FISH OIL (FO) ON SINGLE CP DOSE INDUCED ACUTE NEPHROTOXIC AND OTHER ADVERSE EFFECTS

(a) Serum/Urine Parameters:

Single CP injection resulted in marked nephrotoxicity as manifested by increased serum creatinine and BUN. However, serum cholesterol, phospholipids, inorganic phosphate and glucose were significantly declined by CP treatment compared to control rats. These changes were associated with massive phosphaturia, proteinuria
and glucosuria accompanied by decreased creatinine clearance. FO supplementation alone caused increase in serum glucose and Pi but decrease in BUN, serum creatinine, PLP and cholesterol. Urinary glucose, Pi and protein excretions declined significantly accompanied by increased creatinine clearance. CP elicited increase in serum creatinine and BUN was markedly lowered by dietary FO supplementation to CP co-administered rats. CP induced alterations in other serum and urine parameters were also prevented by FO supplementation.

(b) Marker enzymes of BBM and lysosomes:
CP treatment to control rats caused significant reduction in the specific activities of ALP, GGTase and LAP in cortical homogenate. The activities of BBM enzymes as in the cortex were also lowered in medulla by CP administration. Feeding of FO diet alone, however, caused increase in GGTase activity both in renal cortex and medulla. The consumption of FO in combination with CP treatment prevented/reduced CP induced decrease in ALP, GGTase and LAP in the cortex as well as in the medulla. The effects of CP and FO were more apparent in BBMVs isolated from renal cortex than observed in the respective cortical homogenates. The magnitude of enzyme activities increased (4-8 folds) in the membrane preparations compared with respective values in the homogenates in control and treated groups for renal cortex indicating that the quality of the membrane prepared by the procedure was uniform for the various experimental groups.

The effect of CP, FO alone and in combinations was also determined on the activity of lysosomal enzyme, ACPase in renal homogenates. The activity of ACPase was significantly increased by CP treatment in cortical and medullary homogenates, however the increase was significantly reduced/prevented by FO diet.

(c) Enzymes of carbohydrate metabolism:
CP treatment to control rats significantly increased LDH, a marker of anaerobic glycolysis and HK whereas decreased MDH, an enzyme of TCA cycle, G6Pase, FBPase (gluconeogenesis) and G6PDH (source of NADPH). When CP treatment was extended to FO-fed rats, CP-induced alterations in the metabolic enzyme activities are prevented. FO alone caused increase in MDH, G6Pase, FBPase and G6PDH activities in cortex as well as in medulla. The activities of LDH, MDH, G6Pase and FBPase in
medullary homogenates were similarly affected by CP treatment albeit to different extent than in the cortical homogenates. Similar to cortex, G6PDH activity decreased whereas ME and HK activity increased by CP in the medulla. Feeding of FO containing diet to CP treated rats prevented CP induced alterations in various enzyme activities in the cortex and medulla.

(d) **Enzymatic and non-enzymatic parameters of antioxidant defense mechanism:**

CP caused significant decrease in the activities of SOD, catalase and GSH-Px in the cortex and medulla which was associated with marked increase in LPO and decrease in total-SH levels, indicating that CP caused severe damage to the renal tissues by generating free radicals and/or by suppressing the activities of antioxidant enzymes. CP treatment caused marked decrease in SOD, GSH-Px activities but slight decrease in catalase activity in the renal cortex. In medulla the activity of SOD, catalase and GSH-Px also showed significant decline by CP administration alone. CP induced increase in LPO and decrease in total-SH was prevented by feeding FO diet to CP treated rats. Dietary FO consumption protected well against CP induced perturbation of oxidants/antioxidants or CP induced oxidative damage. CP induced decrease in SOD, catalase and/or GSH-Px activity was markedly reversed/reduced by FO consumption in the renal cortex and medulla. CP induced increase in LPO was also lowered by FO supplementation in the renal tissues.

**PART-IB: EFFECT OF DIETARY FISH OIL (FO) ON MULTIPLE CP DOSE INDUCED NEPHROTOXIC AND OTHER ADVERSE EFFECTS**

(a) **Serum Parameters:**

Multiple dose CP administration caused severe nephrotoxicity as characterised by significant increase in serum creatinine, cholesterol, phospholipids and BUN accompanied by significant decrease in serum glucose and Pi. Prolonged FO consumption prior to and along with CP treatment significantly lowered CP induced increase in serum creatinine and BUN, indicating reduction in CP nephropathy. FO given to CP treated rats markedly prevented/retarded CP-induced increase of serum creatinine, cholesterol, and BUN.
(b) **Marker enzymes of BBM and lysosomes:**

CP treatment to control rats caused significant decrease in the activity of ALP, GGTase and LAP in the homogenates of renal cortex and medulla and in BBMV isolated from renal cortex. FO consumption in general significantly increased the activities of GGTase and LAP in both cortex and medulla. CP induced decrease in most BBM enzymes in the cortex and medulla was markedly prevented/reduced by FO supplementation to CP treated rats. The activity of ACPase, a lysosomal enzyme was significantly increased by CP treatment. CP-induced increase in ACPase activity was significantly reduced by FO diet both in cortical and medullary homogenates albeit to different extent.

(c) **Enzymes of carbohydrate metabolism:**

The activities of various enzymes involved in glycolysis, TCA cycle, gluconeogenesis and HMP-shunt pathways were variably affected by CP, FO and CP+FO in rat kidney. CP treatment to control rats significantly increased LDH, HK and ME activities whereas decreased MDH, an enzyme of TCA cycle and G6PDH (source of NADPH). When CP treatment was extended to FO-fed rats, CP-induced decrease of metabolic enzyme activities was prevented. CP elicited decrease of MDH activity was normalized to near control values by FO supplementation. However, FO alone caused increase in MDH, G6Pase, FBPase and G6PDH activities.

The activities of LDH, MDH, G6Pase and FBPase in medullary homogenates were similarly affected by CP treatment albeit to different extent than in the cortical homogenates. Similar to cortex, G6PDH activity decreased whereas ME and HK activities were increased by CP in the medulla. Prolonged feeding of FO supplemented diet to CP treated rats prevented CP induced alterations in various enzyme activities in medulla.

(d) **Enzymatic and non-enzymatic parameters of antioxidant defense mechanism:**

CP caused significant decrease in the activities of SOD, catalase and GSH-Px in cortex and in catalase and GSH-Px in medulla which was associated with marked increase in LPO and decrease in total-SH, indicating that CP caused severe damage to these tissues by generating free radicals and by suppressing the activities of antioxidant enzymes. Prolonged FO consumption protected well against CP induced
perturbation of oxidants/antioxidants or CP induced oxidative damage. CP induced decrease in SOD, catalase and GSH-Px was markedly reversed/reduced by FO consumption in both renal cortex and medulla. CP induced increase in LPO was also lowered by FO in the renal tissues.

**Interpretation:** In general, CP caused severe adverse effects on various serum/urine parameters and on the enzymes of BBM, carbohydrate metabolism and antioxidant defense system in rat kidney. FO similarly protected against single and multiple CP dose induced nephrotoxic and other adverse effects most likely due to its intrinsic biochemical and antioxidant properties.

**PART-IIA: EFFECT OF DIETARY FLAXSEED OIL (FXO) ON SINGLE CP DOSE INDUCED ACUTE NEPHROTOXIC AND OTHER ADVERSE EFFECTS**

(a) **Serum/Urine Parameters:**

Single dose CP administration caused severe nephrotoxicity as characterized by significant increase in serum creatinine, BUN, cholesterol and PLP but decrease in glucose and inorganic phosphate accompanied by massive glucosuria, proteinuria, phosphaturia, polyuria and decreased creatinine clearance. Dietary FXO supplementation to CP treated rats markedly prevented/retarded CP-induced increase of serum creatinine, cholesterol and BUN. CP-induced polyuria, phosphaturia and glucosuria were significantly prevented by FXO diet.

(b) **Marker enzymes of BBM and lysosomes:**

CP treatment to control rats caused significant decrease in the activities of ALP, GGTase and LAP in renal cortex and medulla indicating CP induced damage to the kidney. However, dietary FXO consumption caused significant reversal in CP induced decrease in BBM enzyme activities in the cortex and medulla. The activity of ACPase (a lysosomal enzyme) was variably increased by CP in the renal cortex and medulla whereas FXO consumption prevented the CP-induced increase in ACPase.
(c) **Enzymes of carbohydrate metabolism:**

CP treatment caused significant decline in the activities of MDH, G6Pase, FBPase and G6PDH whereas activities of LDH, HK and ME increased significantly in renal cortex and medulla. However, the effect of CP on HK and LDH activities was more marked in medulla while the effect on MDH activity was greater in cortex than medulla. As shown in “Part-IIA”, some of these enzyme activities were enhanced by FXO alone. FXO supplementation similarly prevented/retarded CP-induced decrease of MDH, G6Pase and G6PDH activities in the cortex and medulla.

(d) **Enzymatic and non-enzymatic parameters of antioxidant defense mechanism:**

CP treatment significantly decreased the activities of SOD, catalase and GSH-Px activities, accompanied with marked elevation of LPO and decrease in total-SH levels in the cortex and medulla. FXO alone significantly increased SOD, catalase and GSH-Px activities in the renal cortex and catalase activity in the renal medulla. FXO-diet when given to CP treated rats significantly ameliorated the CP-induced imbalance in the antioxidant defense system. LPO was decreased, whereas total-SH increased and the activities of the antioxidant enzymes remained significantly higher in FXO fed-CP treated rats compared to CP treated rats.

*PART-II: EFFECT OF DIETARY FLAXSEED OIL (FXO) ON MULTIPLE CP DOSE INDUCED NEPHROTOXIC AND OTHER ADVERSE EFFECTS*

(a) **Serum/Urine Parameters:**

Multiple dose CP treatment to control rats resulted in significant increase in serum creatinine (Scr), BUN, cholesterol and phospholipids but decrease in glucose and inorganic phosphate (Pi). These changes were associated with profound phosphaturia, proteinuria and glucosuria, accompanied by decrease in creatinine clearance. Feeding of FXO diet to CP administered rats resulted in significant reversal of various CP elicited deleterious effects on serum and urine parameters.
(b) **Marker enzymes of BBM and lysosomes:**

Multiple doses of CP caused significant decrease in the activities of BBM marker enzymes *viz* ALP, GGTase and LAP while FXO supplementation alone increased the activities of ALP and GGTase in renal cortical and medullary homogenates. FXO diet fed to CP treated rats prevented/reduced the CP induced decrease in the BBM enzymes while the activities were significantly higher in CPFXO than the CP group. The effect of CP was much more apparent in isolated BBMV preparations than observed in the respective homogenates. The activity of ACPase was however increased by CP in both cortical and medullary homogenates and FXO diet was able to prevent/retard the CP-induced increase in ACPase activity in a similar manner.

(c) **Enzymes of carbohydrate metabolism:**

CP treatment to control rats significantly increased LDH, HK and ME while decreased MDH, G6Pase, FBPase and G6PDH activities. However, FXO alone caused increase in MDH, G6Pase and FBPase activities. The activities of LDH, HK, MDH, G6Pase and FBPase in medullary homogenates were similarly affected by CP treatment albeit to different extent than in the cortical homogenates. Similar to cortex, G6PDH activity decreased whereas ME and HK activities were increased by CP in the medulla. Prolonged FXO feeding to CP treated rats prevented the CP-induced alterations in the metabolic enzyme activities.

(d) **Enzymatic and non-enzymatic parameters of antioxidant defense mechanism:**

Multiple CP doses caused significant decrease in the activities of SOD, catalase and GSH-Px in the cortex and medulla which was associated with marked increase in LPO and decrease in total-SH levels. The dietary supplementation of FXO was able to ameliorate the CP induced oxidative damage in both renal cortex and medulla. CP induced increase in LPO and decrease in total-SH were prevented/reduced by feeding FXO diet to CP treated rats. The activities of SOD, catalase and GSH-Px both in the cortex and medulla were significantly ameliorated by FXO supplementation to CP treated rats. These results indicate marked protection by FXO diet against single and multiple CP dose induced oxidative damage to renal tissues.
**Summary**

**Interpretation:** Single as well as multiple CP dose treatments caused severe damage to the kidney as reflected by increase in serum creatinine and BUN and decrease in biomarker BBM enzymes. There appeared to be a shift from aerobic to anaerobic metabolism most likely due to CP induced mitochondrial damage. CP also increased oxidative stress as indicated by increased LPO and decreased total-SH levels associated with suppressed antioxidant enzyme activities. FXO supplementation diet similarly prevented/retarded single and multiple CP dose induced alterations in various biochemical parameters and enzyme activities most likely by empowering the antioxidant defense system and improving cellular membrane integrity.

In conclusion, single or multiple dose CP administration elicited deleterious nephrotoxic effects by causing major damage to mitochondria, lysosomes and in particular to BBM of renal proximal tubules as reflected by significant decrease in the activities of the specific biomarker enzymes, confirming the morphologic and toxicogenomic observations that showed these organelles as prime CP target. CP seems to enhance glycolytic enzyme LDH in order to increase energy dependence on glycolysis due to mitochondrial damage and depressed TCA cycle enzymes. Gluconeogenic enzymes, G6Pase and FBPase also appeared to decrease due to mitochondrial dysfunction as oxaloacetate produced by MDH is not available. These nephrotoxic and other adverse effects appeared to be mediated in part due to CP elicited oxidative damage. The significant lowering of LPO by FO/FXO in renal cortex and medulla suggests that ω-3 PUFA enriched FO/FXO play a major role in reducing oxidative damage induced by CP. The decrease in oxidative damage by dietary FO/FXO supplementation appears to be due to the synergistic increase in the activity of both SOD and catalase and GSH-Px. The present results imply that FO/FXO exhibited a nephroprotective effect probably by empowering the biological defense system. Dietary ω-3 PUFA enriched fish oil and flaxseed oil to a larger extent prevented CP-induced nephrotoxicity parameters by enhancing nutrition/energy metabolism, by strengthening antioxidant defense mechanism and by altering membrane organization. Further FO and FXO offered renoprotection similarly against single and multiple dose CP treatments. Based on our present observations and already known numerous health benefits, we propose that ω-3 PUFA enriched FO/FXO may provide a cushion for a prolonged therapeutic option against CP induced nephropathy without harmful side effects. Similar protective effects of FO and FXO provide a much needed relief for millions of vegetarians who do not eat fish/fish oil for religious or other reasons.