Introduction
INTRODUCTION

1. GENERAL:
The kidney is a vital organ, which plays an essential role in health, disease and overall development and growth. The main function of the 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 Barcaly, 1995; Priyamvada et al., 2009). Platinum-based drugs are widely used anticancer agents with a broad range of antitumor activities (Alois et al., 2008). 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. However, its therapeutic utility is limited by acute and chronic nephrotoxicity along with some untoward side effects including nausea, vomiting, diarrhea, myleosuppression, ototoxicity and neurotoxicity (McKeage, 1995, Markman, 2003). The kidney accumulates cisplatin (CP) to a greater degree than other organs and is the major route for its excretion (Yao et al., 2007). Dose dependent nephrotoxicity is the major limitation of this compound, sometimes requiring a reduction in dose or discontinuation of treatment (Schrier, 2002). Approximately 25–35% of patients develop evidence of nephrotoxicity following a single dose of cisplatin (Saad et al., 2007). Cisplatin nephrotoxicity is chiefly characterized by tubular damage, primarily affecting the proximal tubules. Tubular damage may range from a mere loss of brush border of epithelial cells to an overt tubular necrosis in severe cases (Meyer and Madias, 1994). Light and electron microscopy have shown that the CP-induced injury and necrosis in the rat kidney are predominantly localized in S3 subsegments of proximal tubule (Townsend et al., 2003). Mitochondria, lysosomes and microsomes are critical CP targets (Zhang et al., 1993; Leibbrandt et al., 1995). Functional alterations are characterized by change in urine volume, increase in blood urea nitrogen and serum creatinine (Khan et al., 2009). In addition to kidney, the structure and function of other major tissues e.g intestine and liver are also reported to be affected (Naqshbandi et al., 2011; 2012a, b; Khan et al., 2009; Bearcroft et al., 1999; Neife et al., 2007). Continued aggressive
high-dose CP chemotherapy necessitates the investigation of ways for prevention of the dose-limiting side effects that inhibit the CP administration at tumoricidal doses. 

As man has moved over the centuries from a hunter-gatherer diet to greater intake of saturated and trans-fatty acids, the world appears to be now increasingly interested in the health benefits of foods and have begun to look beyond the basic nutritional benefits of food stuffs to disease prevention and health enhancing ingredients of the same. Dietary fat is one environmental variable that affects our health in different ways. Saturated fats mostly from animal based foods have been blamed for various forms of cancers and heart disease (Simopoulos, 2002a) whereas unsaturated fats in the diet were shown to have numerous health benefits (Deckelbaum et al., 2006). 

Evidences supporting the possible importance of unsaturated fatty acids especially polyunsaturated fatty acids (PUFA) came from studies showing that young humans and experimental animals experienced impaired growth when all fatty acids were removed from the diet. Small amounts of ω-3 (α-linolenic acid) or ω-6 (linoleic acid) fatty acids prevented that impaired growth and thus were termed as “essential” (Burr and Burr, 1930). Highly unsaturated/polyunsaturated fatty acids (HUFA/PUFA) were relatively found to be several-fold more effective (Turpeinen, 1937). 

Epidemiological and clinical trial evidence suggests that both ω-3 and ω-6 PUFA have numerous health benefits. However, ω-3 PUFA rich foods were found to be more effective in lowering incidence of various pathologies compared to ω-6 PUFA rich diets (Simopoulos, 2001). This stems from the observations of the different prevalence of coronary heart disease and other chronic diseases (including psoriasis, bronchial asthma, diabetes mellitus and thyrotoxicosis) in the Greenland Inuit (Eskimo) population relative to Western populations (Kromann and Green, 1980). PUFA from marine fish and mammals were indicated as the main dietary factor responsible for such differences (Dyerberg et al., 1975; Bang et al., 1976). The consumption of fish has now been linked with the rate of depression among various populations. In countries where people eat the least fish the rate of depression is highest, and vice versa (Hibbeln, 1998; Small, 2002). This correlation holds true across the world. The most dramatic change in what we eat has happened in the past century, with industrialization and development of the food industry, the intake of saturated fat, trans fat and especially ω-6 enriched refined oils have
enormously increased whereas the consumption of ω-3 PUFA has considerably declined. These dietary imbalances in fat intake, in fact, are prime cause of modern sufferings like cancer, hypertension, diabetes, depression, cardiovascular and renal disorders.

Nutritional recommendations have recently promoted the increased need to consume omega-3 (ω-3/n-3) polyunsaturated fatty acids (PUFAs) (Simopoulos, 2000). The most common way to consume omega 3 fatty acids has been in the form of marine oils like fish oil. Recently flaxseed has been identified as a significant alternative source of omega 3 fatty acids. Role of ω-3 PUFA enriched fish oil has been extensively investigated and has received a great deal of attention in recent times as therapeutic options in a variety of clinical situations ranging from cardiovascular disorders (CVD) to hyperlipidemia, cancer, inflammatory and immune disorders, respiratory diseases, depression and diabetes (De Caterina et al., 1994; Kakar et al., 2008). Lately, ω-3 PUFA from certain plants/seeds e.g. walnut, canola and most notably from flaxseed (linseed) showed many similar health benefits as demonstrated by ω-3 PUFA from fish/fish oil (Nannicini et al., 2006; Vijaimohan et al., 2006).

Although ω-3 PUFA have been shown as deterrents for environmental pollutants, studies on their possible beneficial effects against drug/chemical induced nephrotoxicity are very limited (Watkins et al., 2007; Priyamvada et al., 2008). Considering profound beneficial health effects of ω-3 PUFA from marine or plant foods against various pathologies, the present work embodied in this thesis was undertaken to study the detailed mechanisms of CP induced nephrotoxic alterations and possible protection/prevention by ω-3 fatty acids enriched fish oil (FO) and/or flaxseed oil (FXO) diets against CP nephropathies and other alterations. The effect of dietary supplementation of fish oil (FO) and flaxseed oil (FXO) was examined on single dose and multiple dose CP treatment induced alterations.

2. OMEGA FATTY ACIDS - “FATS OF LIFE”:

“We are what we eat”. This adage represents the force behind research efforts in diet manipulation as preventive measures and treatments of various diseased states. The nature of the relation between diet and disease is the subject of great controversy and
the last fifty years have been characterized by the understanding of the impact of nutrition and dietary patterns on health (Caballero, 2003).

The dietary and body fat are essential for life. The concept of the essentiality of the long chain PUFA was made by Burr and Burr (1930) at the University of Minnesota Medical School (Holman, 1998). They were first to suggest that unsaturated fatty acids: linolenic and linoleic acids play a bioeffector role, in the growth and development of young humans and animals. Thus, the generic term “essential fatty acid” was used for them.

2.1 Definition, classification, distribution and sources of dietary polyunsaturated fatty acids (PUFA):

Unsaturated fatty acids consist of monounsaturates (MUFA) and polyunsaturates (PUFA). PUFAs are further divided between two classes omega-3 and omega-6. The only fatty acids known to be essential for complete nutrition of many species of animals including humans are linoleic (LA) and \( \alpha \)-linolenic acid (\( \alpha \)-LA), as mammals lack the ability to synthesize them de novo and must obtain them in their diet. Moreover, if supplied in the diet these two can be elongated and further desaturated by the mammals. However, certain plants and marine mammals possess the ability to synthesize them (Simopoulos, 1991; Teitelbaum and Walker, 2001). Marcel et al. (1968) pioneered the cascade for LA and Klenk and Mohrhauer (1960) for ALA. Both LA and ALA are metabolized by the same microsomal enzyme system, by alternating desaturation and elongation to make two cascades of metabolic products up to 22 or more carbons long (Figure 1) which occur in tissue structural lipids in animals and in man (Donadio 1991; Holman, 1998).

Nomenclature: PUFA are commonly classified according to a shorthand nomenclature which designates the chain length, number of double bonds and position of the double bond nearest to the terminal methyl group (Willis, 1987). This terminal carbon atom is often referred to as the omega (\( \omega \)) as it occurs at the opposite end of the molecule to carbon-1 which bears the carboxyl function. The use of the “omega” nomenclature was proposed by Holman (1964). Alternatively, the n-x notation is used instead of \( \omega \)-x to describe the position nearest to the methyl group (Tinoco, 1982).
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Figure 1: Metabolic cascade of ω-3 and ω-6 fatty acids.
fatty acids are represented by alpha-linolenic acid (ALA, C18:3 cis\(\Delta^{9,12,15}\)) while \(\omega-6\) fatty acids by linoleic acid (LA, C18:2 cis\(\Delta^{9,12}\)).

The general formulas for \(\omega-3\) and \(\omega-6\) fatty acids are,

\[\text{CH}_3-\text{CH}_2-\text{CH}=\text{CH}-\text{R} \quad \text{CH}_3-\text{CH}_2-\text{CH}_2-\text{CH}=\text{CH}-\text{R}\]

respectively. Where 'R' represents rest of the carbon chain and the number of conjugated double bonds may vary.

**Distribution:** LA and ALA as well as their long chain derivatives (e.g. EPA and DHA), are important components of animal and plant cell membranes. LA is found in triglycerides, cholesterol esters and in very small amounts in phospholipids. EPA is found in cholesterol esters, triglycerides and phospholipids. DHA is found mostly in phospholipids and is one of the most abundant components of the brain’s structural lipids (Teitelbaum and Walker, 2001). Both these essential fatty acid families have distinctive nutritional and metabolic effects and each has a direct precursor relationship with specific class of eicosanoids. Thus, all eicosanoids formed in the human body are generated from PUFA that must be derived from the diet (Willis, 1987).

**Sources:** The omega fatty acids are found in fish and marine foods, green leafy vegetables and in certain plant seeds and walnut.

(a) **Gifts from the sea:** omega-3 fatty acids (EPA and DHA)

The long chain \(\omega-3\) PUFA, eicosapentaenoic acid (EPA, C20:5 \(\omega-3\)) and docosahexaenoic acid (DHA, C22:6 \(\omega-3\)) (Figure 2) are predominantly found in certain fish and sea mammals (tuna, trout, herring, salmon, sardine etc.). In fact, the origin of EPA/DHA in aquatic systems are the plants of the sea, microalgae and seaweed. They produce high levels of EPA and DHA which are acquired by the marine fish/mammals through the food chain. Fish oils are concentrated source of EPA and DHA (Simopoulos *et al.*, 1986). Eggs can also provide small amount of DHA (<50 mg/egg) (Davis and Kris-Etherton, 2003).
(b) **Gifts from the land:** (i) omega-3 fatty acids (ii) omega-6 fatty acids

(i) **Omega-3 fatty acids: ALA**

α-Linolenic acid (ALA, C18:3 ω-3) (Figure 2) is found in chloroplast of green leafy vegetables (purslane, spinach etc.) and in oily seeds of canola, hempseed, walnuts and most notably in flaxseeds (richest source). (Davis and Kris-Etherton, 2003). Flaxseed (*Linum usitatissimum* or linseed) contains 32-45% of its mass as oil of which 51-55% is ALA and is the richest source of dietary fibre, both soluble and insoluble; and the highest content of phytoestrogenic lignans (Lay and Dybing, 1989; Harris and Haggerty, 1993; Prasad, 2000; Simopoulos, 2002b; Vijaimohan *et al.*, 2006). The oils from these seeds are the only source of ω-3 PUFA for vegetarians who do not eat fish for religious or other reasons.

(ii) **Omega-6 fatty acids: LA**

Linoleic acid (LA, C18:2 ω-6) (Figure 2) is plentiful in nature and is found in the seeds of most plants except coconut, cocoa and palm. It is most predominant PUFA in western diet. Cooking oils with the greatest ω-6 fatty acid content include safflower oil; grapeseed oil; sunflower oil; corn oil; cottonseed oil and soybean oil. Processed foods, convenience foods and snack foods are also significant contributors to ω-6 intake (Davis and Kris-Etherton, 2003).

Some of the major ω-3 and ω-6 sources of PUFA are summarized in Table 1 (Udo Erasmus, 1993; USDA nutrient data laboratory).

**Table 1: Fats and Oil Composition**

<table>
<thead>
<tr>
<th>Fatty acid Components (%)</th>
<th>Vegetable Based*</th>
<th>Marine Based*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sunflower</td>
<td>Corn</td>
</tr>
<tr>
<td>ALA</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>LA</td>
<td>68</td>
<td>58</td>
</tr>
<tr>
<td>ALA+LA</td>
<td>69</td>
<td>59</td>
</tr>
<tr>
<td>EPA</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>DHA</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*The values are expressed as percent of total fatty acids.
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(A) \( \alpha \)-linolenic acid (ALA)  
Chemical formula: **C18:3\( ^{\Delta 9,12,15} \)**

(B) Eicosapentaenoic acid (EPA)  
Chemical formula: **C20:5\( ^{\Delta 5,8,11,14,17} \)**

(C) Docosahexaenoic acid (DHA)  
Chemical formula: **C22:6\( ^{\Delta 4,7,10,13,16,19} \)**

Figure 2: The structures of \( \alpha \)-3 fatty acids
2.2 Beneficial health effects of ω-3 PUFA from marine sources (EPA and DHA): "Medicinal fatty acids"

The health benefits of fish oil have been extensively studied since the observation that incidence of cardiovascular diseases was lower among population that consumed large amounts of fish (Kris-Etherton et al., 2002). Fish oil contains a high proportion of ω-3 PUFA e.g. eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Efficacy and potential clinical utility of these fatty acids range from cardiovascular disorders (CVD) to hyperlipidemia, cancer, inflammatory disorders of renal and gastrointestinal systems, respiratory diseases, diabetes etc. (Connor, 2000; Kakar et al., 2008). A number of investigations have demonstrated that ω-3 fatty acids present in fish oil have profound beneficial effects on murine function including both cell and humoral mediated immunity in autoimmune diseases such as lupus nephritis (Grande and Donadio, 1998) and rheumatoid arthritis (Yusufi et al., 2003). Recently it has been shown that FO may have a therapeutic potential as an adjunct therapy for human AIDS (Xi and Chen, 2000). DHA has been found to be essential in normal functional development of retina and brain (Simopoulos, 2000). The intake of ω-3 PUFA/fish oil or consumption of fish was shown to have inverse association with growth and development and rate of depression and other mental deficiencies (Simopoulos, 1991). Fish oil therapy has been used in cancer of the digestive tract, breast and prostate (Karmali, 1989). Intake of fish oil has been reported to have anti-ulcer actions (Lugea et al., 1991). Several animal and human studies demonstrate that FO may act as free radical scavenger (Fisher an Levine, 1991; Chautan et al., 1990; Sarsilmaz et al., 2003; Barbosa et al., 2003). Thus dietary ω-3 fatty acids have moved from speculation about their functions to solid evidence that they are not only essential nutrients but they also favorably lower the incidences and progression of various chronic diseases whereas ω-6 PUFA are known to produce pro-inflammatory factors promoting inflammation, tumor growth and most degenerative disorders (Simopoulos, 2001; Berquin et al., 2008). Some of the beneficial effects of ω-3 PUFA in detail are described as follows:

(A) Blood lipids and cardiovascular effects: The potential role of FO in CVD risk reduction first came from the observation involving Inuits in Greenland (Mouratoff et al., 1967). Epidemiological data indicate a very low incidence of ischemic heart
disease in Eskimos and Japanese population that consume a diet rich in seafood (Bang et al., 1976; Dyerberg and Bang, 1982). Burr et al. (1989) showed that patients who had myocardial infarcts had reduction in mortality over 2 years by eating 2 or 3 fish meals a week. It has been reported that fish consumption reduce the incidence of deaths from coronary artery disease via effects on blood pressure, atherosclerosis and thrombogenesis (Kinsella et al., 1990; Connor, 1994; Leaf, 2008). One of the most remarkable and consistent effects of ω-3 fatty acid supplementation in humans is their ability to decrease plasma concentration of triglycerides and VLDL (Harris, 1997; Foulon et al., 1999). PUFA have been shown to reduce total plasma cholesterol and improves LDL to HDL ratio (Dupont et al., 1990; Ruiz-Gutierrez et al., 1999). Numerous epidemiological studies suggest that people who subsist on PUFA rich diets have lower blood pressure than people whose diets have high content of saturated fatty acids (Smith-Barbaro and Pucak, 1983; Iacono et al., 1984). Dietary ω-3 FAs retard the development of hypertension and related proteinuria (Rayner and Howe, 1995). Beneficial effects of ω-3 fatty acids from both plant (flaxseed oil) and marine sources have been demonstrated on all coronary artery disease, fatal and non fatal myocardial infarction, stroke, sudden cardiac death (Schmidt, 1993; Lanzmann, 2001; Nannicini et al., 2006; Posta et al., 2006). Thus, ω-3 fatty acids are “Heart healthy”.

(B) Dietary lipids and cancer: During the past several decades, research surveys and animal studies have indicated that high-fat diets are associated with increased incidence and accelerated development of certain tumors (Fernandis and Venkatraman, 1993). However, unsaturated dietary fats are inversely related to the development and progression of various forms of cancers. Although dietary lipid itself is not usually considered a true carcinogen, it is postulated to enhance or promote development of tumor cells. Results from experiments with carcinogen-induced and transplant tumor models showed that diet containing higher levels of marine oil-derived ω-3 PUFA affects tumorigenesis in a different manner from equivalent vegetable oil-derived ω-6 PUFA diets. In animal models atleast, ω-6 PUFA diets have stimulated development of four types of solid tumors (breast, colon, pancreas and prostrate), whereas ω-3 PUFA diets have diminished them (Cave, 1991). It has been found that ω-3 fatty acids prevent the worsening of colon cancer while ω-6 fatty acids
promote the growth of colon tumors (Anti et al., 1994). \(\omega-3\) fatty acids in combination with other nutrients (namely vit C, vit E, beta-carotene, selenium) may prove to be of particular value for preventing and treating breast cancer (Zhu et al., 1995). DHA and EPA can inhibit the growth of prostate cancer (Aronson et al., 2001).

It has also been shown that changes in the lipid composition of the diet, readily induce fatty acid alteration in both the neoplastic and non-neoplastic cell membranes, hence the cell's subsequent physiological and pathological behaviour. EPA and DHA have been shown to inhibit carcinogenesis by several molecular mechanisms including decreased/increased production of free radicals and ROS; suppression of arachidonic acid derived eicosanoid biosynthesis, gene expression (decreasing the expression of AP-1) and cell cycle regulation (Siddiqui et al., 2003), influences on transcription factor activity, signal transduction pathways and alteration of estrogen metabolism (Hardman, 2002; Larsson et al., 2004).

(C) Depression and other mental disorders: There are several lines of evidence relating diet with depression and other mental conditions. After all, brain is all fat and fats in the brain could easily be altered by diet. At present in the western societies the diets contain 15-20 times as much \(\omega-6\) as \(\omega-3\) PUFA and this imbalance was considered to be responsible for altered mental health. According to Hibbeln (1998) lack of \(\omega-3\) PUFA early in life may forever alter the way the brain develops and operates. Low levels of DHA are linked to suboptimal visual acuity and reduced brain development in infants, for this reason DHA is now added to infant formula milk (Uauy et al., 2001). Diminished levels of \(\omega-3\) PUFA, especially DHA have been associated with several neurological and behavioral disorders, such as depression, schizophrenia, Alzheimer's disease and attention deficit hyperactivity disorder (Conquer and Holub, 1997; Zamaria, 2004; Bourre, 2005). Depressed patients showed significant progress within two weeks of taking fish oil. Most remarkably cross culture studies demonstrate a strong relationship between native's consumption of \(\omega-3\) PUFA and its level of depression. In countries where people eat the least fish the rate of depression is highest, and vice versa. The average New Zealander, for example, eats only 18 kg of fish a year and 6% of the population suffers from depression. In Japan, where they eat 64 kg of fish a year, depression accounts <1% of population. This correlation holds true across the world. Growing evidence support the essential
role of PUFA in the development, growth and overall functions of neuronal cells, as 
20% of neuronal cell membranes are essential fatty acids. DHA and other ω-3 PUFA 
boost serotonin and affect mood (Small, 2002). In animal models, ω-3 deficiency 
caused memory deficit (Gamoh et al., 1999), learning disability (Carrie et al., 1999) 
and visual acuity (Neuringer, 2000). Thus, while these long-chain fatty acids are not 
technically “essential” nutrients, it is important to ensure that they should be 
sufficiently present in the diet for “happy and healthy brain”.

(D) Omega-3 fatty acids and renal diseases: Recent studies have demonstrated that 
dietary supplementation with ω-3 PUFA retards disease progression in human and 
experimental renal disease (Donadio et al., 1994; Grande and Donadio, 2001; 
Donadio, 2001). Fish oil enriched in EPA and DHA has been shown to reduce blood 
pressure, serum lipid levels, decrease eicosanoid and cytokine production and reduce 
proteinuria in human and experimental renal disease (Goldstein et al., 1975; Engler 
and Engler, 2000). In IgA nephropathy, the most common glomerulonephritis 
worldwide (D’Amico, 1987), the rate of renal disease progression was significantly 
reduced in patients given a fish oil supplement (Donadio et al., 2001). Dietary ω-3 
competes for arachidonic acid oxygenation pathway resulting in formation of 
biologically inactive end products thus exhibiting a favourable effect on glomerular 
injury, various forms of acute and chronic renal failure, allograft rejection, structural 
and functional renal damage in cyclosporine nephrotoxicity, hepatorenal syndrome, 
diabetic nephropathy, renal hypertension, vascular access thrombosis of end stage 
renal disease, idiopathic calcium urolithiasis, idiopathic immunoglobulin A 
nephropathy and lupus nephritis (Azar et al., 1989; De Caterina and Craven, 1993; 
De Caterina et al., 1994; Donadio, 2001; Baggio et al., 2002). Fish oil was also linked 
to the management of type 2 diabetes (Montori et al., 2000; Mostad et al., 2006). ω-3 
PUFA are “renoprotective” fatty acids and portray as potential new treatments for 
immune renal diseases.

(E) Ωmega-3 fatty acids and gastrointestinal pathologies: In past few years there 
have been many advances in the area of small bowel physiology, pathology and 
therapy. Dietary PUFA influence the natural history of Inflammatory bowel diseases 
(IBD) including: Crohn’s disease (CD) and ulcerative colitis. CD is a chronic, 
transmural, inflammatory process that results in bloody stools and malabsorption
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problems whereas ulcerative colitis is a chronic relapsing inflammatory disease of the colonic and rectal mucosa resulting in ulceration, hemorrhage, edema, and regenerating epithelium. ω-3 fatty acids found in fish oil have an anti-inflammatory activity and have given spectacular results in treatment of patients with CD (Belluzzi et al., 1996; Tsujikawa et al., 2000) and shown marked clinical improvement of active, mild to moderate ulcerative colitis (Salomon et al., 1990; Aslan and Triadafilopoulos, 1992; Barbosa et al., 2003). FO showed significant protective effect in different experimental models (Faust et al., 1989; Al-Harbi et al., 1995; Lichtenberger et al., 1998; Manjari and Das, 2000) of gastric ulceration by inhibition of offensive mucosal factors and oxidative stress, and augmentation of defensive mucosal factors (Bhattacharya et al., 2006). Dietary FO has been shown to protect against ethanol-induced gastric mucosal injury (Leung, 1992). Dietary ω-3 fatty acids are preferentially incorporated into the mucosal phospholipids at the expense of ω-6 fatty acids resulting in immunomodulatory effects including alteration of lymphocyte proliferation, neutrophil function, antigen presentation and induction of apoptosis in addition to changes in intestinal structure and function including changes in cellular glycoproteins and tight junctions. ω-3 fatty acids have been shown to influence the intestinal BBM uptake of glucose, galactose and cholesterol (Thompson et al., 1988; 1989).

2.3 Beneficial health effects of ω-3 PUFA from plant sources (ALA) in health and diseases – “Seeds of hope” for vegetarians:

The health benefits of dietary consumption of food rich in marine ω-3 PUFA are well established. Whether plant ω-3 PUFAs are as beneficial as the ω-3 PUFA from fish/marine food has not been investigated extensively. Lately, flaxseed, Linum usitatissimum has been the focus of increased interest in the field of diet and disease research due to the potential health benefits (Abdel-Moneim et al., 2011a) associated with some of its biologically active components. It contains 32 to 45% of its mass as oil of which 51 to 55% is α-Linolenic acid (ALA) (18:3 n-3, omega-3 fatty acid). α-Linolenic acid (ALA), is a major ω-3 FA in plants (Harris, 2005). It is a precursor of EPA but not DHA and must be converted to EPA for its bioeffector role (Mantzioris et al., 1994). ALA is metabolized to EPA in humans to a significant extent (Valsta et
ALA 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). Although plants, sea microalgae and seaweeds are primary source of EPA and DHA, but most are not concentrated sources. They do not contribute significantly to EPA intakes in the western world but are important sources where people use large quantities of seaweeds on a daily basis (e.g. Japan and other parts of Asia). Thus vegetarians can rely to some extent on eggs/microalgal supplements for the supply of essential fatty acids.

ALA from vegetable oils and EPA and DHA from fish oil have several parallel beneficial health effects (Freese and Mutanen, 1997; Ide et al., 2000; Kim and Choi, 2001). Recent epidemiologic studies have shown that dietary ALA is associated with a lower risk of CVD in men and women (Djousse et al., 2001). ALA has been associated with a lower rate of fatal and non-fatal coronary events (Nannicini et al., 2006). Hypotensive effect of flaxseed oil have been documented lately thus constituting another mechanism for cardioprotective effect of ALA (Paschos et al., 2007). The consumption of ALA rich FXO offers protective effects against cardiovascular disorders compared to LA-rich sunflower oil via their ability to decrease the tendency of platelet to aggregate (Allman, 1995) and inhibition of progression of arterial atherosclerosis (Lorgeril and Salen, 2004; Mozaffarian, 2005). ω-3 ALA has been shown to have greater hypolipidemic effect than ω-6 LA (Kim and Choi, 2001). Role of ALA in flaxseed oil in prevention of colon cancer has also been demonstrated (Kelley et al., 1991; Williams et al., 2007). Utility of flax and flaxseed oil in lupus nephritis and renal failure have also been accounted (Kelley et al., 1991; Basch et al., 2007). Dietary supplementation with 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). Flaxseed oil has been shown to increase life span of irradiated mice suggesting its prophylactic potential against radiation-induced degenerative changes in liver (Bhatia et al., 2007). Recent researches have shown that long term flaxseed oil supplementation diet protects BALB/c mice against streptococcus pneumoniae infection (Archana et al., 2010). Flaxseed oil has received attention for its potential role in lead acetate induced kidney and liver damage and in preventing lipid disorders (Abdel-Moneim et al., 2010, 2011a, 2011b). Dietary
supplementation of flaxseed was found to inhibit the growth and development of prostate cancer in the transgenic adenocarcinoma mouse prostate model (Lin et al., 2002). Flaxseed has also been reported to inhibit human breast cancer growth and metastasis and down-regulate expression of insulin-like growth factor and epidermal growth receptor (Chen et al., 2002). Another study using rat suggested that exposure to flaxseed during suckling inhibited chemically induced mammary tumorigenesis (Chen et al., 2003). Moreover, previous studies have shown that the cyclophosphamide-induced decline in the blood levels of glutathione (GSH), glutathione peroxidase (GSH-Px) and alkaline phosphatase were also significantly reduced by flaxseed oil in mice (Bhatia et al., 2006).

Taken together, it appears that ω-3 PUFA both from marine and plant sources have many similar beneficial effects and that plant ω-3 PUFA are a great relief for those who are largely vegetarians.

3. STRUCTURE AND FUNCTION OF KIDNEY:

The kidney is a vital organ that plays an essential role in health and disease. The main function of the kidney is to maintain total body fluid volume, its composition and pH within physiologic range. This is achieved collectively by the presence of several millions of architectural and functional units of the kidney, known as "nephron". A nephron consists of glomerulus with an extended tubular structure. A rat kidney contains 30,000-35,000 nephrons whereas a human kidney is made up of about 1,30,000 nephrons. All these nephrons contribute to maintain renal functions by selective reabsorption of various ions and solutes.

The structure of the mammalian kidney apparently looks very homogenous, however, can be viewed as a composite of several tissue organs, geometrically, functionally and metabolically (Schmidt and Guder, 1976) (Figure 3). Thus each nephron consists of group of organs arranged in series coursing through four concentric tissue planes, the cortex, outer and inner zones of the outer medulla and the inner medulla (papilla) (Schmidt and Guder, 1976) (Figure 4). Each tissue plane also has individual “organ” characteristics with respect to their ionic contents and metabolic rates (Guder, 1973; Schmidt and Guder, 1976).
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Figure 3: Anatomy of Kidney.

Figure 4: Structure of the nephron.
The luminal brush border membrane (BBM) of renal proximal tubule is the major site for reabsorption of various solutes including amino acids, sugars and other nutrients, ions and minerals such as Na\(^+\) and inorganic phosphate (Pi) (Evan et al., 1983). Reabsorption of most ions and solutes from the tubular lumen is coupled by an active transport with sodium (Na\(^+\)) via a carrier located on apical side and is driven by an electrochemical gradient of Na\(^+\) generated by Na\(^+\)/K\(^+\)-ATPase located on basolateral side (McCrory et al., 1952; Massary and Fleisch, 1980; Bonjour and Caverzasio, 1984). Thus the transport of Na\(^+\) is considered to be a major work function of the kidney upon which all other transports are dependent (Ullrich et al., 1974; 1977). The energy for the sodium transport is mainly provided by the hydrolysis of ATP at antiluminal membrane site involving Na\(^+\)/K\(^+\)-ATPase (Balagura-Baruch et al., 1973; Evan et al., 1983). Since the production of ATP is usually coupled to oxidative metabolism occurring in mitochondria, Na\(^+\) transport appears to be linked with the oxidative metabolism or oxygen tension (pO\(_2\)) of the renal tubular cells. A direct linear relationship between O\(_2\) uptake/utilization and Na\(^+\) reabsorption has been found (Thurau, 1961; Torelli et al., 1986). There appears to be a reverse cortico-medullary gradient for tissue oxygen tension (pO\(_2\)) i.e., pO\(_2\) in inner medulla is far lower than in cortical tissue (Aukland and Krog, 1960; 1961; Aukland, 1962; Aperia and Liebow, 1964).

According to several studies, fatty acids, glutamine, lactate, citrate and in particular glucose are the major substrates which support the transport work of the kidney (Leal-Pinto, 1973; Park et al., 1974; Pitts and Macleod, 1975; Mandel and Balaban, 1981). It is well established that various nephron subsegments located in different zones of the kidney have different functions in solute and fluid transport, as well as in substrate metabolism. For example, the renal cortex is characterized mostly by aerobic oxidative metabolism (Lee et al., 1962) while the renal medulla is the site of anaerobic metabolism and glycolysis. Moreover, the renal cortex is also capable of producing glucose (Guder, 1973; Burch et al., 1978; Maleque, 1980). The oxidation of glucose in kidney may occur by several different metabolic pathways depending on the location and type of a particular nephron segment in the kidney: (i) the tricarboxylic acid (TCA) cycle, in which glucose is first converted to pyruvate by glycolysis which in turn is completely oxidized to CO\(_2\) and H\(_2\)O (ii) the hexose-monophosphate (HMP) shunt pathway; and (iii) the glycolysis in which glucose is
Introduction

partially oxidized to lactate (Figure 5). On the other hand glucose is known to be produced in the kidney by gluconeogenesis perhaps in the proximal tubule of the cortex (Schmidt and Guder, 1976; Maleque, 1980; Burch et al., 1978). The enzymes belonging to the above pathways are found to be present and distributed differentially in the kidney.

The renal medulla is the major region for the production of lactate from glucose by glycolytic enzymes (Hems and Gaja, 1972; Guder, 1973) while the oxidative conversion of glucose to CO$_2$ was shown in renal cortex (Lee et al., 1962; Cohen, 1979).

Nephron, which consists of various subsegments, shows distinct structural and functional differences (Figure 4). Thus, nephron heterogeneity also adds to the variation in the kidney functions. Both inter- and intra-nephronal heterogeneity exists in the mammalian kidney that depends on the origin and location of the nephrons in the cortical region of the kidney (Lise et al., 1987; Francois and Danielle, 1985). The nephron which originates from the glomerulus located in superficial cortex is known as “superficial nephron” while the nephron which originates from deep cortical region is called as “deep” or “juxtamedullary nephron”. These populations of nephrons have been found to be distinct structurally and functionally (Francois and Danielle, 1985).

In inter-nephronal heterogeneity, proximal convoluted tubules of superficial nephrons always touch the surface of the kidney. In intra-nephron or axial heterogeneity, the proximal tubules have been divided into three distinct morphological subsegments namely $S_1$, $S_2$, and $S_3$. The early proximal convoluted tubules (PCT) both in superficial and juxtamedullary nephrons is defined as $S_1$-segment and can be identified by its attachment with glomeruli on one side. $S_2$ is defined as the late superficial proximal convoluted tubule, early superficial proximal straight tubule and late juxtamedullary proximal convoluted tubule. $S_3$ is located principally in the outer stripe of outer medulla and consist of terminal superficial proximal straight tubule and entire juxtamedullary proximal straight tubule. $S_3$ is identified by its medullary location and by its connection with thin limbs on distal part. All $S_3$-subsegments (pars recta), as they descend from cortex into the outer stripe of the outer medulla, change from $S_2$ to $S_3$ cell type. Thus, the outer stripe of the outer medulla contains proximal tubular cells but only the $S_3$ type (Woodhall et al., 1978). These segments can be characterized by their biomarker enzymes (Yusufi et al., 1994).
**Figure 5:** Schematic outline of glucose homeostasis pathways: Glycolysis [I], TCA cycle [II], Gluconeogenesis [III], HMP shunt pathway [IV].
3.1 Acute Kidney Injury:

Acute kidney/renal injury (AKI), previously called acute renal failure (ARF), is a process rather than a state. Often it denotes a reversible insufficiency of the glomerular and tubular excretory functions which may be triggered by renal or extra-renal mechanisms. AKI denotes a dramatic clinical situation in which both the kidneys stop their function within a short period of time or immediately depending on the severity of AKI. It is best characterized by increase of waste products, such as serum creatinine and blood urea nitrogen and appearance of proteinic and enzymic components in the urine.

AKI can be grossly divided into three phases; pathogenic phase, manifestation phase and recovery phase. In the first phase, a progressive disintegration and necrosis especially of tubular epithelial cells has been observed, leading to the functional loss of the kidney which is manifested by the reduction of inulin clearance (Steinhausen and Parekh, 1984). In the second phase, long lasting effects are observed that severely affect the clearance of both creatinine and inulin and which can continue for several days after recovery begins, depending on the degree of renal damage. In the recovery phase, there is an increase in concentrating ability of the kidney with eventual normalization of kidney functions.

A number of environmental variables viz heavy metals chromium, mercury, cadmium, lead, uranium (Bank et al., 1967; Cronin, 1986; Banday et al., 2008b) and chemicals (Yagil, 1990; Weinberg et al., 1990) including drugs like aminoglycosides, cephalosporin, cisplatin etc. (Porter and Bennett, 1981; Harris et al., 1990; Basnakian et al., 2002; Banday et al., 2008a) affect the structure and function of the kidney leading to AKI. Generally, AKI caused by drugs and chemicals is much more severe and irreversible and the recovery sometimes is not possible (Steinhausen and Parekh, 1984). The pathophysiological mechanism of AKI has been investigated extensively in the last few decades. Four major possible causes of AKI have been generalized which include renal vasoconstriction, glomerular permeability, tubular obstruction and tubular leakage (Mason, 1986). For many causes of AKI, ROS have been invoked as a pathway for renal injury. The kidney has been studied as an organ that can generate ROS and is vulnerable to the damaging effects of ROS. That ROS contribute to the pathogenesis of AKI is supported by the presence of oxidative stress in models of
AKI, the amelioration of renal injury in these models by antioxidant maneuvers, and the worsening of renal injury in such models by manipulations that exacerbate sustained oxidative stress (Mason, 1986; Penelope et al., 2011). Oxygen radicals are important mediators of renal damage in AKI causing lipid peroxidation of cell and organelle membranes, disrupting the structural integrity and capacity for cell transport and energy production (Greene and Paller, 1991). Several preventive measures have also been utilized but a definite answer for the pathogenesis and control of acute renal injury, however, remains the topic of future studies.

4. CISPLATIN: PATHOPHYSIOLOGY

Cisplatin (cis-diamminedichloroplatinum II) is an inorganic complex of platinum whose cytotoxicity was first discovered by Rosenberg et al. in 1965. Cisplatin is one of the most potent anti-cancer drugs currently in use and is effective against a wide spectrum of solid cancers. Despite its effectiveness, the dose of cisplatin (CP) that can be administered is limited by its nephrotoxicity. Several other platinum analogues (e.g. carboplatin, explatin, nedaplatin) had entered clinical trials in order to improve the effectiveness and to lesser the toxicity of CP. Cisplatin was first synthesized by Michael Peyrone in 1845, and is historically known as Peyrone’s chloride. The structure was first elucidated by Alfred Werner in 1893 (Figure 6). Cisplatin is a heavy metal platinum co-ordination complex containing a central atom of platinum surrounded by chloride and ammonium atoms in the cis position of a horizontal plane. Cisplatin and other related drugs form strong electrophilic intermediates that act via nucleophillic substitution reaction to form inter and intra strand DNA cross-links. CP remained a major antineoplastic drug for the treatment of solid tumors such as metastatic testicular cancer, advanced ovarian carcinoma (Taguchi et al., 2005), advanced bladder carcinoma and squamous cell carcinoma of head and neck (Nakashima et al., 1990).

\[
\begin{array}{c}
\text{NH}_3 \\
\text{Cl} \quad \text{Pt} \quad \text{NH}_3 \\
\text{Cl}
\end{array}
\]

Figure 6: Structure of Cisplatin (CP)
It is given intravenously or intraperitoneally, binds to serum protein by about 90%, distributes to most tissues and is cleared unchanged from the kidney (Royer et al., 2005). Free CP in the plasma, by virtue of its low molecular weight and uncharged character, is freely filtered at the glomerulus (Safirstein et al., 1984). CP and its analogs accumulate in the kidney to higher degree than other organs probably through energy-mediated transport (Arany and Safirstein, 2003; Kawai et al., 2005). CP concentrations in proximal tubular epithelial cells exceeds plasma concentrations by a factor of five. The disproportionate accumulation of cisplatin in kidney tissue contributes to cisplatin-induced nephrotoxicity (Arany and Safirstein, 2003). Intracellularly, the highest concentration of CP is found in the cytosol, mitochondria, nuclei and microsomes (Kuhlmann et al., 1997).

4.1 Effect of Cisplatin on Kidney:

The toxic effect of the drug in mammals and animals include nephrotoxicity, ototoxicity, neurotoxicity and bone marrow suppression but its chief dose-limiting side effect is nephrotoxicity (Arany and Safirstein, 2003; Boulikas and Vougiouka, 2003; Sastry and Kellie, 2005). Morphologically, the nephrotoxicity induces necrosis of the terminal portion of the proximal tubule and apoptosis, predominantly in cells in the distal nephron. CP treatment also induces extensive death of cells in proximal and distal tubules and loop of Henle (Arany et al., 2004; Taguchi et al., 2005). Light and electron microscopy have shown that the CP induced injury and necrosis in the rat kidney are predominantly localized in the S3 segment of proximal tubule in the corticomedullary region with or without accompanying distal changes (Townsend et al., 2003). Pathological changes were rarely observed in the S1 and S2 segments of pars convoluta of the proximal tubule. Cisplatin toxicity in proximal tubular epithelial cells is morphologically characterized by tubular necrosis, loss of microvilli, alterations in number and size of lysosomes, and mitochondrial vacuolization. These structural alterations are accompanied by functional disturbance of various cell organelles (Kuhlmann et al., 1997). Very few changes were noted 1 to 2 days after the injection of drug, although a focal loss in brush border, increased cytoplasmic vesicles and a necrotic cell were seen occasionally in the proximal tubules in the outer stripe region. More severe lesions became evident after 3 days, and the S3 segment showed a spectrum of morphologic alterations (Dobyan et al., 1980). In some cells, the brush
border was almost completely obliterated with only a few microvilli remaining. Clumping of nuclear chromatin and increased number of cytoplasmic vesicles could be seen in many of the injured cells (Nonclercq et al., 1989).

Functionally, the nephrotoxicity causes reduced renal perfusion and a concentrating defect, and changes in renal hemodynamics. Biochemical, morphological and functional alterations induced by CP in renal mitochondria supports the idea that mitochondria could be the primary target of toxicity as the S3 segment of the proximal tubule has a large number of mitochondria as compared to other parts of the kidney (Santos et al., 2007).

The alterations induced by CP in the kidney functions are characterized by change in urine volume, increase in BUN and serum creatinine levels (Dauggard, 1990). Some biochemical changes due to CP treatment include generation of free radicals, lipid peroxidation (Hannemann and Baumann, 1988; Matsushima et al., 1998; Sadzuka et al., 1992), loss of renal sulfhydryl groups, damage to mitochondria (Zhang and Lindup, 1993) impaired antioxidant enzyme activities (Ulubas et al., 2003) and inhibition of DNA synthesis (Gorneva et al., 1993).

Although the exact mechanism of cisplatin nephrotoxicity remains unclear, biotransformation of cisplatin could play an important role (Fillastre and Raguenez-Viotte, 1989). Conversion of cisplatin to nephrotoxic molecule in the proximal tubule cells is required for cell injury (Townsend and Hanigan, 2002). CP undergoes ligand binding reactions which are virtually irreversible (Daley-Yates and McBrien, 1982). CP is biotransformed through binding to low molecular mass substances such as glutathione, methionine, cysteine and to high molecular mass substances such as albumin and nucleic acids and the resulting metabolites are known as mobile and fixed metabolites respectively (Farris et al., 1985). It has been shown that cisplatin is conjugated to glutathione and then metabolized through a γ-glutamyl transpeptidase (GGTase) and a cysteine S-conjugate β-lyase dependent pathway to reactive thiols, a potent nephrotoxin (Yao et al., 2007; Townsend et al., 2003). A series of studies on the role of the enzyme γ-glutamyl transpeptidase in cisplatin toxicity revealed that in tumor cells GGTase expression increased resistance to cisplatin, while in kidney GGTase expression made the cells sensitive to cisplatin toxicity (Hanigan et al., 1999; Hanigan et al., 2001). The disparate roles of GGTase in the antitumor activity and nephrotoxicity of cisplatin suggest that the mechanism by which cisplatin kills tumor
cells is distinct from the mechanism by which it kills the proximal tubular cells in the kidney.

Once cisplatin is administered to cancer patients intravenously as a sterile saline solution, there are three possible mechanisms involved in CP toxicity. Upon entering the bloodstream, it remains intact due to the relatively high concentration of chloride ions (~100mM). Cisplatin enters cells by diffusion where it is converted to its active form. Inside the cell the neutral cisplatin molecule undergoes hydrolysis in which a chlorine ligand is replaced by a molecule of water, generating a positively charged species (Figure 7). These aquated forms are highly reactive with nucleophiles and can lose hydrogen ions to form cytotoxic hydroxyl radicals. Once inside the cell, cisplatin has a number of possible targets: DNA; RNA; sulfur containing enzymes such as metallothionein, glutathione and mitochondria. The principal target of cisplatin is DNA (Cohen and Lippard, 2001). It causes intrastrand cross-linking probably between N7 and O6 of the adjacent guanine molecules, which results in local denaturation of DNA chain. CP also damages cell mitochondria, arrest cell cycle in the G2 phase, inhibits ATPase activity, alters the cellular transport system, eventually causing apoptosis, inflammation, necrosis and cell death (Boulikas and Vougiouka, 2003; Taguchi et al., 2005).

**Inside the Cell:**

\[
\text{Pt} (\text{NH}_3)_2 \text{Cl}_2 + \text{H}_2 \text{O} \rightarrow [\text{Pt} (\text{NH}_3)_2 \text{Cl} (\text{H}_2 \text{O})]^+ + \text{Cl}^- \\
[\text{Pt} (\text{NH}_3)_2 \text{Cl} (\text{H}_2 \text{O})]^+ + \text{H}_2 \text{O} \rightarrow [\text{Pt} (\text{NH}_3)_2 (\text{H}_2 \text{O})_2]^{2+}
\]

![Figure 7: Cellular uptake of cisplatin and its target](from: Encyclopedia of cancer by Pil and Lippard, 1997)
There are two distinct genomes in the cell; mitochondrial and nuclear. It has been postulated that mitochondrial DNA damage induced by CP causes nephrotoxicity (Singh, 1989), the reasons being, that, mitochondrial DNA is less closely associated with proteins than nuclear DNA (Salazar et al., 1982) and thus will be more accessible to attack by aquated species of CP. Secondly, DNA repair mechanisms play a major role in nucleus than in mitochondria. Damage of the mitochondrial DNA, would result in the inhibition of the de novo synthesis of mitochondrial proteins, leading to the degeneration of the organelles. Cisplatin nephrotoxicity has been demonstrated to be mediated by DNase1 (Basnakian et al., 2005).

Another mechanism proposed is that cisplatin induces renal damage by free-radical generation, by altering arginine metabolism and by increasing the activity of calcium independent nitric oxide synthase (Devipriya and Shyamaladevim, 1999). Glutathione is one of the most important antioxidant systems. The interaction of CP with sulphhydryl groups is an important factor in promoting cytotoxicity. Due to the compound's high affinity to SH groups, its chloride moieties are replaced by sulphhydryl groups (Kuhlmann et al., 1997). The formation of stable protein-S-CP adducts results in dysfunction of membrane associated and cytoplasmic proteins e.g. Na⁺/phosphate and Na⁺/glucose cotransporters (Courjault-Gautier et al., 1995) and decreases the activity of important enzyme systems such as glutathione-S transferase, reductase and peroxidase (Bompart, 1989). In addition, stable glutathione-CP adducts lead to a decrease in the amount of reduced glutathione available to scavenge free reactive oxygen metabolites (Mistry et al., 1991). This may effectively harm the cellular oxidant defense systems, eventually leading to lipid peroxidation. The depletion of renal glutathione level has been observed in rats in response to oxidative stress caused by CP (Nakano and Gemba, 1989). A recent study suggests that apoptosis may also play an important role in development of CP induced acute renal injury (Lee et al., 2001). CP has been reported to induce apoptosis in renal epithelial cells (Lieberthal et al., 1996; Zhan et al., 1999; Lau 1999). Metabolic responses, cell cycle events and the inflammatory cascade seem to be important determinants of the degree of renal injury induced by cisplatin (Arany and Safirstein, 2003). Different studies have demonstrated that the cytotoxicity of CP is probably due to peroxidation of cell membrane, mitochondrial dysfunction, inhibition of protein synthesis and DNA damage in kidney thereby inducing renal dysfunction (Santos et al., 2007).
4.2 Protection or Prevention against Cisplatin toxicity:

Cisplatin (CP) is an antineoplastic agent that has a remarkably broad spectrum of clinical activity in the treatment of solid tumors (Cohen and Lippard, 2001). However, CP nephrotoxicity limits its use in cancer therapy. Hundreds of platinum compounds have been tested over the last two decades in order to improve the effectiveness and to lessen the toxicity of CP (Ali and Al-Moundhri, 2006). Several agents have been tested to ameliorate the nephrotoxicity of platinum drugs (Ali and Al-Moundhri, 2006). The agents that have been shown to prevent/reduce experimental CP nephrotoxicity include antioxidants, modulators of nitric oxide, agents interfering CP metabolism, diuretics and cytoprotective and apoptotic agents. Only few of these have been tested in humans.

Administration of GSH was protective against lethal CP toxicity (Anderson et al., 1990). The antioxidant agents that have been reported to either ameliorate or prevent the nephrotoxicity of cisplatin include melatonin (Sener et al., 2000; Hara et al., 2001; Saad and Al-Rikabi, 2002), selenium (Hu et al., 1997), vitamin E (Naziroglu et al., 2004), N-acetylcysteine (Wu et al., 2005), sodium selenite (Baldew et al., 1989; Franseccato et al., 2001), vitamin C (Appenroth et al., 1997; Antunes et al., 2000), curcumin (Antunes et al., 2000) and carotenoid bixin (Silva et al., 2001). Among the antioxidants that have been tried against nephotoxicity of cisplatin, include those that have been extracted from natural products (Conklin, 2004) and medicinal plants such as *Nigella Sativa* (Ezzats and El-Daly, 1996). An ethyl acetate extract of *Phellinus rimosus*, fungus, has been shown to protect mice against cisplatin nephrotoxicity (Ajith et al., 2002). Shirwaiker et al. (2003) reported that treatment of rats with extract of the flowers of the plant *Pongamia pinnata* ameliorated cisplatin nephrotoxicity in a dose-dependent manner. It has been recently reported that treatment of rats with capsaicin, was effective in protecting against cisplatin-induced nephrotoxicity (Shimeda et al., 2005). The flavanoid naringenin has been shown to have *in vitro and in vivo* antioxidant and antiproliferative actions, which may be the basis of its protective effect against cisplatin nephrotoxicity (Totta et al., 2004). An angiotensin-converting enzyme (ACE) inhibitor containing sulphydryl (-SH) group, captopril can protect against cisplatin-induced nephrotoxicity (El-Sayed et al., 2008). Recent studies have shown that green tea ameliorated CP-induced nephrotoxic effects due to its associated biochemical/antioxidant properties (Khan et al., 2009).
It has been suggested that NO is involved in CP nephrotoxicity (Srivastava et al., 1995, 1996) and it has been reported that the inhibitor of NO synthase, NG-nitro-l-arginine methyl ester, was effective in mitigating lipid peroxidation and other biochemical changes associated with nephrotoxicity caused by cisplatin (Saad et al., 2000). L-Arginine (precursor of NO) was also shown to have nephroprotective effects on CP nephrotoxicity. It has recently been shown that nephrotoxicity of cisplatin can be blocked by inhibiting either of the two enzymes expressed in the proximal tubules, γ-glutamyl transferase or cysteine-S-conjugate-β-lyase (Townsend et al., 2003). Erythropoietin (EPO) has been shown to exert cytoprotective and antiapoptotic effects in experimental cisplatin-induced nephrotoxicity and ischaemic acute renal injury (Yalcin et al., 2003; Vessey et al., 2004).

The in vivo mechanisms of cisplatin nephrotoxicity are complex and involve oxidative stress, apoptosis, inflammation, and fibrogenesis. High concentrations of cisplatin induce necrosis in proximal tubule cells, whereas lower concentrations induce apoptosis through a caspase-9-dependent pathway (Lieberthal et al., 1996). Treatment of rats with anti-inflammatory agent, salicylate reduced cisplatin nephrotoxicity (Li et al., 2002). The broad-spectrum cytoprotective agent, amifostine, has been approved by the US Food and Administration for use in patients receiving cisplatin, with the aim of reducing its nephrotoxicity. Both human and animal studies have shown that the use of diuretics (furosemide, mannitol and others) and hydration substantially mitigate cisplatin nephrotoxicity (Cornelison and Reed, 1993; Yoshizawa et al., 1998; Santoso et al., 2003; Hanigan et al., 2005).

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; Anurag et al., 2007; Estella et al., 2006). From ancient times the physicians and scholars in Asia have understood that foods have both preventive and therapeutic value and are an integral part of health. Renewed interest has been observed in the recent years in ω-3 FA enriched dietary sources. However, studies on the protective effect of ω-3 PUFA enriched diet on drug induced toxicity are limited. Fish oil enriched in ω-3 PUFA has been found to exert protection against gentamicin and uranyl-nitrate induced nephrotoxicity (Priyamvada et al., 2008, 2009). ω-3 PUFA enriched FO and FXO have been shown to offer
significant protection from cisplatin induced oxidative damage in rat liver and intestine (Naqshbandi et al., 2011, 2012a, 2012b). However, it is important that the prospective nephro-protective agent should not affect the drug efficacy while reducing its side effects.

In light of these findings, the present investigation was undertaken to find an appropriate approach to reduce/prevent cisplatin associated nephrotoxic effects.
"Let food be thy medicine and medicine be thy food" this philosophy of Hippocrates (460 B.C.) is the underlying concept for the development of functional foods. The concept of functional food is novel to the west and is viewed as a revolution but in Asia, in contrast, functional foods have been part of the culture for centuries. 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.

Diets or "pharmacologically acting nutrients” are garnering more attention and are a potentially important approach to modulate the therapeutic uses of drugs. The recent few years have been rich in information coming from laboratories all around the world concerning the positive impact of nutrition and dietary patterns on human health (Caballero, 2003). Chemoprevention has emerged as a practical approach in reducing risk of various ailments. The concept of chemoprevention of certain diseases using naturally occurring substances that could be included in the diet consumed by human population is gaining attention. At the same time, the factors both endogenous and exogenous that influence the incidence and progression of many chronic diseases are being better defined and understood.

The kidneys play an essential role in the maintenance of total body fluid volume, its composition and acid-base balance by selective reabsorption. A number of environmental contaminants, chemicals and drugs including antibiotics and anticancer drugs dramatically alter the structure and function of various tissues and produce multiple adverse effects in the liver, kidney, heart and intestine (Soberon et al., 1979; Ozturk et al., 1997; Kohn et al., 2005). The antineoplastic nature of liganded platinum compounds, such as cisplatin (CP) has led to its increasing clinical use for the treatment of malignancies (Safirstein, 1984). The therapeutic use of cisplatin however is limited by myelotoxicity, ototoxicity, intestinal toxicity, and most notably renal toxicity (Fillastre and Ranguenez-Viotte, 1989; Mahmood and Waters, 1994). 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; Iraz et al., 2006) and reaction with thiols in protein and glutathione, which could cause cell dysfunction. In general, brush border membrane (BBM) and other major organelles e.g. mitochondria, lysosomes and microsomes are critical CP targets. CP is known to cause alterations in the structure and functions of these organelles that can be demonstrated by determining the associated biomarker enzymes. Evidence points out that CP induces nephrotoxicity partly via oxidative stress due to free radical generations. Considering vital clinical use of CP, it was used as a model of toxic injury in the present studies.

For the effective use of CP, it is necessary that it should exert minimum side effects. Several approaches utilizing different mechanism have been attempted to reduce CP toxicity. Many different agents and strategies have been reported to ameliorate CP toxicity in experimental animals (Liao et al., 2008). Cisplatin induced toxicity in experimental animals has been shown to be protected by prior treatment with various antioxidants such as ebselene (Yoshida et al., 2000), selenium (Francescato et al., 2001; Naziroglu et al., 2004), vitamin C (Antunes et al., 2000; Fatima et al., 2007), green tea (Khan et al., 2009) and methionine (Kroning et al., 2000; Campbell et al., 2003). It has also been reported that NAC (N-acetyl cysteine), low molecular weight antioxidant can protect the cisplatin associated nephro and ototoxicity (Mishima et al., 2006; Nisar and Feinfeld, 2002). However, none of these strategies were found to be suitable/safe for clinical practice.

The quest for protective dietary nutrients for the control and management of various chronic diseases has spanned generations of scientific research. Of late, renewed interest and vigor has been showered on the role of ω-3 fatty acids. Fish oil enriched in ω-3 PUFA (EPA and DHA) provide 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 diseases, diabetes, depression, arthritis, asthma and inflammatory and immune disorders of kidney and intestine. 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. Dietary fatty acids are known to be incorporated in cellular membranes especially plasma membranes affecting membrane fluidity and the organization of various membrane enzymes and transport proteins (Kogteva and Bezuglov, 1998) influencing the functional aspects of various organs especially of kidney; leading to “positive” end points relating to health and disease (Seo, 2005).

Although ω-3 PUFA have been shown as environmental deterrents (Watkins et al., 2007) for environmental contaminants, studies on their possible beneficial effects against drug/chemical induced nephrotoxicity are especially very limited. Recently research in our lab has shown that FO could prevent gentamicin and uranyl nitrate induced nephrotoxicity (Priyamvada et al., 2008, 2009). Considering the profound 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 CP induced nephrotoxic and other adverse effects in rat kidney.

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

In the first part:
Experiments were conducted to study the effect of ω-3 FA enriched dietary fish oil (FO) and cisplatin (CP) alone and in combination on various biochemical parameters in serum/urine and on the enzymes of carbohydrate metabolism, brush border membrane (BBM), lysosomes and antioxidant defense system in the renal cortex and medulla. Both single (acute) and multiple dose (chronic) effects of CP administration were evaluated and the efficacy of dietary ω-3 PUFA in ameliorating there effects was assessed.

In the second part:
Experiments were carried out to determine the effects of ω-3 FA enriched dietary flaxseed oil (FXO) and cisplatin (CP) alone and in combination on various biochemical parameters in serum/urine and on the enzymes of carbohydrate metabolism, brush border membrane (BBM), lysosomes and antioxidant defense
system in the renal cortex and medulla. The possible protective effect of FXO was analyzed both in single and multiple dose CP treatment protocol.

To understand the mechanism of CP induced nephrotoxicity and its possible protection by dietary ω-3 PUFA, the following parameters/biomarkers of energy metabolism/mitochondria/lysosomes/BBM/ and oxidative stress were determined to ascertain their role in drug induced nephropathy and possible protection by dietary FO and FXO.

a) Serum parameters such as cholesterol, creatinine, blood urea nitrogen (BUN), inorganic phosphate, glucose and phospholipids were estimated to determine the effect of CP toxicity.

b) Various enzymes of carbohydrate metabolism e.g. LDH, HK, MDH, G6Pase, FBPase, G6PDH and ME were determined as representative enzymes of glycolysis (LDH and HK), gluconeogenesis (G6Pase and FBPase) and HMP-shunt pathways (G6PDH).

c) Activities of ALP, GGTase and LAP were measured as these enzymes are biomarkers of BBM and were used to assess structural/functional integrity of renal BBM, which is major CP target.

d) ACPase was measured to assess lysosomal dysfunction, as lysosomes are major targets of CP toxicity.

e) The activities of the indicators of oxidative stress such as SOD, catalase, GSH-Px, LPO and total-SH were evaluated to ascertain the role of GSH, which is a major cellular defense mechanism in CP induced oxidative stress.

The results of the present studies showed that single as well as multiple CP dose administration caused specific alterations in various biochemical parameters in serum/urine and in the enzyme activities of various systems in renal tissues confirming nephrotoxic effects caused by CP. The results further demonstrate that dietary FO and FXO, largely prevented/ameliorated CP induced alterations in various parameters. The studies would help us in furthering our understanding of CP induced nephropathy and its possible prevention or protection by dietary ω-3 PUFA from fish oil and flaxseed oil for the safe use of CP as an important antineoplastic agent. The studies put forth a good basis for the clinical use of omega-3 fatty acids both as dietary components and future drugs/adjuvants which are relatively safe and inexpensive, confirming the dicta-“Nutrition is the foundation for all health".