SUMMARY

A number of environmental contaminants and pharmaceutical agents including heavy metals and antibiotics dramatically alter the structure and function of various tissues and produce multiple adverse effects in the liver, kidney, intestine and heart (Soberon et al., 1979; Ozturk et al., 1997; Kohn et al., 2005). These environmental variables are also known to influence the incidence and expression of various diseases. The heavy metals such as mercury, lead, chromium, platinum and uranium have been shown to cause severe damage to kidney leading to acute renal failure (ARF) (Sanchez et al., 2001; Fatima et al., 2004, 2005; Barbier et al., 2005).

Cisplatin (CP) is an anti-neoplastic agent that has a remarkably broad spectrum of clinical activity in the treatment of solid tumors, while gentamicin (GM), an aminoglycoside is used in a variety of infections caused by Gram negative bacteria. The limiting side-effect of both these drugs is the nephrotoxicity associated with their use (Humes, 1988; Tulkens, 1989; Barry, 2000).

The specificity of GM for renal toxicity is apparently related to its accumulation in the renal proximal convoluted tubule causing a number of morphological and biochemical alterations in humans and experimental animals (Kacew and Bergeron, 1990; Ali, 1995; Mingeot-Leclercq and Tulkens, 1999). GM has been shown to cause marked histological damage in particular to renal proximal convoluted tubules (S1 and S2 subsegments) (Humes and Connor, 1988; Abdel-Gayoum et al., 1994) resulting in swelling, vacuolization and necrosis of epithelial cells and accumulation of myelin-like bodies (Ali and Bashir, 1994; Pedraza-Chaverri et al., 2000). The adverse interaction of the drug with critical intracellular processes leads to renal cortical phospholipidosis disrupting functions of membranes and organelles including BBM, BLM, mitochondria, lysosomes and microsomes (Laurent et al., 1990; Cronin and Henrich, 1996; Mingeot-Leclercq et al., 1999).

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 kidneys, 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. In tubular cells a primary target for CP is probably the genomic DNA.
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 (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 disturbance of various cell organelles (Kuhlmann et al., 1997).

Although both GM and CP cause alterations in the structure and functions of the kidney and other major tissues, these appear to be two dissimilar models of ARF (Annie et al., 2005). GM appears to primarily affect the structure and function of membrane phospholipids (Cronin et al., 1986) whereas CP causes renal damage due to interactions with enzyme protein thiol groups in the membrane (Nakano and Gemba, 1989). GM produces marked histological damage in particular to S1 and S2 subsegments whereas CP primarily affects the S3 segment of renal proximal tubule (Abdel-Gayoum et al., 1994; Townsend et al., 2003; Banday et al., 2008a). A relationship between oxidative stress and nephrotoxicity has been well-demonstrated in many experimental animal models (Devipriya and Shyamaladevim, 1999). Evidence points out that cisplatin and gentamicin induce nephrotoxicity partly via oxidative stress. 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). Gentamicin activates phospholipases and alters the lysosomal membrane in addition to causing oxidative stress (Lindquist, 1986).

There is a continuous search for agents which provide nephroprotection against the renal impairment by the drugs, GM and CP. Several approaches, utilizing different mechanisms, have been attempted to reduce GM nephrotoxicity and related aminoglycoside antibiotics. These mechanisms include decreasing or preventing drug accumulation by the kidneys or decreasing binding to BBM (Mingeot-Leclercq et al., 1989). Many different agents and strategies have been reported to ameliorate GM nephrotoxicity in experimental animals (Ali, 2003; Nagai and Takano, 2004). Among them protection against GM nephrotoxicity was mainly focused on the use of various antioxidant agents including the extracts from medicinal plants with antioxidant properties (Ali, 2003). Similarly several agents have been tested to see whether they
could ameliorate the nephrotoxicity of platinum drugs (Ali and Al-Moundhri, 2006). The agents that have been shown to prevent experimental CP nephrotoxicity include antioxidants, modulators of nitric oxide, agents interfering with metabolic pathways and cytoprotective and antiapoptotic agents. Only few of these agents have been tested in humans. However, none of these approaches have been found safe/suitable for clinical practice due to known and unexplored side effects.

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 (Alschuler, 1998; Weisburger, 1999; Connor, 2000). 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 and green tea provide such dietary sources of biologically active components that has been shown to be co-preventive and co-therapeutic in a wide variety of ailments (Doughman et al., 2007; Khan and Mukhtar, 2007).

Since ancient times GT consumption has been known to maintain and improve health. It has been shown to have extensive health benefits. GT consumption has been linked to lowering of various forms of cancers (Katiyar and Mukhtar, 1997; Ahmad and Mukhtar, 1999). GT and its constituents have been shown to have cardioprotective, neuroprotective, antidiabetic, anti-inflammatory, antiatherosclerotic, anti-microbial and anti-aging properties (Alschuler, 1998; Liao, 2001). In addition, GT has been found to be useful in the treatment of arthritis, high cholesterol levels, and impaired immune function (Van het Hof et al., 1999). GT consumption has also resulted in improved kidney function in animal models of renal failure (Yokozawa et al., 1996, 2005). GT polyphenols have been shown to exhibit strong antioxidant properties (Alschuler, 1998). Most beneficial health effects ascribed to GT are considered to be mediated by potential antioxidant properties of GT polyphenols that scavenge free radicals and reduce oxidative damage (Augustyniak et al., 2005).

In a preliminary report, GT has been shown to mitigate gentamicin and CP induced nephrotoxicity by lowering the level of serum urea, creatinine and tissue LPO content (Upaganlawar et al., 2006). Green tea extract has also been found to offer significant protection from cisplatin induced oxidative damage in rat kidney and testes (Leena
Although there are several studies supporting the preventive potential of green tea, a proper understanding of the mechanisms by which they reduce the risk is necessary to establish the efficacy. In view of numerous beneficial health effects of GT we hypothesized that: "Green tea enriched in polyphenols would be able to prevent/reduce GM/CP induced nephrotoxicity and other adverse effects caused by them in rat kidney, intestine and liver".

To address this hypothesis the present work was undertaken to study the detailed biochemical events/cellular response/mechanism of GM and CP induced nephrotoxic and other adverse effects in rat kidney and intestine. The effect of GT was also determined to observe any protection provided by it against GM/CP nephrotoxicity.

The following parameters were determined under various experimental conditions:

(a) Certain biochemical parameters in serum.

(b) The activities of BBM and lysosomal marker enzymes in the renal cortical, medullary, mucosal and liver homogenates and BBM marker enzymes in isolated BBM preparations from renal cortex and intestinal mucosa.

(c) The transport of $^{32}$Pi in renal cortical BBM.

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

(e) The enzymic and non-enzymic parameters of antioxidant defense system in the renal cortex, medulla, small intestine and liver.

THE RESULTS OBTAINED ARE SUMMARIZED AS FOLLOWS:

Part I:

Effect of Green tea (GT) consumption via diet (GTD) or via drinking fluid (GTE)

(a) Serum parameters:

In general, GT consumption either via diet (GTD) or drinking GT extract (GTE) resulted in slight loss of body weight and BUN that was associated with decrease in serum glucose, cholesterol and Pi and increase in phospholipids.
(b) Marker enzymes of BBM and lysosomes:

The effect of GT consumption was determined on marker enzymes of BBM and lysosomes in different rat tissues. GT consumption caused significant increase in the activities of BBM enzymes, ALP, GGTase, LAP and sucrase and lysosomal enzyme ACPase in mucosal homogenates. The activity of BBM enzymes except ALP was also increased in BBM preparations isolated from intestinal mucosa. The activities of BBM and lysosomal enzymes were differentially altered by GT in the cortex, medulla and liver homogenates. In the liver, the activity of ALP and GGTase were significantly decreased whereas LAP and ACPase were not changed. However, the activities of ALP and ACPase increased but GGTase and LAP decreased in cortical homogenates whereas the activities of ALP, GGTase and ACPase also slightly increased but LAP decreased in renal medulla. The activities of ALP and GGTase markedly increased but LAP significantly lowered in BBM vesicles isolated from renal cortex by GT consumption. The differential effects of GT on BBM and lysosomal enzymes observed in different tissues can be attributed to their different organization in the thickness of BBM and/or to differential accumulation/accessibility of GT in different tissues.

(c) Enzymes of carbohydrate metabolism:

GT consumption caused significant increase in the activities of various enzymes of carbohydrate metabolism, however, to different extent in different rat tissues. The activity of HK, LDH (glycolysis), MDH (TCA cycle) significantly increased to much greater extent in the intestine and renal cortex but to a lesser extent in the liver. The activity of HK and LDH increased, whereas MDH activity decreased in the medulla by GT consumption. The activity of G6Pase and FBPase, enzymes of gluconeogenesis significantly increased in the intestine, renal cortex and medulla but decreased in the liver. The activity of G6PDH (HMP-shunt pathway) significantly lowered by GT consumption in the intestine, liver and medulla whereas profoundly increased in the cortex. The activity of NADP-malic enzyme (ME), however, decreased in all the tissues although to much different extent.
(d) Enzymic and non-enzymic parameters of antioxidant defense mechanism:

GT consumption appeared to perturb antioxidant defense system although differentially in different rat tissues. The activity of Cu, Zn superoxide dismutase (SOD) significantly decreased in the intestine and liver but significantly increased in the renal cortex and medulla. In contrast, catalase activity markedly increased in the intestine and liver but did not change in renal tissues by GT ingestion. The alterations in the activity of SOD and catalase were associated with significant lowering of lipid peroxides (LPO), measured as malondialdehyde in the liver and renal cortex. However, LPO levels were enhanced in the intestine and renal medulla by GT consumption. Taken together, the results imply that biological defense system perturbed by GT as a result of free radical scavenging properties chiefly of its polyphenols.

⇒ Interpretation: In general, GT consumption significantly enhanced the activities of the enzymes of carbohydrate metabolism involved in glycolysis, TCA cycle, gluconeogenesis, BBM and oxidative stress in the intestine and kidney. GT consumption appeared to exert numerous beneficial health effects by improving nutrition/energy metabolism and in part by strengthening cellular antioxidant defense mechanism.

Part II:

Influence of green tea (GT) consumption on gentamicin (GM) induced nephrotoxic and other adverse effects

(a) Serum parameters:

GM administration caused severe nephrotoxicity as characterized by significant increase in serum creatinine, cholesterol, phospholipids and BUN accompanied by significant decrease in serum glucose and Pi. Prolonged GT consumption prior to and together with 10 day GM treatment significantly lowered GM induced increase in serum creatinine and BUN, indicating reduction in GM nephropathy. GM elicited increase in serum cholesterol was also lowered whereas serum glucose remained depressed but serum Pi and phospholipids increased.
(b) Marker enzymes of BBM:

GM treatment to control rats caused significant decrease in the activities of ALP, GGTase and LAP in renal cortex and medulla, indicating GM induced damage to the kidney. However, the activities of these enzymes along with sucrase increased in the intestine. GM caused small decrease in BBM enzymes in liver homogenates. GT consumption caused significant reversal in GM induced decrease in BBM enzyme activities in the cortex and medulla, whereas the enzyme activities further enhanced in the intestine by GT in GM co-administered rats. GM induced alterations and GT elicited changes in BBM enzyme activities were more apparent in isolated renal and mucosal BBM vesicles compared to the effect observed in the respective homogenates. The activity of ACPase (a lysosomal enzyme) variably decreased by GM whereas GT partially restored ACPase activity in various tissues.

(c) Na-dependent transport of $^{32}$Pi in the BBMV isolated from renal cortex:

The rate of concentrative uphill uptake of $^{32}$Pi in the presence of a Na-gradient measured at 30 s was markedly decreased by GM but significantly increased by GT. GM induced decrease in $^{32}$Pi uptake was not only prevented by GT consumption but remained significantly higher in GM and GT co-administered than in control rats. The uptake of $^{32}$Pi in the absence of Na-gradient and after equilibrium at 120 min did not change under any experimental conditions, indicating the specificity of the effect on $^{32}$Pi transport by GM or GT or their combination.

(d) Enzymes of carbohydrate metabolism:

The activities of various enzymes involved in glycolysis, TCA cycle, gluconeogenesis and HMP shunt pathway were variably altered by GM/GT and GM+GT in different tissues. The activity of HK and LDH significantly increased but MDH activity decreased in the intestine, cortex and medulla by GM treatment. In the liver, GM increased the activity of HK and MDH but decreased LDH activity. The activity of G6Pase and FBPase increased in the intestine and liver but decreased in renal tissues. However, G6PDH activity decreased in the liver but increased in the intestine and renal tissues whereas ME activity decreased in all the tissues.
As shown in Part I, many of these metabolic enzymes were enhanced by GT. GT consumption by GM treated rats resulted not only in the prevention of GM induced decrease in many of the enzyme activities but their activities remained significantly higher compared to control/GM treated rats. The activity of LDH was especially enhanced to much greater extent by GT in GM treated rats.

(e) Enzymic and non-enzymic parameters of antioxidant defense mechanism

GM caused significant decrease in the activities of SOD and catalase in the intestine, liver and renal cortex and medulla which was associated with marked increase in LPO and decrease in total-SH, indicating that GM caused severe damage to various tissues by generating free radicals and by suppressing the activities of antioxidant enzymes. GT consumption protected well against GM induced perturbation of oxidants/antioxidants or GM induced oxidative damage. GM induced decrease in SOD and/or catalase activity was markedly reversed/reduced by GT consumption. GM induced increase in LPO was also lowered by GT components at least in the liver and renal tissues.

⇒ Interpretation: GM caused severe damage to kidney and produced multiple adverse effects to other tissues as reflected by nephrotoxicity parameters, decrease in BBM and lysosomal enzymes, certain enzymes of carbohydrate metabolism and suppression of antioxidant enzymes SOD and catalase. GT consumption markedly reversed/reduced GM induced oxidative damage and cellular functions by enhancing carbohydrate metabolism and antioxidant defense mechanism most likely due to intrinsic biochemical and antioxidant properties of GT polyphenols.

Part III:

Influence of green tea (GT) consumption on cisplatin (CP) induced nephrotoxicity and other adverse effects

(a) Serum parameters:

CP administration resulted in marked nephrotoxicity as manifested by increased serum creatinine and BUN. Serum cholesterol, phospholipids significantly increased
but serum glucose and Pi significantly declined by CP treatment. GT significantly decreased glucose, cholesterol and Pi but phospholipids increased. CP elicited increase in serum creatinine and BUN was markedly lowered by GT consumption, co-administered to CP treated rats. CP induced increase in serum cholesterol was also prevented by GT.

(b) Marker enzymes of BBM and lysosomes:

CP treatment to control rats caused significant decrease in the activity of ALP, GGTase, LAP and sucrase (only in the intestine) in the homogenates of intestine, liver, renal cortex and medulla and in BBMV isolated from renal cortex and intestinal mucosa. GT, in general significantly increased the activities of ALP, GGTase, LAP and sucrase in the intestine; the activity of ALP in the cortex and ALP and GGTase in the medulla were increased by GT consumption. CP induced decrease in most BBM enzymes in the intestine and ALP and GGTase in the cortex and medulla was markedly prevented/reduced by GT co-administration to CP treated rats. The changes observed by CP and/or GT were more apparent in the BBMVs isolated from renal cortex and intestinal mucosa than observed in the respective homogenates. The protection provided by GT against CP induced alterations was also more marked in isolated BBM preparations.

(c) Na-dependent transport of $^{32}$Pi in the BBM$^+$ isolated from renal cortex:

The rate of concentrative uphill uptake of $^{32}$Pi in the presence of a Na-gradient measured at 30 s was markedly decreased by CP but significantly increased by GT. CP induced decrease in $^{32}$Pi uptake was not only prevented by GT consumption but remained significantly higher in CP and GT co-administered than in control rats. The uptake of $^{32}$Pi in the absence of Na-gradient and after equilibrium at 120 min did not change under any experimental conditions, indicating the specificity of the effect on $^{32}$Pi transport by CP or GT or their combination.

(d) Enzymes of carbohydrate metabolism:

The activities of various enzymes involved in glycolysis, TCA cycle, gluconeogenesis and HMP shunt pathway were variably affected by CP and GT and CP+GT in different rat tissues. The activity of LDH and to some extent that of HK significantly
increased, whereas MDH activity significantly decreased in the intestine, cortex and medulla by CP treatment. However, the activity of HK and MDH increased but LDH activity decreased in the liver. The activity of G6Pase (enzyme of gluconeogenesis) significantly increased in the intestine but significantly decreased in the liver, cortex and medulla by CP. In contrast to GM, CP significantly decreased the activity of G6PDH in all the tissues along with significant increase in NADP-malic enzyme (ME) activity in the intestine, cortex and medulla. As shown in Part I, many of these metabolic enzymes were increased by GT consumption. Prolonged GT consumption by CP treated rats markedly prevented CP induced decrease in MDH, G6Pase, G6PDH activities in the cortex, medulla and liver. The decrease of MDH activity by CP was also prevented by GT in the intestine. However, the activity of LDH remained significantly higher in the intestine, cortex and medulla in CP treated rats after GT consumption.

(d) Enzymic and non-enzymic parameters of antioxidant defense mechanism:

CP caused significant decrease in the activities of SOD and catalase in the intestine and renal cortex and 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. In the liver, the increase in LPO was however associated with a slight decrease in SOD but increase in catalase activity. GT consumption protected well against CP induced perturbation of oxidants/antioxidants or CP induced oxidative damage. CP induced decrease in SOD and/or catalase activity was markedly reversed/reduced by GT consumption in most tissues. CP induced increase in LPO was also lowered by GT in the intestine, liver and renal cortex.

⇒ Interpretation: CP caused severe damage to kidney and produced multiple adverse effects to other tissues as reflected by increase in nephrotoxicity parameters, decrease in BBM and lysosomal enzymes, certain enzymes of carbohydrate metabolism and suppression of antioxidant enzymes, SOD and catalase. GT consumption markedly reversed/reduced CP induced oxidative damage and cellular functions by enhancing carbohydrate metabolism and antioxidant defense mechanism most likely due to intrinsic biochemical and antioxidant properties of GT polyphenols.
Conclusion:

In conclusion, GM/CP elicited deleterious nephrotoxic effects by causing major damage to mitochondria, lysosomes and in particular to BBM of renal proximal tubule and intestinal mucosa as reflected by significant decrease in the activities of their specific biomarker enzymes, confirming the morphologic and toxicogenomic observations that showed these organelles as prime GM/CP targets. Both GM and CP seem to enhance glycolytic enzyme LDH in order to increase energy dependence on glycolysis due to mitochondrial damage and depressed TCA cycle enzymes. These nephrotoxic and other adverse effects appeared to be mediated in part due to GM/CP elicited oxidative damage. GT consumption to a larger extent prevented GM/CP induced nephrotoxicity parameters by enhancing nutrition/energy metabolism and $^{32}$Pi transport capacity, by strengthening antioxidant defense mechanism and by altering membrane organization. It is apparent that green tea is a source of a wide range of phytochemicals that are digested, absorbed and metabolized by the body and that tea constituents exert their effects at the cellular level. Tea’s status as a functional food lends credibility to what has been believed by tea drinkers for centuries. Based on our present observations and already known numerous health benefits, we propose that GT polyphenols may provide a cushion for a prolonged therapeutic option against drug/chemical induced nephropathy without harmful side effects.