Introduction
INTRODUCTION

GENERAL

A number of environmental contaminants, chemicals and drugs including antibiotics 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). These environmental variables also influence the incidence and expression of various diseases. The heavy metals such as mercury, lead, chromium, platinum and uranium have been linked to certain forms of cancers, depression, cardiovascular and renal disorders (Sanchez et al., 2001; Fatima et al., 2004, 2005; Banday et al., 2008b). Histopathological studies revealed that exposure to uranium, chromium and platinum cause severe damage to kidney leading to acute renal failure (ARF) (Dobyan et al., 1980; Kaloyanides, 1984; Humes and Connor, 1988). Aminoglycoside antibiotics including gentamicin (GM) frequently used in the treatment of life threatening bacterial infections also cause nephrotoxicity (Nagai and Takano, 2004). Loss of microvilli, increased apical vacuolization, mitochondrial swelling and eventual cell necrosis at specific sites within renal proximal tubule have been reported in nephrotoxic models induced by GM (Ali and Bashir, 1994; Pedraza-Chaverri et al., 2000) and/or heavy metals including uranium, chromium, mercuric chloride and cisplatin (CP) (Kuhlmann et al., 1997). These structural changes result in profound alterations in cell integrity, metabolism and reabsorption properties of the kidney. GM is known to damage the S1 and S2 segments of the pars convoluta (Humes and Connor, 1988; Abdel-Gayoum et al., 1994), whereas cisplatin (CP) preferentially injures the S3 segment of the pars recta (Dobyan et al., 1980; Safirstein et al., 1984). The drug and chemical-induced nephrotoxicity can be visualized by increased serum creatinine and blood urea nitrogen (BUN) (Banday et al., 2008a, 2008b). In addition to the kidney, the structure and function of other major tissues e.g. gastrointestinal tract and liver, are also reported to be affected (Farooq et al., 2007).

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. Since ancient times, green tea (GT) consumption has been considered as Nature’s gift for
promoting health. Important progress has been made in the last five years concerning the effects of green tea on health (Mukhtar and Ahmad, 2000; Khan and Mukhtar, 2007). Experimentation with new accurate tools provide useful information about the metabolism of green tea components in the body, their mode of action as antioxidants at the cellular level and their protective role in the development of cancer, cardiovascular disease and other pathologies. The use of green tea components as nutraceuticals and functional foods is also gaining importance (Dufresne and Farnworth, 2001). Green tea is a “non-fermented” tea and contains more catechins than any other tea type. Catechins are in vitro and in vivo strong antioxidants (Wiseman et al., 1997). In addition, its content of certain vitamins and minerals increases the antioxidant potential of this type of tea (Graham, 1999). Recent human studies suggest that green tea may contribute to a reduction in the risk of cardiovascular disease and some forms of cancer, as well as to the promotion of oral health and other physiological functions such as anti-hypertensive effect, body weight control, antibacterial and antiviral activity, solar ultraviolet protection, bone mineral density increase, anti-fibrotic properties, and neuroprotective power (Cabrera et al., 2006). Thus medical research is confirming the ancient oriental wisdom that therapy of many diseases resides in a teapot.

The kidneys are another area where green tea has shown to have protective effects. Green tea extract consumption has been found to ameliorate cyclosporine A and cisplatin-induced renal dysfunction in experimental rats (Mohamadin et al., 2005; Leena and Balaraman, 2005). It has also been found to exert a protective effect on the gastrointestinal mucosa and was found to prevent atrophy of the intestinal mucosa (Safar et al., 2003).

Several clinical studies have revealed physiological responses to tea which may be relevant to the promotion of health and the prevention or treatment of some chronic diseases (Khan and Mukhtar, 2007). Studies on possible beneficial effect of GT on drug/chemical induced gastro- and nephrotoxicity are very limited. A proper understanding of the mechanisms by which green tea reduces the risk of several human diseases is necessary to establish its efficacy for the population where it could be most useful.
In view of the numerous health benefits of GT, the present work was undertaken to study detailed mechanisms of GM and CP induced tissue injury and possible mechanisms of protection/prevention by means of green tea consumption against GM/CP nephropathies. A comparative effect of green tea as extract and green tea in diet was also made to rule out any discrepancies that could arise due to different mode of intake. The effects of GM/CP and GT were also studied in small intestine and liver, which are known targets of the toxic insult of these agents. The results embodied in this thesis showed that GT consumption via diet or drinking fluid resulted in enhanced metabolic activity and antioxidant defense parameters. The results further demonstrated that GT consumption markedly reversed GM/CP induced nephrotoxicity parameters in the serum and GM/CP induced alterations in the enzymes of carbohydrate metabolism, BBM and antioxidant system. The present results suggest that GT consumption can be an option for long-term clinical use of GM/CP without nephrotoxic and other harmful side effects.

**BENEFICIAL EFFECTS OF GREEN TEA:**

"Better to be deprived of food for three days, than tea for one"

This ancient Chinese Proverb amply describes the importance of tea in our day-to-day lifestyle and the enormous health benefits ascribed to tea.

**Green tea and its composition:**

Tea is one of the most widely consumed beverages in the world today second only to water (Chantre and Lairon, 2002). Tea brewed from the plant *Camellia sinensis*, a member of Theaceae family is consumed in different parts of the world as green, black and oolong tea. Of the tea produced worldwide, 78% is black tea, which is usually consumed in Western countries, 20% is green tea, which is commonly consumed in Asian countries mostly in China and Japan, and 2% is oolong tea which is produced by partial fermentation in southern China. Green and black teas are processed differently during manufacturing. Green tea (GT) is prepared by steaming fresh leaves to prevent any oxidation to catechins, major polyphenols. By contrast black tea is produced by crushing fresh tea leaves and allowing enzyme-mediated oxidation to occur in a process known as fermentation. In the process, major polyphenols such as catechins are converted to unique oxidants such as theaflavins,
bisflavanols and thearubigens (polymers of simple polyphenols). The chemical composition of green tea is similar to that of the young shoots initially cultivated. It contains many polyphenolic compounds, which account for 30-40% of the extractable solids of green tea leaves, with most of the polyphenols being flavanols more commonly known as catechins. The primary catechins (Fig 1) are epicatechin (EC), epicatechin-3-gallate (ECG), epigallocatechin (EGC) and epigallocatechin-3-gallate (EGCG) (Graham, 1999). EGCG is the major catechin in GT and may account for 50-80% of total catechin in tea. GT also contains caffeine (3.5%), theanine (4%), lignin (6.5%), organic acids (1.5%), protein (15%) and chlorophyll (0.5%). A typical tea beverage prepared in a proportion of 1 g leaf to 100 ml water in 3-min brew, usually contains 250-300 mg tea solids, comprised of 30-42% catechins and 3-6% caffeine (Mukhtar and Ahmad, 1999).

The potential health effects of GT polyphenols depend on the amount consumed and on their bioavailability. Following oral administration of GT polyphenols to rats, the four principal catechins have been identified in the portal vein indicating that tea catechins are readily absorbed in the intestine (Okushio et al., 1996). Yang et al (1998) demonstrated a dose dependent increase in plasma polyphenols accompanied by dose dependent increase in the excretion of these compounds and/or their metabolites in the urine and feces. It has been shown that GT catechins are more stable in a fasted stomach than in a fed stomach and that the oral bioavailability can be enhanced in the fasting conditions (Safar et al., 2003).

Substantial amounts of tea polyphenols were found in esophagus, large intestine, kidney, bladder, lung and prostate and relatively low amounts in the liver, spleen, heart and thyroid after oral administration (Higdon and Frei, 2003). Holding of GT solution in the mouth or slow repeated ingestion showed high concentration of GT polyphenols in the saliva and oral cavity. Such local high levels may support the use of GT in the prevention of oral cancer and carries (Yang et al., 1999). It appears that GT polyphenols can be variably accumulated in certain tissues and thus can exert biological effects accordingly.
Figure 1: Structure of green tea (GT) catechins
Since ancient times GT consumption has been known to maintain and improve health (Weisburger, 1997). It has been shown to have extensive health benefits. GT consumption has been linked to lowering of various forms of cancers (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). Several lines of evidence suggest that prooxidant and antioxidant actions of plant (GT) polyphenols may be important mechanisms for their anticancer properties (Azam et al., 2004).

**Health Benefits of Green tea:**

The various health benefits of GT have been reviewed extensively (Mukhtar and Ahmad, 1999; Higdon and Frei, 2003; Khan and Mukhtar, 2007), and are described in detail in the following pages.

**Anticancer properties of Green tea:**

It is believed that almost one third of the cancers are caused by dietary habits and the manipulation in the diet is increasingly being recognized as a potential strategy against cancer (Katiyar and Mukhtar, 1997; Weisburger, 1999). The epidemiological as well as laboratory studies have shown an inverse association between GT consumption and the development of certain cancer types. Animal studies have shown that both green and black tea have cancer preventive activity against ultraviolet light, chemically induced and genetic models of carcinogenesis. These include various types of cancers e.g. skin, lung, breast, oral cavity, esophagus, stomach, pancreas, liver, small intestine, bladder, colon and prostate (Weisburger, 1999; Katiyar and Elmets, 2001; Yang et al., 2002). The majority of studies assessing the usefulness of tea in prevention of cancer have been conducted with GT whereas in few studies the
chemopreventive potential of black tea was assessed (Katiyar and Mukhtar, 1997; Ahmad et al., 1997).

Green tea polyphenols, known as catechins, are strong antioxidants and are believed to be responsible for most of the chemopreventive properties of GT. The anticarcinogenic and anti-mutagenic activity of GT polyphenols was first reported almost two decades ago (Khan et al., 1988). The ability of GT to prevent cancer is so well established that new studies are testing GT polyphenols and EGCG as chemotherapeutic agents (Khan et al., 2006; Khan and Mukhtar, 2007) against various types of cancers. In humans, most of the studies showing an inverse relationship between tea consumption and development of cancer were conducted on gastrointestinal cancers in Japan and China where GT is the main form of tea consumed (Wang et al., 1999; Mu et al., 2003). Studies in northern Italy have suggested a protective effect of GT against oral, pharyngeal and laryngeal cancer. Frequent consumption of GT in China has been shown to be associated with lower incidence of esophageal cancer (Wang et al., 1999; Gao et al., 1994). A protective effect against gastric cancer by tea has been suggested from studies in Japan, Turkey and Sweden (Wang et al., 1999). In Japan women consuming more than 10 cups of GT daily were found to have lower risk for all cancers including breast cancer metastasis and recurrence (Nakachi et al., 1998; Wu et al., 2003). Tea drinking was shown to be associated with lower risk for digestive tract cancers and urinary tract cancers in women from Iowa, USA (Lambert and Yang, 2003a, 2003b). Individuals who consumed over 10 cups of GT per day showed remarkable reduction in risk for lung, colon and liver cancers (Sueoka et al., 2001). The preventive effect of GT against prostate cancer was also demonstrated (Jian et al., 2004). Oral administration of GT, black tea or EGCG inhibits the growth of well established skin tumors and in some cases tumor regression was also observed (Mukhtar and Ahmad, 2000; Mantena et al., 2005). Recent research has suggested that dietary chemopreventive agents including EGCG may prevent cancers by modulating one or more cell signaling pathways including apoptosis in a manner that interrupts the carcinogenic process (Surh, 2003; Khan et al., 2006).
Cardioprotective properties of Green tea:

Cardiovascular diseases, principally heart disease and stroke are the leading cause of mortality in Western countries, among both men and women in all racial and ethnic groups. The risk of atherosclerosis is increased by high blood pressure, diabetes, stress and lipid disorders. Underlying mechanisms for the beneficial effects of GT include vasculoprotective, antioxidative, antithrombogenic, anti-inflammatory and lipid-lowering properties of flavanoids (Stangl et al., 2006). The intake of GT has been inversely associated with the development and progression of atherosclerosis and it has been reported that dietary GT intake preserves and improves arterial compliance and endothelial function (Murakami and Oshato, 2003). GT has been shown to mitigate risk factors associated with heart disease and stroke (Khan and Mukhtar, 2007). GT catechins have been shown to affect lipid metabolism by different pathways and prevents the appearance of atherosclerotic plaques (Crespy and Williamson, 2004). Animal experiments have also studied the protective effects of GT against cardiovascular disease (Young et al., 1967; Sano et al., 1986; De Whalley et al., 1990). Ingestion of extract of GT in rats and humans has been shown to significantly decrease plasma cholesterol and triacylglycerol concentrations (Muramatsu et al., 1986; Chisaka et al., 1988; Ikeda et al., 1992; Deng et al., 1998; Tokunaga et al., 2002). Possible mechanisms of action include down regulation of liver fatty acid synthase, 3-hydroxy-3-methyl glutaryl coenzyme A reductase, a key enzyme in cholesterol synthesis and intestinal acyl CoA: cholesterol acyltransferase (Van het Hof et al., 1999; Maron et al., 2003). Increased consumption of GT was associated with decreased serum concentration of total cholesterol and triacylglycerols and an increased proportion of high density lipoprotein cholesterol together with a decreased proportion of low and very low lipoprotein cholesterol, which resulted in a decreased atherogenic index (Imai and Nakachi, 1995; Yang et al., 1997). An inverse association of GT intake and myocardial infarction and its genetic variation has also been reported (Hirano et al., 2002; Mukamal et al., 2002). GT is a natural angiotensin-converting enzyme (ACE) inhibitor and has been shown to lower blood pressure in humans and animals (Imai and Nakachi, 1995). The use of tea to control hypertension and obesity may also have an impact on the incidence of cardiovascular diseases (CVD). The protective activities of tea and tea polyphenols
against the oxidation of lipoproteins have been proposed to contribute to the prevention of atherosclerosis and other cardiovascular diseases.

**Antioxidant properties of Green tea:**

The potential health benefits associated with tea consumption have been partially attributed to the antioxidative property of tea polyphenols (Mukhtar and Ahmad, 2000). Tea catechins and polyphenols have been found to be efficient scavengers of free radicals in a number of *in vitro* systems (Rice-Evans, 1999). Recently, it has been reported that GT consumed within a balanced controlled diet improves the overall antioxidative status and protects against oxidative damage in humans (Erba et al., 2005). Tea preparations have been shown to trap reactive oxygen species, such as superoxide radical, singlet oxygen, hydroxyl radical, peroxyl radical, nitric oxide, nitrogen dioxide and peroxynitrite, reducing their damage to lipid membranes, proteins and nucleic acids in cell free systems (Paquay et al., 2000; Higdon and Frei, 2003). The radical quenching ability of GT is usually higher than that of black tea. Among tea catechins, EGCG is most effective in reacting with most reactive oxygen species. Overproduction of nitric oxide and peroxynitrite, has been associated with chronic inflammation and may be associated with the etiology and pathology of a number of chronic diseases. GT has been found to be about five times more potent than black tea in scavenging of these radicals (Paquay et al., 2000). Additionally, EGCG was found to inhibit the ONOO⁻-mediated formation of 8-OHdG in calf thymus DNA more potently than vitamin C or glutathione (Fiala et al., 1996). The chemical structures contributing to effective antioxidant activity of catechins include the vicinal dihydroxy or trihydroxy structure, which can chelate metal ions and prevent the generation of free radicals. This structure also allows electron delocalization, conferring high reactivity to quench free radicals. GT catechins have been found effective in inhibiting *in vitro* and *in vivo* lipid peroxidation (Kondo et al., 2001; Leena and Balaraman, 2005). A significant rise in plasma antioxidant capacity was detected after brewed GT was consumed (Leenen et al., 2000). GT may also increase the activity of antioxidant enzymes. Researchers found a significant increase in the activity of glutathione peroxidase, glutathione reductase, glutathione-S-transferase, quinine reductase and superoxide dismutase, all of which are antioxidant
and detoxifying enzymes in the small intestine, liver, kidney and lungs (Serafini et al., 1996).

**Neurological and Psychological effects of Green tea:**

GT has been shown to protect the brain from oxidative stress and hence green tea might be useful in preventing age-related brain degeneration. Neurodegenerative diseases have been linked both to free radical damage and to excessive breakdown of neurotransmitters caused by high monoamine oxidase (MAO) activity. GT has been found to be effective at inhibiting monoamine oxidase (MAO) and lowering peroxide levels in glial cells in brain (Mazzio et al., 1998). Recently, it has been reported that catechins possess divalent metal chelating, antioxidant and anti-inflammatory activities to penetrate the brain barrier and protect neuronal death in a wide array of cellular and animal models of neurological diseases (Mandel et al., 2006). It has been shown that EGCG reduces focal ischemia/reperfusion-induced brain injury in a rat model (Choi et al., 2004). An excess of the neurotransmitter, nitric oxide, may lead to neural death and neurodegenerative disorders. GT polyphenols possibly protect against Parkinson’s disease and have demonstrated neuroprotectant activity in cell cultures and animal models, such as the prevention of neurotoxin-induced cell injury (Pan et al., 2003). Japan has a much lower rate of Alzheimer’s disease than Western countries (Juneja et al., 1999). GT polyphenols have been found to inhibit or diminish iron-induced epileptic seizures and also the hyperactivity of dopaminergic neurons. A study done on hypertensive rats has confirmed that epigallocatechin gallate, EGCG, reduces the incidence of stroke and prolongs lifespan (Sato et al., 1989). The amino acid L-theanine is unique to tea. Theanine acts as a neurotransmitter in the brain and decreases blood pressure significantly in hypertensive rats (Khan and Mukhtar, 2007). Theanine is absorbed, modulates brain serotonin and dopamine levels, improving memory, learning ability and affects emotions (Juneja et al., 1999). There are some other results which indicate that daily consumption of GT in sufficient amounts will have life prolonging effects by avoiding premature deaths caused by various chronic ailments such as cancer, CVD and neurological disorders (Nakachi et al., 2003).
Obesity, Thermogenesis and Green tea:

It has been reported that the body weights of rats and their plasma triacylglycerol, cholesterol and LDL-cholesterol have been significantly reduced by feedings of oolong, black and green tea leaves to the animals (Lin and Lin-Shiau, 2006). In vitro experiments have demonstrated that GT inhibits lipogenesis (Hasegawa et al., 2002; Mori and Hasegawa, 2003). EGCG purified from GT when given to mice in diet decreased diet-induced obesity in mice by decreasing energy absorption and increasing fat oxidation (Klaus et al., 2005). Supplementation with tea catechins resulted in a significant reduction of high-fat diet-induced body weight gain, visceral and liver fat accumulation, and the development of hyperinsulinemia and hyperleptinemia in C57BL/6J mice (Murase et al., 2002). EGCG also significantly reduced or prevented an increase in body weight in lean and obese male and female Zucker rats (Kao et al., 2006). On the basis of the in vivo effects of EGCG on body weight loss, body fat, serum lipid nutrients, thermogenesis and fat oxidation and of the in vitro effects of EGCG on fat cell functions, long term consumption of GT may decrease the incidence of obesity and perhaps, GT components such as EGCG may be useful in treating obesity. It has been reported that GT extract rich in catechins and caffeine has thermogenic properties and promotes fat oxidation beyond than those explained by its caffeine content per se; the GT extract may play a role in the control of body composition via sympathetic activation of thermogenesis, fat oxidation, or both (Dulloo et al., 1999). The increased and prolonged sympathetic stimulation of thermogenesis by the interaction between polyphenols and caffeine could be of value in assisting the management of obesity (Dulloo et al., 2000).

Antibacterial properties of Green tea and GI tract:

It is well established that GI tract plays a very significant role in the absorption, metabolism and conjugation of GT polyphenols (Spencer, 2003). In the GI tract, GT is found to activate intracellular antioxidants, inhibit pro-carcinogen formation, suppress angiogenesis and cancer cell proliferation (Mukhtar and Ahmad, 1999). GT is effective to prevent dental carries and reduce cholesterol and lipid absorption in GI tract as well as benefits subjects with CVD (Koo and Cho, 2004). Studies have shown that GT catechins interact and bind endogenous proteins in the small intestine limiting their absorption (Carbonaro et al., 2001). These protein binding properties of
catechins have been linked to their bactericidal abilities and other beneficial properties in the digestive tract. Tea may modify the intestinal microflora, by selectively increasing the growth of bifidobacteria and lactobacilli in the gut wall (Yamamoto et al., 1997; Weisburger, 1999) while decreasing levels of numerous potential pathogens. Levels of pathogenic bacterial metabolites were also decreased (Goto et al., 1998). GT is an antimicrobial agent against a variety of gram positive and gram negative pathogenic bacteria that causes cystitis, pyelonephritis, diarrhea, dental caries (You, 1993), pneumonia and skin infections (Chou et al., 1999). The health promoting properties of GT have the potential to prevent gastrointestinal diseases (Suganuma et al., 1999). GT has been shown to reduce inflammation associated with Crohn’s disease and ulcerative colitis, the two types of inflammatory bowel disease (IBD) (Alic, 1999; Setiawan et al., 2001; Sano and Sasako, 2001). GT may have beneficial effect against viral infection (Khan and Mukhtar, 2007).

**Antidiabetic properties of Green tea and kidney health:**

The kidneys are another area where GT has been shown to have protective effects. In one particular study, the antioxidant effect of GT was observed in rat kidney (Sano et al., 1995). In another study, GT supplementation decreased urinary oxalate excretion and calcium oxalate deposit formation and thus was seen to protect against calcium oxalate urolithiasis (Itoh et al., 2005). The concomitant administration of GT extract to Cyclosporine A (CsA) receiving rats markedly prevented the generation of thiobarbituric acid-reacting substances (TBARS) and significantly attenuated CsA-induced renal dysfunction (Mohamadin et al., 2005). Kidney problems are often associated with high blood sugar and consequent glycosylation of various proteins (hence the stray link between kidney failure and diabetes). Since GT has the ability to lower serum glucose, this is another way by which it protects against kidney failure (Sabu et al., 2002). A study by Waltner-Law et al. (2002) provided compelling in vitro evidence that EGCG decreases glucose production in rat hepatoma cells. Both green and black teas were shown to possess antidiabetic activity and were found to be effective both in the prevention and treatment of diabetes (Serafini et al., 1996; Yokozawa et al., 1996; 2005). EGCG was shown to induce antioxidant enzymes in the kidneys, as well as to reduce uremic toxins in the blood, suggesting improved kidney function in an animal model of renal failure (Yokozawa et al., 1996).
The kidney is a vital organ that plays an essential role in health and diseases. 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 is consisting 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). 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). Each tissue plane also has individual "organ" characteristics with respect to their ionic contents and metabolic rates (Guder, 1973; Schmidt and Guder, 1976). 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, certain ions and minerals such as Na⁺ and inorganic phosphate (Pi) (Hammerman and Schwab, 1984; Kempson and Dousa, 1986). 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 (McCrorry 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 transport are dependent (Ullrich et al., 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 (pO₂) of the renal tubular cells. A direct linear relationship between O₂ uptake/ utilization and Na⁺ reabsorption has been found (Thurau, 1961; Torelli et
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 (Auckland and Krog, 1960, 1961; Auckland, 1962; Aperia and Leibow, 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 et al., 1973; Park et al., 1974; Pitts and MacLeod 1975; Pitts, 1975; Mandel and Balaban, 1981). It is well established that various nephron subsegments located in different tissue 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 et al., 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: (1) the tricarboxylic acid (TCA) cycle, in which glucose undergoes glycolysis to pyruvate, which in turn may oxidize to $CO_2$; (2) the hexose-monophosphate (HMP) shunt pathway; and (3) the glycolysis in which glucose is partially oxidized to lactate. 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; Burch et al., 1978; Maleque et al., 1980). 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) whiles the oxidative conversion of glucose to $CO_2$ was shown in renal cortex (Lee et al., 1962; Cohen, 1979).

Nephrons, which are consisting of various subsegments, showed distinct structural and functional differences (Francois and Danielle, 1985; Lise et al., 1987). Thus nephron heterogeneity also contributes 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 (Francois and Danielle, 1985; Lise et al., 1987). The nephron which originates in the glomerulus located in superficial cortex is known as "superficial nephron" while the nephron...
Figure 2: Structure of 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 in structure as well as in function (Francois and Danielle, 1985). In inter-nephronal heterogeneity, the 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 S1, S2, S3. The early PCT both in superficial and juxtamedullary nephrons is defined as S1-segment and can be identified by its attachment with glomeruli on one side. S2 is defined as the late superficial proximal convoluted tubule, early superficial proximal straight tubule and late juxtamedullary proximal convoluted tubule. S3 is located principally in the outer stripe of outer medulla and is terminal superficial proximal straight tubule and entire juxtamedullary proximal straight tubule. S3 is identified by its medullary location and by its connection with thin limbs on distal part. All S3-subsegments (pars recta), as they descend from cortex into the outer stripe of the outer medulla change from S2 to S3 cell type. Thus the outer stripe of the outer medulla contains proximal tubular cells but only the S3 type (Woodhall et al., 1978). These segments can be characterized by their biomarker enzymes (Yusufi et al., 1994).

**Acute Renal Failure:**

A number of environmental variables and heavy metals like chromium, mercury, cadmium, lead, uranium (Bank et al., 1967; Sanchez et al., 2001) including drugs like aminoglycosides, cephalosporin, cisplatin etc. (Humes, 1988; Mahmood and Waters, 1994; Mingeot-Leclercq and Tulkens, 1999) affects the structure and function of the kidney leading to acute renal failure (ARF). The term “Acute Renal Failure” 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 ARF. Acute Renal Failure is a process rather than a state. Often it denotes a reversible insufficiency of the glomerular and tubular excretion functions which may be triggered by renal or extrarenal mechanisms. ARF is characterized by increase of nitrogenous waste products, such as serum creatinine and blood urea nitrogen and appearance of proteinic and enzymic components in the urine.

ARF 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. 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 function.

Generally ARF caused by drugs and chemicals is much more severe and irreversible and the recovery sometimes is not possible (Loghman-Adham et al., 1987). The pathophysiologic mechanism of ARF has been investigated extensively in the last few decades (Maleque et al., 1980). Four major possible causes of ARF have been generalized which include renal vasoconstriction, glomerular permeability, tubular obstruction and tubular leakage (Maleque et al., 1980).

THE STRUCTURE AND FUNCTION OF INTESTINE:

The digestion and absorption of food components are major functions of the intestinal mucosa. It is the most metabolically active tissue in the body. Many complex compounds while passing through the small intestine are degraded into simple compounds which cross the intestinal epithelium before reaching the various body organs. Thus this epithelium not only regulates diverse absorptive and secretory processes but also process the substances that traverse it. The small intestine extends from the pyloric orifice where it is continuous with the stomach to the ileocecal junction where it continues to the large intestine. It is divided into three distinct structural and functional parts:-

(i) Duodenum
(ii) Jejunum
(iii) Ileum

To perform all these functions, the absorptive surface is greatly amplified by transverse folds. The small intestine is consisting of four layers

(i) serosa (outermost)
(ii) muscularis mucosa
(iii) submucosa
The absorptive cells of the small intestine are highly polarized tall columnar cells with a general architecture and structure that is similar to a number of other epithelial cell types whose major function is absorption (Grosser et al., 1992). They are distinguished by the presence of an apical striated border which forms the absorbing surface in contact with luminal contents. The plasma membrane of these enterocytes consists mainly of two different regions; brush border and the basolateral membrane which are morphologically, structurally and functionally different.

**Brush Border Membrane**

The small intestinal mucosa is one of the largest areas of contact of the human organism with the environment (Schummann et al., 1999). The mucosa of the small intestine has transverse folds known as villi, which are finger like projections 0.5-1.5 mm in length. The villus is lined with a single layer of epithelial cells, containing a network of capillaries and lymphatic vessels (Lacteals). The plasma membrane covering the microvilli is covered with a polysaccharide coat referred to as glycocalyx and consists of glycolipids and glycoproteinic enzymes that hydrolyze carbohydrates and peptides. The cytoplasm beneath the microvilli contains fine filaments known as terminal web. The products of digestion may go through the microvillar membrane traversing the terminal web into cytoplasm.

Brush Border membrane (BBM) lining the enterocytes (intestinal cells) constitutes one of the most important cellular membranes owing to its role in terminal digestion and absorption functions which are carried out by certain hydrolytic enzymes e.g. disaccharidase, dipeptidases, oligopeptidases, γ-glutamyl transpeptidase, maltase, lactase, sucrase and alkaline phosphatase (Kenny and Booth, 1978). These enzymes are differentilly distributed in the thickness of BBM e.g. sucrase being superficially located (Brasitus et al., 1979) whereas alkaline phosphatase and ATPase are more deeply embedded within the membrane (Sigrist-Nelson et al., 1977).

The absorption of digestive food and various ions occurs by passive as well as active transport (Hopfer et al., 1973; Schultz, 1977). The transport of water, sugars and amino acids is a Na⁺ dependent secondary active transport energized by an
Figure 3: Structure of intestinal villi
electrochemical gradient due to differences in Na\(^+\) concentrations across the BBM (Hopfer et al., 1973; Schultz, 1977; Hopfer, 1978; Ramaswamy et al., 1991). The role of proton motive force as a source of energy (in terms of ATP) has also been demonstrated (Rajendran et al., 1987; Vorum et al., 1988; Ganapathy et al., 1991). This is carried out by sodium pumps (Na\(^+\)K\(^+\)ATPase); which are highly conserved integral membrane protein.

The activities of certain enzymes belonging to glycolysis, TCA cycle, HMP shunt pathway, have been reported under various experimental conditions (Farooq et al., 2004). The presence of hexokinase, glucokinase, 6-phosphofructokinase, G-6-phosphatase, FBPase, oxoglutarate dehydrogenase, citrate synthase, PEP carboxykinase, NADP-malic enzyme and glutaminates indicate that gluconeogenesis is also operational in the small intestine (Farooq et al., 2004). The function of small intestine appears to be metabolic as opposed to be absorptive. Thus the studies involving metabolic activities may in some way reflect the absorption properties of the intestinal mucosa.

The process of digestion as well as absorption is a regulatory process which is regulated by various drugs, hormones, and is also affected by nutritional and dietary status (Farooq et al., 2004, 2006). The small intestine is also the primary site for great exposure to hazardous and life threatening environmental toxicants such as heavy metals (uranium) and drugs (aminoglycoside antibiotics and antineoplastic agents). Recent studies have clearly demonstrated that the enzymes present in the small intestine are not only altered by dietary stress such as starvation and fasting (Farooq et al., 2004, 2006) but also by antibiotics such as gentamicin (Farooq et al., 2007).

Most prominent pathophysiological mechanism of gastrointestinal toxicity include direct effects on cell membrane, inhibition or stimulation of mucosal proliferation, nerve damage, activation of emetic pathways, disruption of intracellular signal transduction, generation of reactive oxygen substances, activation or inhibition of metabolic enzymes, intracellular toxicity etc. Reactive oxygen metabolites/species are the major causative factors for the mucosal lesions through oxidative stress. The major toxic effects of ROS include direct cytotoxicity towards epithelial cells, net fluid secretion into the lumen and alteration in functions of intestinal
microvasculature that lead to increased permeability and membrane damage (Grisham et al., 1990).

**STRUCTURE AND FUNCTION OF LIVER:**

The liver weighing roughly 1.2-1.6 kg, performs many of the functions necessary for staying healthy. The liver is the first organ that comes into contact with enterally absorbed nutrients and xenobiotics via the portal blood (Kahl, 1999). Two large vessels carry blood to the liver: the hepatic artery comes from the heart and carries blood rich in oxygen, whereas the portal vein brings to the liver, blood rich in nutrients from the small intestine. These vessels are divided into smaller and smaller vessels, ending in capillaries, which further divide into thousands of lobules. Each lobule is composed of hepatocytes, and as blood passes through, they are able to monitor, add or remove substances from it (Sherwood, 1997).

Among the most important liver functions are:

- Removing and excreting body wastes and hormones as well as drugs and other foreign substances. Enzymes in the liver alter some toxins so they can be more easily excreted in the urine.
- Synthesizing plasma proteins, including those necessary for blood clotting. Most of the 12 clotting factors are plasma proteins produced by the liver.
- Producing immune factors and removing bacteria, helping the body fight infection.
- Producing bile to aid in digestion. Bile salts aid in fat digestion and absorption.
- Excretion of bilirubin. Bilirubin is one of the waste products excreted in bile. Macrophages in the liver remove worn out red blood cells from the blood. Bilirubin results from the breakdown of hemoglobin and is excreted by hepatocytes.
- Storing certain vitamins, minerals and sugars. The liver stores enough glucose in the form of glycogen to provide a day’s energy.
The Liver

Blood returning to the heart
hepatic vein
diaphragm
left lobe
of liver
hepatic artery
Bringing fresh blood
from the heart
portal vein
Bringing blood from the intestines
Bile draining to the intestines
gall bladder
common bile duct
right lobe
of liver

Figure 4: Structure of Liver
One of liver’s most interesting ability is self-repair and the regeneration of damaged tissue. In clearing the body of toxins, the liver is damaged by exposure to harmful substances, demonstrating why this capability is important (Tzanakakis et al., 2000).

Toxic damage to the liver parenchymal cells presents histologically as degenerative alterations of the cell or as abnormal storage, usually of fat (steatosis) (Arias et al., 1994). Cell death in liver injury may be of two types; it can be programmed cell death, or necrosis, a toxic lysis of the cell (Zimmerman, 1993).

**GENTAMICIN: PATHOPHYSIOLOGY**

Aminoglycosides such as gentamicin (GM) are the most commonly used antibiotics worldwide in the treatment of gram-negative bacterial infections (Nagai and Takano, 2004). The high incidence of associated nephrotoxicity and ototoxicity (Lang and Liu, 1997) represents an important concern in the use of aminoglycoside antibiotics which have been implicated as one of the primary causes of drug induced acute-renal failure (Appel, 1990).

Gentamicin was isolated in 1963 by Weinstein and colleagues from the soil fungus Micromonospora purpura (of the Actinomycete group) (Sande and Mandell, 1985). It was introduced in the USA in 1969 and is a complex of gentamicins C1, C1a and C2 and gentamycin A. Structurally, gentamicin comprises three aliphatic six-membered rings, two tetrahydropyrane rings and one cyclohexane ring (Fig. 5). Aminoglycosides are polycationic and consist, for the most part, of a central 2-deoxystreptamine molecule with glycosidic linkages to two or more amino sugars. They react basically (pKa ≥ 8), are highly water-soluble, have low lipid solubility, and have a low capacity to penetrate membranes. The number of amino groups per aminoglycoside molecule, and their cationic structure, appears to be correlated to the nephrotoxic potential (Bennet, 1997).

**Figure 5: Structure of Gentamicin**
Effect of Gentamicin on Kidney:

Aminoglycosides are low protein-binding drugs and are freely filtered through the glomerulus in the kidney without being metabolized in the body. Most of the intravenously administered dose is excreted into the urine, whereas a relatively sizeable portion is selectively accumulated at high concentrations in the renal cortex leading to renal damage such as structural changes and functional impairments of the plasma membrane, mitochondria and lysosomes (Mingeot-Leclercq and Tulkens, 1999).

The cellular uptake and accumulation of GM in the proximal tubule is a multistep process initiated with the binding of the cationic drug to the anionic sites on the brush border membrane (BBM) of the proximal tubular cells. This binding is mediated in part by electrostatic interaction with acidic phospholipids, predominantly to phosphatidylinositol (PI) which act as the principal receptor (Kaloyanides and Pastoriza-Munoz, 1980; Humes et al., 1982; Walker and Duggin, 1988; Hori and Inui, 1989; Mingeot-Leclercq et al., 1989). More recently, megalin, a giant endocytic receptor protein, abundantly expressed at the apical membrane of renal proximal tubules, has been found to play an important role in the binding and endocytosis of the drug (Moestrup et al., 1995; Nagai and Takano, 2004). The adverse interaction of GM with one or more critical intracellular processes leads to renal cortical phospholipidosis and disruption of functions of membranes and organelles including mitochondria, lysosomes, microsomes, BBM and basolateral membrane (BLM) (Sundin et al., 2001).

GM accumulates in the renal cortex, mainly in the cells of the proximal convoluted tubule (Cojocel et al., 1983). The morphologic changes incurred by GM predominantly occur in S1 and S2 segments of PCT and to lesser extent in pars recta (S3-subsegments) in animals and human kidneys. Histopathological studies strongly support the concept that tubular necrosis is the primary cause of aminoglycoside toxicity (Kaloyanides, 1984). The ultrastructural changes in the proximal tubular cells include the loss of the brush border, proliferation and multimembranous restructuring of lysosomes, formation of myeloid bodies, distension of the endoplasmic reticulum and swelling of the mitochondria (Kaloyanides, 1991) leading to total disorganization.
and disruption of cellular organelles, with cellular necrosis (Kaloyanides and Pastoriza-Munoz, 1980; Humes et al., 1982).

Aminoglycoside nephrotoxicity is characterized by increase in serum creatinine and blood urea nitrogen (BUN) associated with a marked decrease in tubular transport of electrolytes, calcium and magnesium, glucose, protein and organic anion transport (Cojocel and Lock, 1999). This was accompanied by massive proteinuria, enzmurina, glucosuria and phosphaturia (Cojocel and Hook, 1983). Gentamicin has been reported to inhibit the oxidative phosphorylation in the isolated renal cortex and to decrease the total ATP content of rat kidney (Simmons et al., 1980). Gluconeogenesis was found to be depressed in rabbit renal tubules incubated with gentamicin (Michalik et al. 1989).

Recently GM has been shown to affect various enzymes of carbohydrate metabolism including glycolysis, TCA cycle, HMP shunt pathway and gluconeogenesis (Banday et al., 2008a). The damage caused by GM to lysosomes, BBM and BLM was demonstrated by decrease in the activities of their biomarker enzymes in renal cortex associated with their increase in the urine (Banday et al., 2008a). GM also interacts with components of the protein and phospholipid biosynthesis machinery from the lumen of golgi and endoplasmic reticulum resulting in inhibition of renal protein and phospholipid metabolism (Sundin et al., 2001). Decreased expression of multiple genes by GM treatment has been elucidated by toxicogenomic studies (Amin et al. 2004; Kramer et al., 2004).

Various nephrotoxins have been shown to generate ROS and produce oxidative stress leading to cellular injury. Reactive oxygen species (ROS) are considered important mediators of GM nephropathy (Walker et al., 1999; Cuzzocrea et al., 2002; Ali, 2003). Gentamicin has been shown to cause release of iron from renal cortical mitochondria (Baliga et al., 1997). Formation of ROS following bioactivation of GM has been reported (Sha and Schacht, 1999). GM induces superoxide anion (O$_2^-$), hydrogen peroxide and hydroxyl radical production from renal mitochondria (Yang et al., 1995; Walker et al., 1999). It has been reported that GM suppresses antioxidant enzymes whereas H$_2$O$_2$ generation, lipid peroxidation, and protein carbonyl content are increased in the renal cortex (Sandhya et al., 1995; Ali and Bashir, 1996; Walker et al., 1999; Naidu et al., 2000; Cuzzocrea et al., 2002; Pedraza-Chaverri et al., 2000).
and 2003; Maldonaldo et al., 2003; Parlakpinar et al., 2004; Karahan et al., 2005; Banday et al., 2008a).

**Effect of Gentamicin on Intestine:**

Nutritional stress and a number of environmental variables including certain chemicals, metal ions and antibiotics, dramatically alter the digestive and absorptive function of the intestinal mucosa, as well as dramatically alter the activities of enzymes of intestine (Farooq et al., 2004, 2006, 2007). Aminoglycoside antibiotics are frequently used in the treatment of severe infections of the abdomen and urinary tract including bacteremia and endocarditis (Nagai and Takano, 2004). Although kidney is the major GM target, it has been shown to accumulate in various tissues including intestine, liver, heart and ear causing multiple adverse effects in these tissues (Soberon et al., 1979; Parker, 1990; Ozturk et al., 1997; Kohn et al., 2005).

Aminoglycoside antibiotics such as neomycin and kanamycin, were shown to alter mucosal morphology and absorption of nutrients concomitant with nausea, vomiting, diarrhea (Jacobson et al., 1960; Faloon et al., 1966; Paes et al., 1967). They are known to interact with the intestinal epithelium, its innervations causing alterations in intestinal motility and contribute to the pathogenesis of antibiotic-associated diarrhea and colitis (Goldhill et al., 1996). Study on the impact of GM on absorptive function of small intestine in patients with chronic bronchitis revealed that GM inhibits intestinal fat, carbohydrate and protein absorptions (Akimovo and Shteingardt, 1992).

GM is widely used in the organ culture of the intestine and caused decrease of intestinal lactase and alkaline phosphatase activity (Shimizu et al., 1991). Farooq et al. (2007) have recently reported that activities of certain enzymes involved in terminal digestion and glucose metabolism were selectively altered in a time dependent manner by GM treatment.

The proposed mechanisms include a) the direct action of antibiotic on intestinal function b) inducing predisposition to infection with an entire pathogen c) and factors secondary to disturbance of the normal intestinal flora that do not involve infection with a known pathogen (Borriello, 1992).

ROS mediated injury to the small intestine has been demonstrated in several conditions such as ischemia/reperfusion, inflammatory bowel disease, surgical stress,
drug usage etc (Halliwell and Gutteridge, 1999; Arivarasu et al., 2007). GM administration also affects the antioxidant defenses in small intestine, indicated by elevated lipid peroxidation and depressed SOD and catalase activities (Farooq et al., 2007).

**Protection or prevention against Gentamicin toxicity:**

Gentamicin, an effective and widely used aminoglycoside antibiotic is known to be potentially nephrotoxic despite close attention to the pharmacokinetics and dosing schedules of the drug (Ishikawa et al., 1985). GM has been shown to cause marked histological damage in particular to renal proximal convoluted tubules (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). The proximal tubule injury and subsequent renal dysfunction has been attributed to the accumulation of the drug (Hori and Inui, 1989) in the tubule cells and its interactions with brush border and basolateral membranes and other organelles leading to disruption of their functions. GM-induced renal cortical phospholipidosis was also suggested to disrupt the functions of various organelles including mitochondria, lysosomes, microsomes and plasma membranes (Cronin and Henrich, 1996; Mingeot-Leclercq and Tulkens, 1999).

Several approaches, utilizing different mechanisms, have been attempted to reduce the nephrotoxicity caused by GM and related aminoglycoside antibiotics. The mechanisms involved include (1) decreasing or preventing drug accumulation by the kidney (2) competition of decreasing aminoglycoside binding to brush border membrane (3) protection against vascular and glomerular effects (Ali, 1995; Mingeot-Leclercq and Tulkens, 1999). Have all helped in reducing the possibility of GM-nephropathy. In human medicine, the single daily injection is the only approach actually used to reduce the renal toxicity of aminoglycosides. In experimental animals several strategies to ameliorate the toxicity have been attempted (Ali, 2003). These include controlling the time of administration of the antibiotics (Beauchamp et al., 1994), modification of the diet (Paquette et al., 2002) and co-administration of antibiotics and certain agents such as thyroid hormone (Cronin et al., 1986) to mitigate renal toxicity. However all of the different agents and strategies used were
not found suitable for clinical practice for reasons related to safety, practicality or effectiveness.

Several workers have suggested that oxygen free radicals are considered to be important mediators of GM-mediated nephrotoxicity (Baliga et al., 1997; Walker et al., 1999). It has been shown that GM enhances the production of hydrogen peroxide by rat renal cortical mitochondria, and suggested therefore, that hydroxyl scavengers such as metallothionein and iron chelators could prevent GM induced acute renal failure (Yang et al., 1991; Shah and Walker, 1992; Yang et al., 1995). It has been asserted that, among the main approaches used to ameliorate or protect against GM nephrotoxicity, the most consistent effects have been observed with the use of antioxidant agents (Mingeot-Leclercq and Tulkens, 1999). Examples of antioxidant agents that have been used to ameliorate GM nephrotoxicity in rats include deferrioxamine, methimazole, saireri-to, vitamin E, vitamin C and selenium. Other agents which have recently been studied include superoxide dismutase, SOD (Ali and Bashir, 1996), lipoic acid (Sandhya et al., 1995); dimethyl sulphoxide, DMSO (Ali and Mousa, 2001); the hormone melatonin (Ozbek et al., 2000; Shifow et al., 2000; Sener et al., 2002); the antihyperlipidemic drug probucol (Kumar et al., 2000), taurine (Erdem et al., 2000), N-acetylcysteine (Mazzon et al., 2001) and trans-reservatrol (Morales et al., 2002). Of special interest is the finding that the agent, M40403 (a SOD mimetic) protects against experimental GM nephrotoxicity (Cuzzocrea et al., 2002). However, M40403 has several advantages over native SOD enzyme that includes stability, better penetration into cells and lack of immunogenicity of non-human enzyme. DMSO is another promising agent to ameliorate GM nephrotoxicity (Ali and Mousa, 2001).

A recent interesting development is the attempt to use extracts from medicinal plants with antioxidant properties to ameliorate/protect against GM-induced nephrotoxicity in rats. Such medicinal plants include garlic (Pedraza-Chaverri et al., 2000), as well as diallyl sulfide, a compound isolated from garlic (Pedraza-Chaverri et al., 2003) and Nigella sativa oil (Ali, 2003). It may appear that these natural antioxidants may offer comparatively safer alternatives to the other antioxidants (e.g. the beta-blocker carvediol), however, their adverse/side effects have not been studied. In addition to the above-mentioned agents, some other miscellaneous drugs have been tested for
their protective/ameliorative effects in GM-induced nephrotoxicity. It has been suggested that the major pathway responsible for renal uptake and accumulation of GM is via megalin (Schmitz et al., 2002). Mice lacking megalin receptors were found to be completely resistant to GM nephrotoxicity. It was suggested that megalin could be considered a unique target for prevention of GM nephrotoxicity.

In view of its excellent safety and efficacy profiles, antioxidant agents were found to produce the best nephroprotection. These agents, especially the natural antioxidants, seem to possess the highest potential for use in the clinic. Of these natural antioxidants present in fish oil and green tea may be another option which is gaining interest in recent times. Fish oil at high doses has been found to exert protection against gentamicin nephrotoxicity (Ali and Bashir, 1994; Abdel-Gayoum et al., 1995). Treatment with green tea modified the biochemical changes that occurred during gentamicin nephrotoxicity and thus was shown to have potential protective affect (Upaganlawar et al., 2006).

CISPLATIN: PATHOPHYSIOLOGY

Cisplatin (cis-diamminedichloroplatinum (II)) is an effective agent against various solid cancers. Despite its effectiveness, the dose of cisplatin (CP) that can be administered is limited by its nephrotoxicity. Hundreds of platinum compounds (e.g. carboplatin, explatin, nedaplatin) have been tested over the last two decades 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 [Fig. 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.

\[
\text{NH}_3 \quad \text{Cl} \quad \text{Pt} \quad \text{NH}_3 \quad \text{Cl}
\]

Figure 6: Structure of Cisplatin (CP)
Cisplatin and other related drugs form strong electrophilic intermediates that act via nucleophilic 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). It is given intravenously or intraperitoneally, binds to serum protein by about 98%, 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 a higher degree than other organs probably through energy-mediated transport (Arany and Safirstein, 2003; Kawai et al., 2005). Cisplatin concentrations in proximal tubular epithelial cells exceed plasma concentrations by a factor of five. Intracellularly, the highest concentrations of CP are found in the cytosol, mitochondria, nuclei and microsomes (Kuhlmann et al., 1997).

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 effects 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. Cisplatin 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 support 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 (Tisher, 1982; 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 (Matsushima et al., 1998), loss of renal sulfhydryl groups, damage to mitochondria (Zhang and Lindup, 1993) 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). CP undergoes ligand binding reactions which are virtually irreversible (Daley-Yates and McBrien, 1982). CP is biotransformed through binding to lower 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).

Once cisplatin is administered to cancer patients intravenously as a sterile saline solution, there are three possible mechanisms involved in CP toxicity. Once it enters 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 (Fig. 7), the neutral cisplatin molecule undergoes hydrolysis in which a chlorine ligand is replaced by a molecule of water, generating a positively charged species.

**Inside the cell:**

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

![Diagram of cellular uptake of cisplatin (CP).](image)

**Figure 7:** Cellular uptake of cisplatin (CP).

(From: Encyclopedia of cancer by Pil and Lippard, 1997)

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; Jo et al., 2005; Taguchi et al., 2005).

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 hydrated 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 DNAsel (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 sulph-hydryl groups is an important factor in promoting cytotoxicity. Due to the compounds high affinity to SH groups, its chloride moieties are replaced by sulph-hydryl 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 failure (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 failure induced by
cisplatin (Arany and Safirstein, 2003). Different studies have demonstrated that the cytotoxicity of CP is probably due to combination of insults, including peroxidation of cell membrane, mitochondrial dysfunction, inhibition of protein synthesis and DNA damage in kidney and intestine thereby inducing renal dysfunction and diarrhea (Santos et al., 2007).

**Effect of Cisplatin on Intestine:**

CP a widely used chemotherapeutic agent has profound effect on the kinetics, morphology and function of the mouse/human small intestine. Studies on CP distribution in experimental animals showed the highest concentrations not only in the excretory organs but also in ovary and uterus, whereas the lowest were in brain (Litterst et al., 1979).

Nausea and emesis are important factors that reduce drug compliance in patients receiving anticancer chemotherapeutic drugs such as cisplatin (McCarthy and Borison, 1984; Kris et al., 1985). CP is thought to be absorbed by simple passive diffusion through the peritoneum, and evidence has been found for active transport after ingestion of an oral dose (Gale et al., 1973; Andrews et al., 1987; Casey et al., 1989; Binks and Dobrata, 1990) although some evidence exists for carrier-mediated uptake of cisplatin by an amino acid mediated transport system (Byfield and Calabro-Jones, 1982).

The first obvious effect of CP toxicity is marked degeneration and desquamation of intestinal villi in jejunum in cisplatin treated rats/mice along with significant reduction in villus height (Ikuno et al., 1995). Destruction of the crypt cells containing large Paneth (secretory) granules in the ileum and the changes in the crypt height resulting from CP toxicity are related to the loss of regenerative capacity of crypts owing to the cytotoxic insult of CP. Histological analysis of small intestine of CP treated experimental animals has revealed that cisplatin also impairs the mucosal structure of the ileum by causing acute epithelial necrosis (Choie et al., 1983)

Cisplatin elicited oxidative stress increased free fatty acids in intestinal mucosal cells, thereby inducing permeability transitions in plasma membrane and subcellular membranes and subsequent release of cytochrome C from mitochondria, leading to apoptosis (Chang et al., 2002).
In studies assessing the gastrointestinal toxicity of cytotoxic chemotherapeutic agent administration it is evident that severe morphological changes occur during drug-induced epithelial damage in the mucosa of the intestines, thus resulting in necrosis and/or haemorrhagic enterocolitis (Schaeppi et al., 1973). Thus, CP toxicity-induced diarrhea may be caused by abnormalities of intestinal absorption or secretion. CP-induced diffuse mucosal damage in the intestine from the jejunum to colon in mice may cause malabsorption and subsequently diarrhea (Ikuno et al., 1995). Crypt cell damage by cisplatin administration reduces the disaccharidase activity, resulting in a decrease in water reabsorption causing diarrhea. Several studies have demonstrated a reversal of CP induced intestinal damage by 5-HT3 receptor antagonists (De Mulder et al., 1990; Marty et al., 1990; Gandara et al., 1993), suggesting a role of 5-HT in cisplatin-induced GI symptomatology (Bearcroft et al., 1999). Cisplatin has been found to induce mitochondrial oxidative stress with impairment of energetic metabolism, membrane rigidification and apoptosis in rat liver (Martins et al., 2007).

**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-induced 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 lesser 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 with metabolic pathways of CP, diuretics and cytoprotective and apoptotic agents. Only few of these have been tested in humans.

The nephrotoxicity of cisplatin is closely related to the activity of reactive oxygen species (ROS). Platinum complexes are very reactive towards the cysteine residue of GSH, which detoxifies these enzymes by a rapid binding mechanism (Jansen et al., 2002). 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) and N-acetylcysteine (Wu et al., 2005). Among the antioxidants that have been tried against nephrotoxicity of cisplatin, include those that have been extracted from natural products (Conklin, 2004). 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 strong in vitro and in vivo antioxidant and antiproliferative actions, which may be the basis of its protection against cisplatin nephrotoxicity (Totta et al., 2004).

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 the 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).

Recent evidence suggests that apoptosis and inflammatory mechanisms play an important role in the pathogenesis of cisplatin nephrotoxicity (Jo et al., 2005). 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 Drug 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 (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. Green tea is one such dietary source of biologically active components that has been shown to be co-preventive and co-therapeutic in a wide variety of ailments (Khan and Mukhtar, 2007).

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 and Balaraman, 2005).
SCOPE OF THE THESIS

The concept of disease prevention by administration of naturally occurring or synthetic compounds is gaining increasing attention. The past five years have been rich in information coming from laboratories all around the world concerning the positive impact of food on human health. Much interest has been centered on the role of oxidant/antioxidant activity in regards to ageing and degenerative diseases like cancer, cardiovascular and diabetes (Khan and Mukhtar, 2007).

Tea produced from the leaves of the plant *Camellia sinensis*, is, next to water, the most widely consumed beverage in the world. It provides a dietary source of biologically active compounds that help prevent a wide variety of diseases (Alschuler, 1998). For many generations, tea has been considered to possess health-promoting potential in some parts of the world (Weisburger, 1997). Tea’s status as a functional food lends credibility to what has been believed by tea drinkers for centuries. Among all teas, green tea (GT) has been best studied for health benefits (Khan and Mukhtar, 2007).

Epidemiological studies strongly suggest that the consumption of green tea is associated with a lower risk of several human diseases, including cancer, cardiovascular disorders, obesity, neurological disorders, diabetes etc (Sueoka et al., 2001; Mukamal et al., 2002; Lambert and Yang, 2003a, 2003b). GT is an excellent source of polyphenolic compounds, known as catechins, with epigallocatechin-3-gallate (EGCG) being the major catechin present in green tea. GT catechins and their derivatives are known to contribute beneficial health effects ascribed to tea (Zloch, 1996) by their antioxidant (Higdon and Frei, 2003), antimutagenic and anticarcinogenic properties (Mukhtar and Ahmad, 1999, 2000). The cancer preventive effect of GT has been observed in pancreatic, rectal and colon cancers (Ji et al., 1997). GT has been found to decrease the risk of esophageal cancer (Gao et al., 1994) as well as improved prognosis in breast cancer has been reported (Nagata et al., 1998). GT has been shown to mitigate risk factors associated with heart disease and stroke, and has been inversely associated with the development and progression of atherosclerosis (Murakami and Oshato, 2003). In addition, GT has been found useful in the treatment of arthritis, high cholesterol levels, infection and impaired immune function (Van het Hof et al., 1999).
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). Gentamicin (GM), an aminoglycoside antibiotic, is still considered to be an important antibiotic against life threatening infections despite its known nephro and ototoxicity (Humes, 1988; Tulkens, 1989; Mingeot-Leclercq and Tulkens, 1999). The antineoplastic nature of liganded platinum compounds, especially 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 Raguenez-Viotte, 1989; Mahmood and Waters, 1994). Recent studies indicate an important role of reactive oxygen species (ROS) in GM and CP induced tissue injury (Masuda et al., 1994; Matsushima et al., 1998; Baliga et al. 1998; Walker et al, 1999).

Several approaches, utilizing different mechanisms, have been attempted to reduce GM and CP toxicity. Many different agents and strategies have been reported to ameliorate GM as well as CP toxicity in experimental animals (Ali, 2003; Nagai and Takano, 2004; Ali and Al-Moundhri, 2006). Cisplatin induced toxicity in human and experimental animals has been shown to be protected by prior treatment with various antioxidants such as ebseleen (Yoshida et al., 2000), Vitamin C (Antunes et al., 2000; Fatima et al., 2007) and selenium (Caffery and Frenkel, 2000; Antunes et al., 2001). However, none of these strategies were found to be suitable/safe for clinical practice.

GT consumption has been found to improve kidney function in animal models of renal failure (Yokozawa et al., 1996). 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). GT extract has also been found to offer significant protection from cisplatin induced oxidative damage in rat kidney and testes (Leena and Balaraman, 2005). 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. Exploration at the cellular level allows a better understanding of the underlying mechanisms regulating functions in normal and pathologic states.

Considering potential beneficial health effects of GT, we hypothesized that: *Green tea consumption would be able to prevent/reduce GM/CP induced nephrotoxic and other adverse effects in various rat tissues.*

To address this hypothesis, the present work was undertaken to study detailed biochemical events/cellular response/mechanisms of GM and CP induced nephrotoxic and other adverse effects in rat kidney, intestine and liver. The effect of GT consumption was determined to observe any protection provided against GM/CP nephrotoxicity for their long-term clinical use without harmful side effects. The specific objectives of the planned research included:-

**Part I**

Experiments were conducted to study the effect of green tea (GT) given to rats in the diet or drinking water as extract on various biochemical parameters in the serum and on the enzymes of carbohydrate metabolism, brush border membrane, lysosomes and oxidative stress in renal cortex, medulla, intestine and liver of normal rats.

**Part II**

Experiments were carried out to determine the effects of GM and GT alone and in combination on various serum biochemical parameters and on the enzymes of carbohydrate metabolism, BBM, lysosomes and antioxidant defense mechanism in the kidney tissues (cortex and medulla), intestine and liver. The effect of GM and GT was also determined on transport of $^{32}$Pi in BBMV isolated from renal cortex.

**Part III**

Experiments were carried out to determine the effects of CP and GT alone and in combination on various serum biochemical parameters and on the enzymes of carbohydrate metabolism, BBM, lysosomes and antioxidant defense mechanism in the kidney tissues (cortex and medulla), intestine and liver. The effect of CP and GT was also determined on transport of $^{32}$Pi in BBMV isolated from renal cortex.
The results of the present studies showed that GM and CP administration caused specific alterations in various serum parameters and in the enzyme activities of various systems in different tissues confirming nephrotoxic and other adverse toxic effects caused by GM/CP. The results further demonstrated that GT consumption largely prevented/ameliorated GM/CP induced alterations in various parameters. The studies would help us in furthering our understanding of GM/CP induced nephropathies and in possible prevention/protection by GT consumption for the safe use of GM, as an important antimicrobial and CP as antineoplastic agents.