CHAPTER 1

INTRODUCTION

General introduction

Cisplatin, *cis*-diamminedichloroplatinum (II), is a platinum complex consisting of two molecules of ammonia and two groups of chloride. Cisplatin is one of the most effective chemotherapeutic agents used for the treatment of several human malignancies including testicular, ovarian, bladder, cervical, head, neck, and small and non-small-cell lung cancers. The most common adverse effect of cisplatin is acute renal failure (ARF).

The oxidative mechanisms responsible for the ARF induced by cisplatin include 1) free radical generation, 2) antioxidant enzyme inhibition, and 3) renal tissue lipid peroxidation. Several antioxidants such as, vitamins C and E, and selenium have been reported to play a protective role against cisplatin-induced ARF. The aqueous extract of *Hibiscus sabdariffa* Linn. (HS), which previously demonstrated free radical scavenging and lipid peroxidation inhibiting properties in some *in vitro* and *in vivo* experiments, has not yet been shown to prevent cisplatin-induced ARF. However, owing to the nature of antioxidant compounds, it is possible that HS aqueous extract may exert both antioxidant and prooxidant effects depending on study circumstances. The aims of this study were to evaluate the protective effect of HS aqueous extract on cisplatin-induced kidney dysfunction, the optimal dosage

regimen and its relationship with renal protective effect, and the antilipid peroxidation on renal tissues.

Literature review

1. Oxidative stress

In the body, the disturbance of the balance between the produce of reactive oxygen species (ROS), including oxygen free radical and non-radical oxygen (Table 1.1), and antioxidant defenses against them produces oxidative stress which then amplifies tissue damage (Gutteridge, 1995). The lipids, proteins and nucleic acids are major targets of free radical damage. Peroxidation of lipids in cell membranes can disrupt their fluidity and permeability. Lipid peroxidation can also interfere the function of membrane bound proteins such as enzymes and receptors (Machlin and Bendich, 1987). Free radicals that directly attack proteins can interfere enzyme activity and cellular structure. Moreover, ROS can cause damages the chromosome and nucleic acids that may initiate the abnormal growth of cells or the DNA fragmentation. The precise site of tissue damage by free radicals is dependent on the tissue type and the reactive species involved. The overall damage caused by oxidative stress is often an accumulation of damage to many sites. Extensive damage can lead to cell death; this may be necrosis or apoptosis depending on the type of cellular damage.

Table 1.1 Examples of reactive oxygen species (ROS).

Oxygen free radical		Non-radical oxygen	
Alkoxyl radical	$RO^{\cdot}$	Hydrogen peroxide	H_2O_2
Hydroperoxyl radical	$HOO^{\cdot}$	Hypochlorous acid	HOCl
Hydroxyl radical	$HO^{\cdot}$	Peroxynitrite	$ONOO^{-}$
Nitric oxide radical	$NO^{\cdot}$	Ozone	O_3
Peroxyl radical	$ROO^{\cdot}$	Singlet oxygen	$^1O_2$
Superoxide anion radical	$O_2^{\cdot -}$		

1.1. Definition of free radical

A free radical is defined as “any atom or molecule which contains one or more unpaired electrons in the outer ring”. It can be any of the most chemically reactive molecules known that has a very strong tendency to pick up electrons from neighbouring molecules, which in turn converts those molecules into secondary free radicals. In living cell, $HO^{\cdot}$ is a principal actor in the toxicity of partially reduced oxygen species. It is very reactive with all kinds of biological macromolecules by abstracting a hydrogen atom that consequently initiates the formation of many more free radical species. The resulting secondary free radical is usually more stable and hence stays longer than the $HO^{\cdot}$. This self-propagating ability in producing more and more free radical is inevitably toxic to living cells (Gutteridge, 1995).

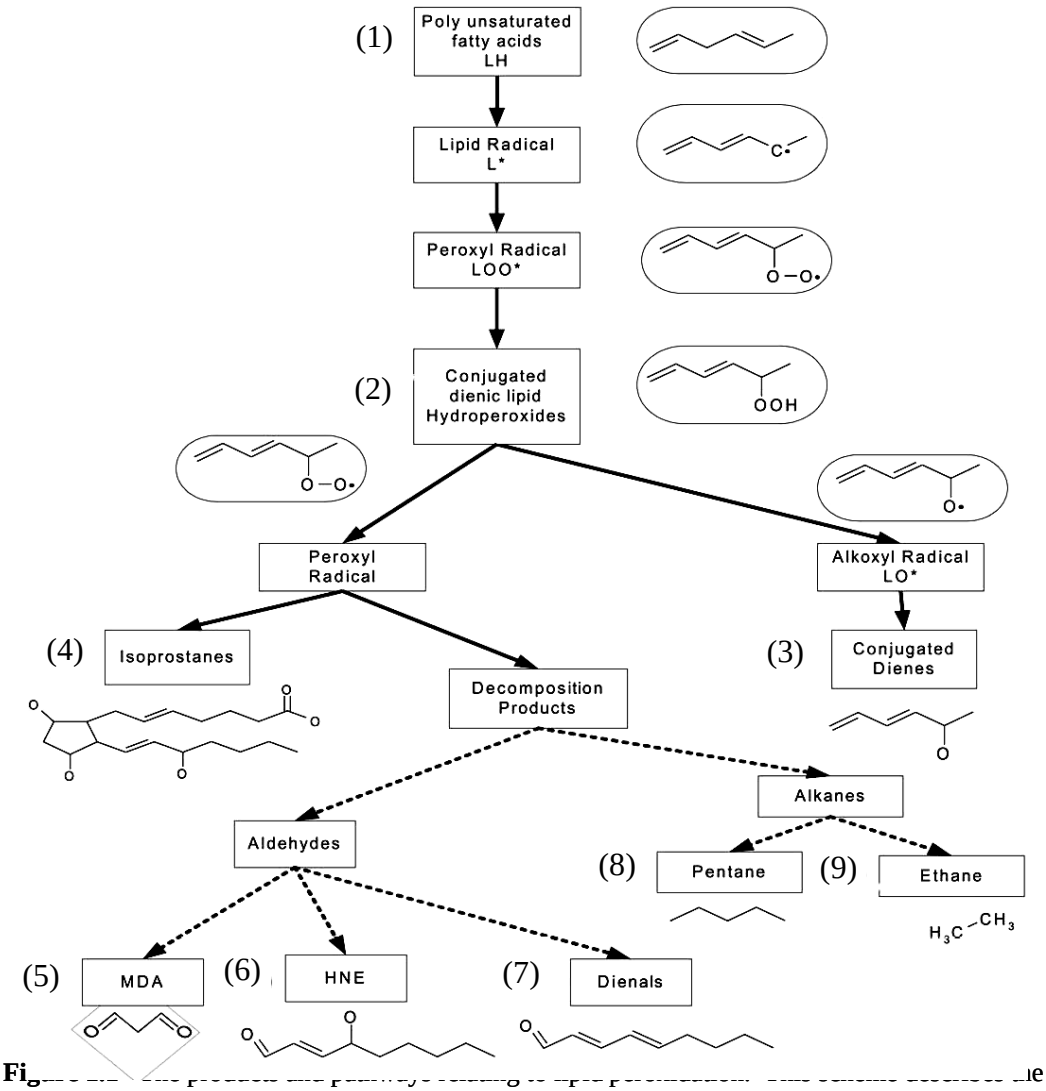
Free radicals can originate endogenously from normal metabolic reactions or exogenously from components of cigarette smoke and air pollutants.

Moreover, they can originate indirectly from the certain solvents, drugs, pesticides and radiation exposure (Gutteridge, 1995).

1.2. Malondialdehyde content as a marker of free radical damage cellular lipid

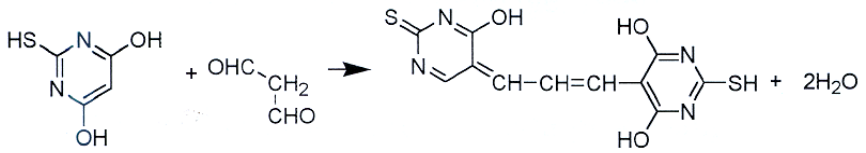
The levels of free radical are determined to identify the substances with prooxidative and antioxidative properties. Direct measurement of free radical formation in biological tissues is difficult, however, there are many indirect manifestations of oxidative stress. These include DNA and protein oxidation, lipid peroxidation, and a shift in the redox states of thiol/disulfide redox couples such as glutathione (GSH) and cysteine (McCall and Frei, 1999; de Zwart *et al*., 1999).

Polyunsaturated lipid molecules of the cell membrane are particularly susceptible to free radicals damage through lipid peroxidation processes. Consequently, lipid peroxidation is frequently used to indicate the involvement of free radical mediated disease (Gutteridge, 1995). As shown in Figure 1.1, the initial products of lipid peroxidation are conjugated dienic lipid hydroperoxides. These hydroperoxides can undergo decomposition either into various aldehyde products of different chain lengths or, if the original fatty acid is arachidonic acid, into isoprostanes. All these products are important biological evidences that can be used to assess the extent of free radical mediated cell damages. These include lipid hydroperoxide and a widely used end product malondialdehyde (MDA) (Dotan *et al*., 2004).



Fi[illegible] various products as well as several methods used to assess their concentrations. Polyunsaturated fatty acids (1). Lipid hydroperoxides (2) can be measured by the FOX assay, HPLC or iodometric assay. Conjugated dienes (3) are measured via UV spectroscopy (234 nm). Isoprostances (4) are created through cyclization of arachidonic acid and can be assessed by either GC/MS or immunological assays. MDA (5) concentrations are measured by variations on the TBARS assays, GC/MS or HPLC. HNE (6) is assessed by using HPLC. Dienals (7) are assessed through the use of spectroscopy. Alkanes (8,9) are usually measured as breath gases, using GC/MS. GC/MS = gas chromatography/mass spectrometry, HNE = 4-hydroxynonenal, MDA = malondialdehyde, TBARS = thiobarbituric acid-reactive substances. (Dotan *et al.*, 2004)

MDA is a highly reactive three carbon/dialdehyde produced from decomposition of lipid hydroperoxides. The concentrations of MDA are often determined either by combination of high-performance liquid chromatography (HPLC) and gas chromatography/mass spectrometry or by spectroscopic technique (Dotan *et al*., 2004). The spectroscopic technique is the most widely used according to the fact that the assay is inexpensive and easy to perform. This latter technique determine the concentrations of pink compound that produced by one molecule of MDA reacts stoichiometrically with two molecules of thiobarbituric acid (TBA) as shown in Figure 1.2 (Burdon and van Knippenberg, 1991).



2TBA MDA Pink colored product absorb light at 532 nm

Figure 1.2 The reaction of thiobarbituric acid (TBA) and malondialdehyde (MDA). (Burdon and van Knippenberg, 1991)

2. Induction of nephrotoxicity by cisplatin

2.1 General introduction to cisplatin

Cisplatin or *cis*-diamminedichloroplatinum (II) [$(NH_3)_2$ Cl_2 Pt] is a heavy metal (platinum) complex that consists of a central atom of platinum, two chloride atoms and two ammonia molecules (NH_3) in the *cis* position, as shown in Figure 1.3. The molecular weight of cisplatin is 300.1.

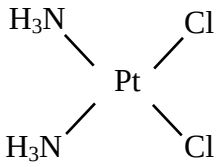


Figure 1.3 Structure of cisplatin or *cis*-diamminedichloroplatinum (II).

Cisplatin is an antineoplastic drug, administered to cancer patients intravenously (i.v.) as a sterile solution. It is widely used for the treatment of several human malignancies including ovarian, testicular, bladder, cervical, esophageal, head, neck, and small and non-small-cell lung cancers (Gonzalez *et al*., 2001). After intravenous administration, cisplatin is rapidly and extensively bound to several plasma proteins such as albumin, transferrin and gamma-globulin. Unionized form of cisplatin is relatively less reactive in the extracellular space where the concentration of chloride ions (Cl^-) is higher than in the intracellular space (Dale-Yates and McBrien, 1984). As shown in Figure 1.4, when cisplatin moves through the cell membrane (by either passive diffusion or active uptake by the cell), it dissolved in aqueous solution where water displaces its chloride ligands thus forming a positively charged platinum

complex, $[Pt(NH_3)_2(H_2O)_2]^{2+}$. This complex is the reactive form of the compound. Intact cisplatin and its metabolites are excreted primarily in the urine. A small amount of cisplatin is excreted via the bile and saliva (Gonzalez *et al*., 2001).

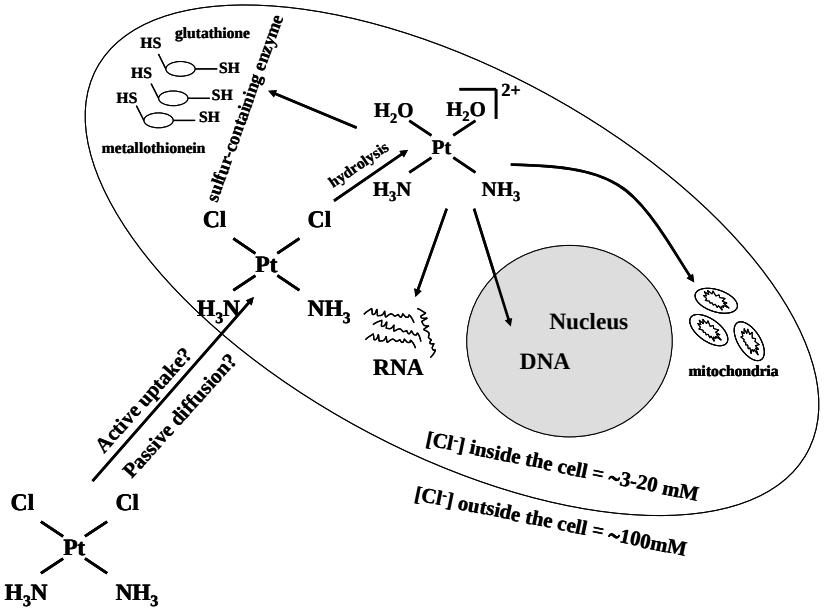


Figure 1.4 The cellular uptake of cisplatin and its targets. Cisplatin react with many cellular components that have nucleophilic sites such as DNA, RNA, proteins, membrane phospholipids and sulfur-containing enzymes. (Modified from http://www.chemcases.com/cisplat/cisplat12.htm)

2.2 Cellular mechanisms of action of cisplatin

Activated cisplatin $[Pt(NH_3)_2(H_2O)_2]^{2+}$ is a potent electrophile for subsequent interaction with nucleophilic groups on nucleic acids. Several types of cisplatin-DNA adducts can be formed such as intrastrand (most common adduct) and interstrand DNA cross-links and DNA-protein cross-links as shown in Figure 1.5 (Gonzalez *et al*., 2001). The mechanisms whereby these DNA adducts destroy cancer cells are not fully understood. However, the formation of these adducts may produce a several local distortion in the DNA double helix. The DNA then becomes unwind and

kinked, resulting in an inhibition of replication and transcription by blocking the action of DNA polymerase (Chu, 1994). These adducts would not be recognized by the repair machinery and thus, become more persistent. This process could interfere with the normal functions of the cell and possibly initiation of cell death. Although genomic DNA is generally accepted as the critical target of cisplatin mediated apoptosis, there are evidences that other cellular components that have nucleophilic sites may also be involved in the cytotoxicity of the cisplatin. These actions of cisplatin involve 1) binding to mitochondrial DNA causing the release of mitochondrial cytochrome c and caspase-3 activation and leading to apoptosis, 2) interacting with phospholipid and phosphatidylserine in cell membranes, 3) disrupting the cytoskeleton microfilaments and 4) affecting the polymerization of actin (Gonzalez *et al*., 2001).

Besides, the induction of apoptosis by cisplatin can happen via triggering two parallel death-response pathways, the p53 and p73 pathways (Gong *et al*., 1999).

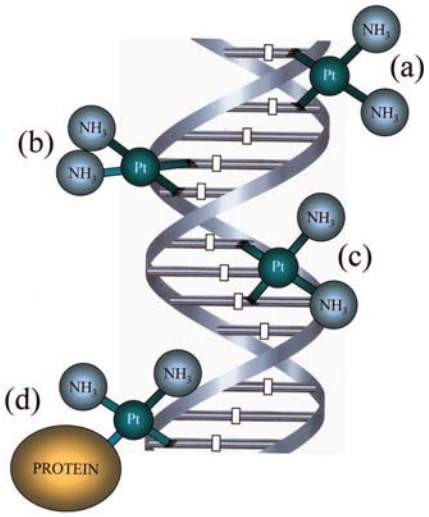


Figure 1.5 Main adducts formed in the interaction of cisplatin with DNA. (a), interstrand cross-link. (b), 1,2-intrastrand cross-link. (c), 1,3-intrastrand cross-link. (d), protein-DNA cross-link. (Gonzalez *et al*., 2001)

2.3 The mechanisms of cisplatin induced nephrotoxicity

Cisplatin has been used to treat various kinds of cancer. Its target is rapidly dividing cells. Unfortunately, cisplatin is a nonselective drug and can attack other types of rapidly dividing cells in the body such as cells in the gastrointestinal tract, hair follicles and bone marrow. For this reason, several adverse side effects of cisplatin including alopecia, gastrointestinal damage (nausea, vomiting and diarrhea), hematologic (anemia and myelosuppression), hepatotoxic, neurotoxic, nephrotoxic (acute or chronic renal failure), ocular toxic, ototoxic and serum electrolyte disturbance (hypocalcemia, hypokalemia, hypomagnesaemia, hyponatremia and hypophosphatemia) have been extensively reported.

The most common adverse effect limiting the use of cisplatin is acute renal failure (ARF) the toxicity may manifest after a single dose or may present with a chronic syndrome of renal electrolyte wasting in patients and various animals. ARF is referred to the sudden and usually reversible loss of renal function determined by the significant reduction of glomerular filtration rate (GFR) (< 50%) and increase in blood urea nitrogen (BUN) which is developed over a period of days or weeks (Rose and Rennke, 1994; Donald and Gerhard, 2000). Cisplatin accumulates in the kidneys to a greater degree when compared to other organs. The cisplatin-induced alterations in kidney functions and biochemical effects have been reported by several investigators.

In experimental animals, an injection of single dose of 5 mg/kg cisplatin caused a reduction in GFR while mean arterial blood pressure (MABP) and intratubular hydrostatic pressure were unaltered suggesting a reduction of renal blood flow (RBF). Winston and Safirstein (1985) reported that 72 hr after administration of cisplatin in Wistar rats, a reduction in whole kidney GFR (from 1.17 to 0.30 ml/min/g kw), RBF

(from 8.25 to 5.30 ml/min/g kw) and single nephron GFR (from 34.5 to 20.2 nl/min/g kw) were observed while intratubular hydrostatic pressure remained unchange. They also found that after volume expansion, whole kidney GFR, single nephron GFR and renal plasma flow (RPF) were increased but intratubular hydrostatic pressure was not different. From these results they suggested that cisplatin-induced GFR reduction is due to reversible changes in RBF and renal vascular resistance. A similar finding was reported by Matsushima *et al*. (1998) who observed that cisplatin treated in Sprague-Dawley rats showed a reduction in body weight (19%), RBF (from 5.91 to 4.67 ml/min) and GFR (from 1.33 to 0.06 ml/min) but an increase in renal vascular resistance (21%), urine volume (138%), serum creatinine (5 folds) and N-acetyl-β-D-glucosaminidase (NAG, a marker of tubular damage) excretion while MABP was unaltered. They also suggested that the mechanism of cisplatin-induced ARF mediated through a reduction of RBF.

Besides a reduction in RBF and GFR, defect of excretory function induced by single dose of 5-7.5 mg/kg cisplatin were reported. In addition, an elevation of kidney weight as a percent of body weight and a reduction of body weight, hematocrit and glutathione-S-transferase (GST) activity also observed. al-Harbi *et al*. (1995) found that administration of cisplatin at the dose of 7.5 mg/kg caused an elevation of BUN, serum creatinine and the kidney weight (as a percent of body weight) but a decrease in serum calcium and albumin. As the same dose of cisplatin Mansour *et al*. (2002) reported that 5 days after injection in Swiss albino rat resulted in an increase in kidney weight as a percent of body weight (77%), serum urea and creatinine, urine volume and urinary excretion of albumin and GST (2.5, 3.3, 2.4 and 5.5 folds of the control values, respectively). Moreover, a reduction in GST activity, serum albumin and urine creatinine and urea (55, 33, 67 and 77%, respectively) were

observed. Appenroth *et al*. (1997) observed that cisplatin treated (6 mg/kg) rat showed significant oliguria (detected from day 2-4 after cisplatin injection) and proteinuria (detected from day 1-5, being maximal on day 3 after cisplatin injection) and increase in BUN (occurred from day 3 to 6 after cisplatin injection). They also found that cisplatin injection caused a reduction of para-aminohippuric acid (PAH) accumulation in renal cortical slices of young (10-day-old) and adult (55-day-old) rats. Badary *et al*. (2005) observed that 5 days after administration of cisplatin (7 mg/kg) caused a reduction in body weight, hematocrit, creatinine clearance and GST activity (46%). They also found that although serum Na^+ and K^+ and urinary excretion of K^+ were not changed but urinary volume, urinary Na^+ excretion and the fractional excretion of Na^+ were increased (2, 3 and 13 folds of the control values, respectively). In addition, serum urea and creatinine were significantly increased 5 and 4 folds, respectively when compared to control values. Other investigators also reported that 3-7 days after cisplatin injection (5 mg/kg), a reduction of creatinine clearance (39-59%) and body weight (17-22%) and an elevation of serum creatinine (0.5-7 folds) and urinary volume (87-279%) were reported (Greggi Antunes *et al*., 2000; Greggi Antunes *et al*., 2001; Francescato *et al*., 2001; Mora *et al*., 2003; Shirwaikar *et al*., 2004).

Alterations in capacity of several active transport systems in renal epithelium which induced by single injection of cisplatin have been reported. Shiraishi *et al*. (2000b) observed that 2 days after injection of 5 mg/kg cisplatin in rats, the H^+-ATPase activity in brush-border membrane significantly decreased while the activity of Na^+/H^+ exchanger was unchanged. Kim *et al*. (1995) reported that injection of 4 mg/kg cisplatin in rabbit caused a decrease in Na^+-K^+-ATPase activity of renal cortical microsomes and basolateral membrane vesicles and the functional Na^+-pump

activity of renal cortical slices. In brush-border membrane vesicles of cisplatin treated rabbit showed a decrease in Na^+-dependent succinate and H^+-dependent tetraethylammonium (TEA) uptake but did not affect the Na^+-dependent uptake of glucose and L-glutamate. They also demonstrated that the ability to accumulate the organic anion PAH and organic cation TEA in renal cortical slices was significantly attenuated by cisplatin. Apart from previously reported this treatment also caused an increase in fractional excretion of Na^+ and K^+ and a decrease in urine flow (45%), GFR (87%), urine osmolarity, free-water reabsorption and urine to plasma creatinine ratio. In addition, glucosuria, phosphaturia and aminoaciduria were also observed. Their results suggested that cisplatin caused an impairment of proximal tubular reabsorptive function and the renal concentrating defect.

There are *in vitro* studies that indicated cisplatin-induced defect in renal cell energetics. Zhang and Lindup (1997) reported that cisplatin led to a time- and dose-dependent decrease in ATP and increase in ADP and AMP in rat renal cortical slices. These authors suggested that the depletion of ATP induced by cisplatin might result from an increase in catabolism of ATP to ADP and AMP. Furthermore, Kruidering *et al*. (1997) observed that incubation of porcine proximal tubular cells with cisplatin (50-500 μM) caused a decrease in activity of complexes I to IV (15-55%) of the mitochondria respiratory chain. This effect of cisplatin resulted in a 70% reduction of intracellular ATP.

Several reports showed the renal histological damages including glomerular and tubular damage after a bolus administration of 5-20 mg/kg cisplatin in experimental animals. In addition, morphological damage in proximal tubular cultures cells which incubated with cisplatin (8-800 μM) also observed. Microscopic

examination of rat renal tissue 5 days after cisplatin injection (7 mg/kg) revealed a remarkable proximal tubular necrosis with extensive epithelial vacuolization, swelling and tubular dilatation and less wall height of proximal tubules when compared to the control rat but no abnormality for the glomeruli histology (Yildirim *et al*., 2003). On the other hand, Sueishi *et al*. (2002) found glomeruli histological damage was found in rat renal section staining with periodic acid-Schiff reagents at the day 4 after 7.5 mg/kg cisplatin injection. In addition, the remarkable vacuolation, necrosis, desquamation of epithelial cell and protein cast in renal tubules were demonstrated. Shirwaikar *et al*. (2004) reported that the presence of peritubular and glomerular congestion, tubular casts, epithelial degeneration, interstitial edema, blood vessel congestion and infiltration by inflammatory cells were observed in rat renal (stained with haematoxylin and eosin) on the day 6 after 5 mg/kg cisplatin injection. Moreover, Choie *et al*. (1981) reported the tubular damages were localized in both proximal and distal tubules of rats with a maximal lesion on the day 7 after 6 mg/kg cisplatin injection. Mainly tubular damage was located in the corticomedullary region, where the platinum concentration was the highest within the kidney. Megyesi *et al*. (1998) used TUNEL technique was used to detect apoptotic cells in mice kidney tissue 3 days after 20 mg/kg cisplatin injection. They found that apoptotic nuclei were identifiable only in cells of the distal nephron, especially in distal convoluted tubules and collecting ducts. Shiraishi *et al*. (2000a) used another staining which performed by using an antibody to Na^{+}-K^{+}-ATPase alpha-1 subunit to localize the site of apoptotic nuclei in mice kidney tissue 3 days after 20 mg/kg cisplatin injection. This technique verified the apoptotic nuclei in both proximal and distal tubules. In addition, mice also exhibited red blood cell extravasation and loss of brush border. In primary cultures of

mouse proximal tubular cells that incubated with cisplatin showed a dose-dependent mechanism of cell death which high dose of cisplatin (800 μM) led to necrotic cell death over a few hours while much lower dose of cisplatin (8 μM) led to apoptosis over several days (Lieberthal *et al*., 1996). The characteristic of necrosis was a cytosolic swelling, early loss of plasma membrane integrity and a smear pattern of DNA due to random DNA degradation. While apoptotic was characterized by cell shrinkage, loss of attachment to the monolayer, nuclear chromatin became condensed and fragmented and a ladder pattern of DNA resulting from internucleosomal DNA cleavage.

The mechanisms underlying the cisplatin-induced ARF have not been fully understood. However, several evidences suggested that oxidative stress may play an important pathogenesis role. Masuda *et al*. (1994) observed the dose dependent generation of $O_2^{\cdot -}$ by cisplatin as measured by chemiluminescence from a Cypridina luciferin analog (CLA) in the cell free system. Moreover, addition of superoxide dismutase (SOD), ascorbic acid and $O_2^{\cdot -}$ and $HO^{\cdot}$ scavengers inhibit intensity of CLA induced by cisplatin, suggesting that cisplatin produced $HO^{\cdot}$ and $O_2^{\cdot -}$ in cell free system. Kruidering *et al*. (1997) reported that exposure of the porcine proximal tubular cells to cisplatin (5-100 μM) led to a time- and concentration-dependent generation of reactive oxygen species as determined by the fluorescent probe Dih 123 with flow cytometry. Baliga *et al*. (1998) found that incubation of renal proximal tubular epithelial (LLC-PK1) cells with cisplatin resulted in a marked generation of $HO^{\cdot}$ and an increase in iron capable of catalyzing free radical reactions (as measured by bleomycin-detectable iron) released into medium. Furthermore, the kidney of rat treated with cisplatin also found a significant increase in iron capable of catalyzing free radical reactions. In addition, Liu and Baliga (2003) reported that iron capable of catalyzing free radical reaction was also

markedly increased in the kidney of mice treated with 10 mg/kg cisplatin. Direct exposure of mice kidney slice to 2 mM cisplatin caused an increase in H_2O_2 and $HO^{\cdot}$ formation (as measured by chemiluminescence from a 2′,7′-dichlorofluorescin and deoxyribose degradation method, respectively) and an increase in bleomycin-detectable iron. Similar results were observed in the microsomes isolated from kidney of mice which incubation with cisplatin (200 μg/ml).

Several investigators have hypothesized the mechanism of cisplatin-induced nephrotoxicity is related to depletion of the antioxidant defense system. Levels of the antioxidants GSH, bilirubin albumin, vitamin A, C and E and β-carotene significantly decreased when compared to control values. On the other hand, an increase in renal GSH level also observed in cisplatin treated (5 mg/kg) rat. These finding may proposed that under oxidative stress condition induced by cisplatin there may be positive regulation in the GSH biosynthesis, resulting in the increased level of renal GSH. Weijl *et al.* (1998) found that plasma antioxidant levels were decreased in 36 cancer patients treated with cisplatin. Eight to 15 days after the start of cisplatin infusion, concentration of vitamin C and E, uric acid and ceruloplasmin levels fell significantly and three weeks after the start treatment, levels of the antioxidants bilirubin albumin and the ratio vitamin E/cholesterol + triglycerides also significantly decreased compared to pretreatment levels. In Wistar rat, Silva *et al.* (2001) reported that 24 hr after administration of cisplatin at the dose of 5 mg/kg resulted in a 29% depletion of GSH level. While Greggi Antunes *et al.* (2000) found that 7 days after injection of 5 mg/kg cisplatin caused a 21% decrease in GSH level. Furthermore, the cisplatin treatment (6 mg/kg, 5 days after injection) led to a reduction in renal vitamin A and E, GSH and β-carotene levels (22, 25, 16 and 16%, respectively) in female

Wistar rat (Naziroglu *et al*., 2004). Five days after treatment with 7 mg/kg cisplatin, renal cortex GSH level in Wistar rat was decreased by 33% when compared to control value (Badary *et al*., 2005) while in Sprague-Dawley rat showed a 16% depletion of renal GSH level (Atessahin *et al*., 2005). Administration of cisplatin at the dose of 7.5 mg/kg resulted in a 20% decrease in renal GSH level 3 days after cisplatin injection (Sueishi *et al*., 2002) while 5 days after injection showed a 22% reduction of renal GSH level (Mansour *et al*., 2002). Additionally, incubation of rat renal cortical slice with cisplatin (2 mM for 60 min) resulted in a 30% depletion of GSH level (Zhang and Lindup, 1993). On the other hand, Francescato *et al.* (2001) reported that 7 days after cisplatin administration as the dose of 5 mg/kg, an elevation of renal GSH level (21%) was observed. This finding was supported by Mora *et al.* (2003) which showed an increase in renal GSH level by 29 and 55%, 24 hr and 7 days after cisplatin injection (5 mg/kg), respectively when compared to the control value.

Another possible mechanism of nephrotoxicity that induced by cisplatin is related to inhibitory effect of cisplatin on antioxidant enzymes activities. Yildirim *et al*. (2003) observed that catalase (CAT), glutathione peroxidase (GSH-Px) and SOD activities in the rat kidney decreased 33, 43 and 25% of control, respectively 5 days after 7 mg/kg cisplatin injection. As the same duration and concentration of cisplatin treatment, Badary *et al*. (2005) reported that administration of cisplatin induced significant decrease in the rat renal CAT, GSH-Px and SOD activities (70, 65 and 55% of control, respectively). In Sprague-Dawley rat, 5 days after cisplatin injection (7 mg/kg) showed a significant decrease in CAT activity (76%) and GSH-Px activity (34%) as compared to control group (Atessahin *et al*., 2005). Mansour *et* al., 2002 reported that 5 days after administration of 7.5 mg/kg cisplatin in Swiss albino rat

caused a significant decrease in renal CAT and GSH-Px activity (41 and 32%, respectively) in comparison to control value. The decreased SOD activity is insufficient to scavenge the $O_2^{\cdot-}$ produced during the normal metabolic process. The decreased CAT and GSH-Px activity resulted in a decrease the ability of the kidney to scavenge H_2O_2 and lipid peroxides.

The increase in free radical generation and the decrease in antioxidant defense system may result in an increase in renal lipid peroxidation and MDA production in renal tissue. Between 24 hr to 7 days after cisplatin injection (5-7.5 mg/kg) in rat, an increase in renal MDA level (29-150% of control values) was observed. In addition, in renal cortical slice which incubated with cisplatin (0.5-3 mM) also showed an elevation of renal MDA level (2.0-3.2 folds of control values). Several investigators have been shown that administration of cisplatin at the dose of 5 mg/kg in rat caused an elevation in MDA level by 29-33% 24 hr after treatment (Silva *et al*., 2001; Mora *et al*., 2003), by 51% 3 days after treatment (Greggi Antunes *et al*., 2001), by 35% 5 days after treatment (Matsushima *et al*., 1998) and by 39-58% 7 days after treatment when compared to the control value (Greggi Antunes *et al*., 2000; Francescato *et al*., 2001; Mora *et al*., 2003). Naziroglu *et al*. (2004) observed that cisplatin treated (6 mg/kg, 5 days after injection) in female Wistar rat showed a rising of renal MDA level (27% of control). While Yildirim *et al*. (2003), Atessahin *et al*. (2005) and Badary *et al*. (2005) reported that 5 days after injection of cisplatin at the dose of 7 mg/kg in rat resulted in an increase in renal MDA level by 13, 159 and 150%, respectively when compared to respective control. In addition, Mansour *et al*. (2002) found that renal MDA level in Swiss albino rat increased 54% of control 5 days after 7.5 mg/kg cisplatin injection. Marked increase MDA formation also observed in *in*

vitro study. Direct incubation of rat renal cortical slices with cisplatin (2 mM for 120 min) resulted in a 2 fold elevation of MDA level (Zhang and Lindup, 1993). Furthermore, Kim *et al*. (1997) reported that exposure of the rabbit renal cortical slices to cisplatin at the doses of 0.5-3 mM led to a dose-dependent formation of MDA (about 500-650 pmol/mg protein).

These evidences may indicate the possible role of antioxidants and free radical scavengers in protecting against cisplatin-induced ARF. It has been reported that several antioxidants and free radical scavengers can protect renal tissues against cisplatin-induced oxidative damage as summarized in Table 1.2.

Table 1.2 Antioxidants and free radical scavengers that are able to protect against the renal oxidative damage induced by cisplatin.

Antioxidants and dose of cisplatin administered	**Protective effect**	**References**
Aminoguanidine (peroxynitrite scavenger) 100 mg/kg + cisplatin 7.5 mg/kg	Decreased serum urea and creatinine concentrations, urine volume, urinary excretion of albumin and GST, renal MDA level and kidney weight and increased GST and GSH-Px activities in the kidney of Swiss albino rats	Mansour *et al*., 2002
Bixin 2.5 or 5 mg/kg + cisplatin 5 mg/kg	Reduced cisplatin-induced abnormal metaphases and total number of chromosome aberrations, inhibited an increase in lipid peroxidation and renal GSH depletion induced by cisplatin in Wistar rats	Silva *et al*., 2001
Catalase 500 U/ml and pyruvate (hydrogen peroxide scavenger) 10 mM + cisplatin 2 mM	Partially prevented necrotic cell death in primary culture of rabbit proximal tubules	Baek *et al*., 2003
Deferoxamine 1 mM (iron chelator) + cisplatin 25 and 75 μM	Completely prevented generation of ROS as determined by the fluorescence probe Dih 123 in porcine proximal tubular cells	Kruidering *et al*., 1997
Dimethylthiourea (hydroxyl radical scavenger) 500 + 125 mg/kg + cisplatin 5 mg/kg	Associated with less accumulation of MDA, less tubular damage and enhanced expression of proliferating cell nuclear antigen in the damaged tubular cells	Matsushima *et al*., 1998

Table 1.2 Antioxidants and free radical scavengers that are able to protect against the renal oxidative damage induced by cisplatin. (continued)

Antioxidants and dose of cisplatin administered	**Protective effect**	**References**
Dimethylthiourea (hydroxyl radical scavenger) 500 + 125 mg/kg + cisplatin 5 mg/kg	Inhibited cytochrome c release from mitochondria and caspase-3 activation in M_1[17] and LLC-PK1 cells, prevented increase in serum creatinine level and fractional excretion of Na^+ in New Zealand white rabbits	Baek *et al*., 2003
30 mM + cisplatin 50 μM and 2 mM	Inhibited apoptotic cell death in primary culture of rabbit proximal tubules	
Edavarone 1-10 mg/kg + cisplatin 5-10 mg/kg	Reversed the cisplatin-induced elevation of BUN and serum creatinine and morphological changes, including vacuolation, necrosis and protein casts in Wistar rats	Sueishi *et al*., 2002
Erdostein 10 mg/kg + cisplatin 7 mg/kg	Reduced depletion in the tissue CAT, GSH-Px and SOD activities, attenuated increase in plasma creatinine, BUN, tissue MDA, nitric oxide levels and provided a histologically-proven protection against cisplatin-induced ARF in Wistar albino rats	Yildirim *et al*., 2003
Isoeugenol 10 mg/kg + cisplatin 3 mg/kg	Prevented body weight reduction and BUN and serum creatinine elevation in rats	Rao *et al*., 1999
Lecithinized superoxide dismutase (superoxide anion scavenger) 3000 U/kg + cisplatin 5 mg/kg	Attenuated the increase in serum creatinine, preservation of RBF and increased urinary cyclic guanosine monophosphate (cGMP) excretion in Sprague-Dawley rats	Matsushima *et al*., 1998

Table 1.2 Antioxidants and free radical scavengers that are able to protect against the renal oxidative damage induced by cisplatin. (continued)

Antioxidants and dose of cisplatin administered	**Protective effect**	**References**
Lycopene 4 mg/kg + cisplatin 7 mg/kg	Decreased plasma creatinine and urea, provided marked normalization in kidney tissue MDA and GSH concentration, increased CAT activity, reduced tubular necrosis in Sprague-Dawley rats	Atessahin *et al*., 2005
Naringenin 20 mg/kg + cisplatin 7 mg/kg	Reduced serum urea and creatinine concentrations, polyuria, body weight loss, urinary fractional excretion of Na^+ and GST activity, increased creatinine clearance, SOD, GSH-Px and CAT activities, improved alteration in renal lipid peroxidation and GST activity in Wistar rats	Badary *et al*., 2005
Diphenyl-*p*-phenylenediamine 20 μM + cisplatin 25 and 75 μM	Completely prevented generation of ROS as determined by the fluorescence probe Dih 123 in porcine proximal tubular cells	Kruidering *et al*., 1997
Salviae radix extract 0.05% + cisplatin 5 mg/kg	Decreased lipid peroxidation, serum creatinine levels, fractional excretion of Na^+ and renal morphological changes and increased GFR in rabbits, reduced lactate dehydrogenase release and lipid peroxidation and increased PAH uptake in renal cortical slices	Jeong *et al*., 2001
Selenium (sodium selenite) 2 mg/kg + cisplatin 15-25 mmol/kg	Reduced BUN and plasma creatinine levels in mice	Satoh *et al*., 1992

Table 1.2 Antioxidants and free radical scavengers that are able to protect against the renal oxidative damage induced by cisplatin. (continued)

Antioxidants and dose of cisplatin administered	**Protective effect**	**References**
Selenium (sodium selenite) 2 mg/kg + cisplatin 5 mg/kg	Decreased the effect of cisplatin on creatinine, renal MDA levels and kidney necrosis in Wistar rats	Francescato *et al*., 2001
1.5 mg/kg + cisplatin 6 mg/kg	Increased renal GSH-Px activity and β-carotene, vitamin E and GSH levels, decreased MDA levels in Wistar rats	Naziroglu *et al*., 2004
Sodium benzoate (hydroxyl radical scavenger) 600 + 300 mg/kg + cisplatin 5 mg/kg	Attenuated tubular damage, as documented by the histologic examination and the measurement of urinary NAG isoenzyme B activity excretion	Matsushima *et al*., 1998
Vitamin C 2.5 g/kg + cisplatin 6 mg/kg	Reduced BUN and urinary protein excretion in Wistar rats	Appenroth *et al*., 1997
50, 100 and 200 mg/kg + cisplatin 5 mg/kg	Decreased serum creatinine levels and increased GSH levels and creatinine clearance	Greggi Antunes *et al*., 2000
Vitamin E 1 g/kg + cisplatin 6 mg/kg	Increased vitamin E concentration in kidney and decreased urinary volume, urinary protein excretion and BUN in Wistar rats	Appenroth *et al*., 1997
1 g/kg + cisplatin 6 mg/kg	Increased renal GSH-Px activity and β-carotene, vitamin E and GSH levels, decreased MDA levels in Wistar rats	Naziroglu *et al*., 2004

3. Pharmacological effects of *Hibiscus sabdariffa* Linn.

3.1 Botanical description and localization

Hibiscus sabdariffa Linn. (HS) is a member of the Malvaceae family and commonly named as karkade (Arabic), l'oiselle (French), roselle, sorrel, Jamaica sorrel, red sorrel (English) and Spanish (Jamaica). The plant is widely distributed both in tropical and temperate regions. HS is an erect annual shrub that grows up to 180 cm tall or more. Leaves are dark green to red, palmately divided into 3-7 lobes, with serrate margins and long petiolate as shown in Figure 1.6. Flowers are white to yellow with reddish centre at the base of the staminal column. Seedpods are enclosed in their maturity enlargement red calyces.

(a)

(b)

Figure 1.6 Leaves, calyces (a) and flower (b) of *Hibiscus sabdariffa* Linn.

3.2 Traditional uses

HS was used in folk medicine as antiseptic, aphrodisiac, astringent, resolvent, cholagogue, digestive, diuretic, stomachic and a remedy for abscesses, heart ailments and hypertension (Perry, 1980). In Thailand, HS is one of the folk medicinal plants which used to lower blood pressure and plasma lipid, as diuretics and for gallstone treatment (Mernwongyard, 1991). Other traditional uses are used to help body refreshment, bile improvement, fever relief, cough cure and thirsty relief (Chaichit, 2004).

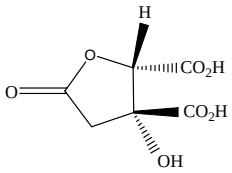
3.3 Chemical constituents of *Hibiscus sabdariffa* Linn. calyces

The HS extract is considered to be a valuable natural substance and may be mainly responsible for the plant biological and pharmacological effects. The chemical constituents of HS calyces are summarized in Table 1.3. The structures of major compounds are shown in Figure 1.7.

Table 1.3 Chemical constituents of *Hibiscus sabdariffa* Linn. calyces.

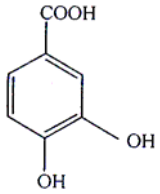
Chemical constituents	Detection method	References
Total anthocyanins (amount)		
(2.5%)	Spectrophotometer method	Chen *et al*., 2003
(0.96 g/kg)	Colorimetric method	Herrera-Arellano *et al*., 2004
(57 mg/kg)	-	Falade *et al*., 2005
(14.74 g/kg)	HPLC	Frank *et al*., 2005
Delphinidin-3-glucoside (0.3 g/kg)	HPLC	Frank *et al*., 2005
Delphinidin-3-sambubioside (8.16 g/kg)	HPLC	Frank *et al*., 2005
Cyaniding-3- glucoside (18 mg/kg)	HPLC	Frank *et al*., 2005
Cyaniding-3- sambubioside (6.26 g/kg)	HPLC	Frank *et al*., 2005
Delphinidin-3-sambubioside	HPLC	Hou *et al*., 2005
L-ascorbic acid	-	Oboh and Elusiyan, 2004
(625 mg/kg)	2,4-di nitro phenyl hydrazine	Falade *et al*., 2005
Total polyphenolic acid (1.7%)	Folin-Ciocalteau method	Chen *et al*., 2003
Total flavonoids (1.43%)	Spectrophotometer method	Chen *et al*., 2003
Protocatechuic acid	-	Liu *et al*., 2005
Hibiscus acid and its 6-methyl ester	HPLC	Hansawasdi *et al*., 2000
Aluminum, chromium, copper, iron, manganese and nickel	Electrothermal atomic absorption spectrometry	Wrobel *et al*., 2000
Zinc, sodium and calcium	-	Oboh and Elusiyan, 2004
Calcium (9.7 g/kg), iron (177 mg/kg), magnesium (2.2 g/kg) and zinc (28 mg/kg)	Atomic absorption spectrophotometry	Falade *et al*., 2005

Organic acid



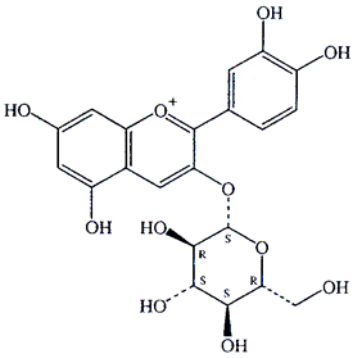
Hibiscus acid

Phenolic acid

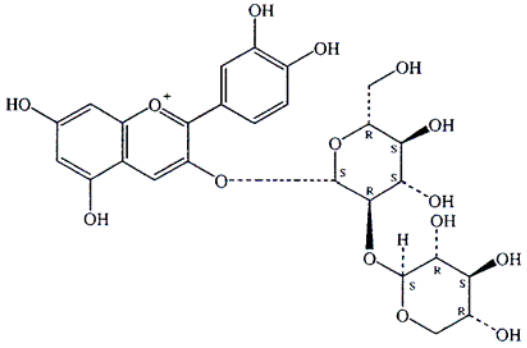


Protocatechuic acid

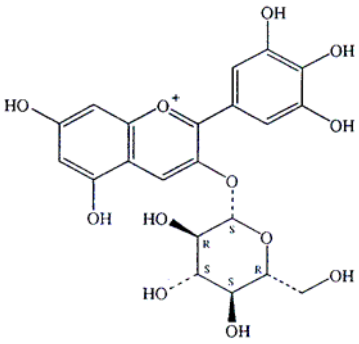
Anthocyanins



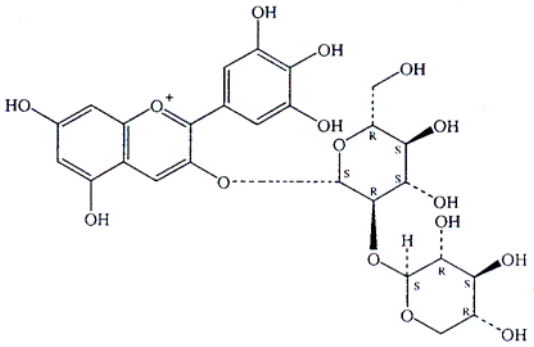
Cyanidin-3-glucoside



Cyanidin-3-sambubioside



Delphinidin-3-glucoside



Delphinidin-3-sambubioside

Figure 1.7 Structures of the major chemical constituents (organic and phenolic acid and anthocyanins) from *Hibiscus sabdariffa* Linn. calyces.

3.1 Effects of *Hibiscus sabdariffa* Linn. extracts on arterial blood pressure and renal functions

It has been reported that HS extract possesses hypotensive and antihypertensive effects and the possible mechanisms that could contribute to these effects have been widely discussed. Adegunloye *et al*. (1996) reported that the hypotensive effect after intravenous injection of HS calyces aqueous extract in anesthetized rats occur in a dose-dependent manner. They also investigated the mechanisms of hypotensive effect by sectioning the left and right vagi nerves, blockade with atropine (cholinergic blocker), cimetidine and promethazine (histaminergic blocker) and bilateral carotid occlusion. It is suggested that the hypotensive effect of HS extract did not appear to be mediated via inhibition of sympathetic nervous system but it could be mediated through acetylcholine-like and histamine-like mechanisms as well as via direct vaso-relaxant effects.

As reported in clinical studies, the administration of two spoonfuls of HS dry calyces as tea preparation in one glass of boiled water, daily for twelve days, in essential hypertensive patients showed a 11% decrease in systolic and diastolic blood pressure (Haji Faraji and Haji Tarkhani, 1999). Another supporting report indicated the antihypertensive activity of the aqueous extract of HS calyces in patients with mild to moderate hypertension (systolic reduction from 139 to 124 mmHg and diastolic reduction from 91 to 80 mmHg) when given daily at the dose of 10 g/0.5 l of boiling water for four weeks (Herrera-Arellano *et al*., 2004). The hypotension caused by HS may happen via either diuretic or vasodilatory effect or both as suggested by these authors.

While antihypertensive effect of HS calyces aqueous extract was observed in spontaneously hypertensive rat (SHR), its toxicity was also reported (Onyenekwe *et al*., 1999). Administration at the doses of 0.5 and 1 g/kg (gavage for 21 days) resulted in a reduction in systolic blood pressure from 153 to 135 mmHg and diastolic blood pressure from 91 to 70 mmHg measuring by tail cuff method. The higher dose (1 g/kg) caused death in SHR. In normotensive Wistar-Kyoto rats, administration of a similar dose of HS decreased systolic and diastolic blood pressure (from 132 to 125 mmHg and from 75 to 66 mmHg, respectively). They also found that continuous administration of this extract for eight weeks lead to a significant decrease in serum creatinine, cholesterol and glucose level but increase in serum uric acid level and urine output in both SHR and normotensive rats. Mojiminiyi *et al*. (2007) also reported that intravenous injection of the aqueous extract of HS calyces at the doses of 1-125 mg/kg also caused a dose-dependent reduction in mean arterial blood pressure in normotensive rats (reduction from 97 to 76 mmHg at the dose of 1 mg/kg), salt-induced hypertensive rats (reduction from 142 to 70 mmHg at the dose of 5 mg/kg) and L-NAME-induced hypertensive rats (reduction from 187 to 105 mmHg at the dose of 1 mg/kg). In addition, they reported that this extract showed the dose dependently lowered heart rate in all experimental models suggesting the negative chronotropic effect may be one of the mechanism(s) in lowering blood pressure.

Another antihypertensive mechanism of HS extract could happen via its antioxidative effect. Odigie *et al*. (2003) reported that administration of HS petals aqueous extract in drinking water at the dose of 250 mg/kg for eight weeks to 2-kidney,1-clip renovascular hypertensive rat resulted in significantly reduced systolic and diastolic blood pressure (from 157 to 128 mmHg and from 146 to 100 mmHg,

respectively). Since the pathogenesis of elevated blood pressure in 2-kidney,1-clip renovascular hypertensive rats is associated with the increased $O_2^{\cdot -}$ (measured by using lucigenin-enhanced chemiluminescence and electron spin resonance spectroscopy), thus this increased $O_2^{\cdot -}$ may subsequently induce an impairment of vascular response to nitrovasodilators (Heitzer *et al*., 1999).

The mechanisms of antihypertension of methanolic extracts of HS calyces have also been evaluated *in vitro*. Ajay *et al*. (2007) examined this effect in pre-contracted (using high K^+ and phenylephrine) endothelium-intact and endothelium-denude aortic rings isolated from SHR by blockade with atropine, L-NAME, methylene blue, indomethacin, acetylcholine and sodium nitroprusside. They concluded that the antihypertensive effect of HS extract is mediated through activation of endothelium-derived nitric oxide/cGMP-relaxant pathway and inhibition of calcium influx through receptor-gated channels. In addition, hydroalcoholic extract of HS calyces has been shown to inhibit angiotensin converting enzyme *in vitro* (Jonadet *et al*., 1990). This may cause a decrease in total vascular resistance and a reduction in arterial blood pressure.

The natriuretic mechanism of HS extract may be responsible for its antihypertensive effect. In patients with mild to moderate hypertension that treated with the aqueous extract from the dried calyces of HS at the dose of 10 g/0.5 l of boiled water for four weeks showed an increase in urinary excretion of Na^+ without substantially modifying urinary pH, K^+ and Cl^- (Herrera-Arellano *et al*., 2004).

The effect of HS extracts on urinary and serum chemical composition also investigated in human and experimental animals suggesting the renal functional alterations and toxicity. In healthy men, after ingestion of HS boiled water extract resulted in a significant decreases in the urinary excretion of creatinine, uric acid, citrate, tartrate, calcium, Na^+, K^+ and phosphate but not oxalate (Kirdpon *et al*., 1994). The aqueous extract from the dried HS calyces were dissolved in the Wistar albino rat's drinking water at the concentrations of 1-4.6 g/kg for twelve weeks. It was showed that serum K^+, Na^+, Cl^- and HCO_3^- levels was decreased but serum urea and creatinine levels was increased significantly (Orisakwe *et al*., 2003). The difference in urinary excretion response after treatments of HS extract may be due to the doses and duration of administration.

3.2 Antioxidative effect of *Hibiscus sabdariffa* Linn. extracts

Many investigations have reported an additional role of HS extracts act as antioxidant in both *in vitro* and *in vivo* systems. *In vitro* study, HS dried flower extract showed the strongest inhibitory effect on xanthine oxidase activity (Tseng *et al*., 1997) and *tert*-butylhydroperoxide (t-BHP) -induced MDA formation in the rat primary hepatocyte cultures (Tseng *et al*., 1996). Hirunpanich *et al*. (2005) reported that the aqueous extract of HS dried calyces at concentrations ranging from 0.1 to 5 mg/ml inhibits the formation of conjugated dienes and TBARs (represents early and late stage in the oxidative process, respectively) in $CuSO_4$-induced LDL oxidation. Duh and Yen (1997) observed that this extract also showed antioxidative activity in both linoleic acid and the liposome model systems, indicating that the extract may protect the cell from damage by lipid peroxidation. Furthermore, this antioxidative effect can also protect

human erythocytes against lipid peroxidation (Suboh *et al*., 2004). Farombi and Fakoya (2005) revealed that chloroform (HSCF) and ethyl acetate soluble fractions (HSEA) of the ethanolic extract of HS dried flowers possess strong free radical scavenging effects on active oxygen species. These two fractions of HS extract were better scavengers of $O_2^{\cdot-}$, $HO^{\cdot}$ and H_2O_2 as compared to butylated hydroxytoluene, quercetin and α-tocopherol. HSCF and HSEA at the concentration of 100 μg/ml exhibited 80 and 89% inhibitory effects on CCl_4-NADPH-induced lipid peroxidation, respectively. In addition, pretreatment with HS extract orally at the concentrations of 100 and 250 mg/kg simultaneously with intraperitoneal injection of $FeCl_2$-ascorbic acid-ADP mixture reduced the formation of rat plasma MDA content.

The *in vivo* studies have been shown to confirm the antioxidant effect of HS extract. Hirunpanich *et al*. (2006) showed that in rat treatment with 0.25, 0.5 and 1 g/kg of the HS dried calyces aqueous extract for six weeks significantly reduced the formation of TBARs and conjugated dienes in $CuSO_4$-induced LDL oxidation. Amin and Hamza (2006) reported that administration of HS dried flower ethanolic extract (1 g/kg/day) for 26 days in cisplatin-induced reproductive toxicity rats caused a reduction in the extent of cisplatin (10 mg/kg)-induced sperm abnormality, enhanced sperm motility (from 22 to 71%) and reversed testicular GSH reduced (from 54.8 to 67.7 nmol/mg protein), SOD (from 0.77 to 1.86 nmol/mg protein), CAT (from 45.9 to 67.4 nmol/mg protein) and MDA (from 2.70 to 1.91 nmol/mg protein) to control levels. These authors suggested that the protective effect of this extract may mediated by their antioxidative activity.

It is possible that the antioxidative effect of HS may depend on the bioavailability of its chemical constituents, anthocyanins and protocatechuic acid.

Tsai *et al*. (2002) showed antioxidative capacity of anthocyanin and brown pigment in the HS dried petals extract elucidating by comparing absorbance with ferric reducing ability of plasma antioxidant assay. Their results revealed that the anthocyanin and brown pigment account for 51 and 24% of the antioxidative capacity, respectively. Wang *et al*. (2000) reported that the anthocyanin isolated from dried flowers of HS at the concentrations of 0.1 and 0.2 mg/ml significantly decreased the leakage of lactate dehydrogenase and the formation of MDA induced by t-BHP in rat primary hepatocyte cultures. Besides, protocatechuic acid that extracted from the dried flowers of HS also has antioxidative characteristics. Lee *et al*. (2002) observed that protocatechuic acid at the doses of 0.1, 0.5 and 1 mM possessed strong potency to inhibit oxidative LDL (as measure by relative electrophoretic mobility and TBARs) induced by copper or a nitric oxide donor.

3.4 Other effects of *Hibiscus sabdariffa* Linn. extracts

Other biological and pharmacological effects of HS extracts apart from those previously mentioned are listed in Table 1.4.

Table 1.4 Other biological and pharmacological effects of *Hibiscus sabdariffa* Linn.

Effects	References
Anti-analgesic and antipyretic activities	Dafallah and al-Mustafa, 1996
Anti-atherosclerotic effect	Lee *et al*., 2002 Chen *et al*., 2003
Antibacterial activity	Oboh and Elusiyan, 2004 Liu *et al*., 2005
Anti-clastogenic effect	Adetutu *et al*., 2004
Anti-hepatoma activity	Lin *et al*., 2002
Anti-inflammation and against ulceration	Cluzel *et al*., 2002
Antimutagenic effect	Chewonarin *et al*., 1999 Farombi and Fakoya, 2005
Antispasmodic effect	Ali *et al*., 1991
Antitumor	Tseng *et al*., 1998 Chang *et al*., 2005 Hou *et al*., 2005 Lin *et al*., 2005
Constipating agent, reducing the intensity of diarrhea (inhibitor of intestinal motility)	Salah *et al*., 2002 Owulade *et al*., 2004

Table 1.4 Other biological and pharmacological effects of *Hibiscus sabdariffa* Linn. (continued)

Effects	References
Enzyme inhibitor	
- α-chymotrypsin and trypsin	Abu-Tarboush and Ahmed, 1996
-porcrine pancreatic α-amylase	Hansawasdi *et al*., 2000 Hansawasdi *et al*., 2001
Hepatoprotective effect	Tseng *et al*., 1997 Wang *et al*., 2000 Ali *et al*., 2003 Lin *et al*., 2003 Amin and Hamza, 2005 Liu *et al*., 2006
Adipogenesis blocker	Kim *et al*., 2003
Hypolipidemic effect	el-Saadany *et al*., 1991 Chen *et al*., 2003 Hirunpanich *et al*., 2006
Stimulate proliferation and differentiation of human keratinocytes	Brunold *et al*., 2004

Hypothesis

According to the antioxidative property of HS calyces water extract reported previously, it is hypothesized that this particular effect may attenuate the acute renal failure induced by cisplatin.

Objectives

To investigate the protective effects of either short or long term oral administration of *Hibiscus sabdariffa* Linn. calyces water extract on renal lipid peroxidation by determining renal malondialdehyde level and renal functions including BUN, glomerular filtration rate, effective renal plasma flow, Na^+ and K^+ excretion using clearance technique in cisplatin-induced acute renal failure rats.