CHAPTER 4

DISCUSSION

The results found in this study supported the hypothesis in which the antioxidative effect of HSE could attenuate the acute renal failure caused by cisplatin. The impairment of kidney function induced by cisplatin is recognized as the main side effect and the most important dose-limiting factor associated with its clinical use. In the present study, three days after the i.p. bolus injection of cisplatin at the two low doses (4.5 and 6 mg/kg) did not result in any significant changes in renal function parameters as compared to vehicle control (Figure 3.1-3.5 and Table 3.1). However, it was found that administration of cisplatin at the two higher doses (7.5 and 9 mg/kg) caused a significant reduction in C_{in} or GFR by 61 and 78% and C_{PAH} or effective RPF by 87 and 94%, respectively (Figure 3.2). During these reductions, the MABP was maintained. The reduction of GFR by 43 and 77% three days after 6 and 9 mg/kg cisplatin injection, respectively have been previously reported by Nualplub and Hiranyachattada (2000-2001) using polyfructosan clearance or by 70% reduction five days after 7 mg/kg cisplatin injection (Badary *et al*., 2005) using creatinine clearance. The reduction of GFR and RPF after 5 mg/kg cisplatin injection in rat was also reported by other investigators. Matsushima *et al*. (1998) observed a decrease in GFR (95%) and RBF (21%) without an altered MABP five days after the injection. Moreover, Winston and Safirstein (1985) found three days after cisplatin administration, a reduction in single nephron GFR (41%), whole kidney GFR (74%) and RBF (36%) while intratubular hydrostatic pressure unchanged. The impaired

glomerular function may be due to the reduction in RBF caused by renal vasoconstriction. This was supported by the finding that six days after 5 mg/kg cisplatin injection caused damage to renal blood vessels (Shirwaikar *et al*., 2004).

Injection of the two higher doses of cisplatin (7.5 and 9 mg/kg) resulted in an increase in BUN (3 and 4.6 folds, respectively). This increase further supported an impaired glomerular function. A similar 3-11 folds increase in BUN level after 3-10 mg/kg cisplatin injection was also reported by al-Harbi *et al*. (1995), Rao *et al*. (1999) and Sueishi *et al*. (2002). Since urea is largely excreted by glomerular filtration, the impaired glomerular filtration is likely resulted in an elevation of BUN. Another possible mechanism involved an increase in BUN is an enhancement of the tissue breakdown caused by cisplatin that leads to the generation of ammonia which is then converted into urea.

A significant increase in urine flow rate by 1.2 and 1.9 folds and FE_{Na} by 3.3 and 8.4 folds were caused by 7.5 and 9 mg/kg cisplatin injection, respectively even thorough a significant reduction in GFR was observed suggested an impairment of reabsorptive capacity and renal concentrating mechanism. It is suggested that these defects may be due to 1) a decrease in tubular reabsorptive capacity and 2) a low renin-angiotensin-aldosterone activity. Histophatological studies in experimental animals by Dobyan *et al*. (1980), Jones *et al*. (1985), Kim *et al*. (1995) revealed an acute tubular necrosis and the loss of proximal tubular brush-border membrane leading to a reduction of the area for water and Na^+ reabsorption 2-7 days after 4-10 mg/kg doses of cisplatin. Field *et al*. (1989), using electron microprobe X-ray analysis technique, found the reduction of Na^+ concentration in proximal tubular cells of cisplatin treated rat suggested the inhibition of Na^+ across the apical membrane into

the proximal tubular cells. Kim *et al*. (1995) reported that Na^+-K^+-ATPase and Na^+-pump activities in renal of 4 mg/kg cisplatin treated rabbit were decreased. The defect in renal cell energetics caused by cisplatin such as reduction in intracellular ATP (Zhang and Lindup, 1997; Kruidering *et al*., 1997) might also interfere the active transport of PAH and Na^+. The possibility that the defect of renin-angiotensin-aldosterone system may contribute to the reabsorptive capacity of Na^+ after cisplatin injection was reported by Hutchison *et al*. (1988) showing low plasma renin and aldosterone activity in cisplatin treated patients.

A significant weight loss (30-34 g) and increase in hematocrit were observed after three days of cisplatin (7.5 and 9 mg/kg). Since cisplatin causes the gastrointestinal toxicity, bloating of stomach, diarrhea, reduction in an ingestion of food and water intake, all of which might have resulted in a fall in body weight. These evidences also found by another investigator. Jacoby *et al*. (2000) also reported the gross appearance of gastrointestinal damage in rat eight days after 9 mg/kg cisplatin injection. Francescato *et al*. (2001) found diarrhea and a reduction of food ingestion and of water intake after 5 mg/kg cisplatin injection. The increase in hematocrit found in this study further supported an impaired reabsorptive capacity and renal concentrating mechanism with a subsequent dehydration.

In this study, administration of all doses of cisplatin caused a significant increase in renal MDA level by 29-52% when compared to vehicle control (Figure 3.6). This increase may be due to 1) an elevation of free radical 2) depletion of antioxidant defense system and 3) a reduction of renal antioxidant enzymes activity. Masuda *et al*. (1994) showed the dose dependent generation of $HO^{\cdot}$ as well as $O_2^{\cdot -}$ by cisplatin. A reduction in renal antioxidant defense system including vitamin

A and E, GSH and β-carotene levels was reported by others (Greggi Antunes *et al*., 2000; Silva *et al*., 2001; Sueishi *et al*., 2002; Naziroglu *et al*., 2004). An inhibition of renal antioxidant enzymes, such as CAT, GSH-Px and SOD, was also observed (Mansour *et al*., 2002; Yildirim *et al*., 2003; Atessahin *et al*., 2005; Badary *et al*., 2005).

The dose of cisplatin that was chosen to induce ARF (including < 50% reduction in C_{in} and an increase in BUN) together with an increase in renal lipid peroxidation was 7.5 mg/kg.

The short term treatment of HSE in this cisplatin treated rat was able to significant increase C_{in} (82%) and C_{PAH} (3.2 folds) and reduce BUN level (40%) while did not alter urine flow rate and FE_{Na}. In addition, this short term treatment exerted antioxidative effect by counteract the increase renal MDA level by cisplatin. The chemical constituents of HSE in this study that may possibly be responsible for the reduction of renal MDA are quercetin and delphinidine-3-sambucoside (anthocyanin compounds) which have been reported to have antioxidative effect *in vitro* (Cao *et al*., 1997; Tsai *et al*., 2002). Other antioxidants or antidotes that are able to protect against an increase in BUN and renal MDA by cisplatin are erdostein (Yildirim *et al*., 2003), selenium (Satoh *et al*., 1992; Francescato *et al*., 2001) and vitamins C and E (Appenroth *et al*., 1997; Naziroglu *et al*., 2004). The reduction of RBF induced by cisplatin has been shown to be preserved by another antioxidant, lecithinized superoxide dismutase (Matsushima *et al*., 1998).

In short term treatment experimental, a non significant improved in urine concentrating ability and Na^+ reabsorptive capacity may be due to the insufficient dose and duration of HSE treatment or binding of cisplatin directly to the

transporter that cause the reduction in its function. However, the dose of HSE in this study is sufficient to returned MDA back to control value. Similarly, Kruidering *et al*. (1997) reported that an antioxidants diphenyl-*p*-phenylene-diamine and deferoxamine completely prevented ROS formation in porcine proximal tubular cells but did not prevent cell death and could not completely recover the activity of complexes I to IV of mitochondria respiratory chain, ATP and GSH levels. Francescato *et al*. (2001) found that orally administered sodium selenite (2 mg/kg) provided partial protection of an elevation in acute tubular necrosis and serum creatinine observed in the cisplatin treated (5 mg/kg) rat and reduced renal MDA level but did not change creatinine clearance, body weight loss, urinary volume and renal GSH level. From these reports it is suggested that ROS generated during cisplatin-induced renal toxicity is not the direct cause of renal failure. An interfering of cisplatin with normal cellular function and an induction of cell death by cisplatin are likely to be the main causes of ARF.

The long term treatment of HSE in this study seemed to have no improving effect on the ARF caused by cisplatin as seen in Figure 3.7-3.10 and Table 3.2. However, the decrease in BUN level is also observed. This improvement may be due to the decrease in body tissue damages although the impaired renal function is still observed. It is noted that hyponatremia is seen after long term administration of HSE although cisplatin alone does not cause any change in plasma Na^+. It is possible that this hyponatremia may be due to the natriuretic effect of HSE since this effect was previously observed in patients with mild to moderate hypertension (Herrera-Arellano *et al*., 2004). Moreover, the hyponatremia was also reported after long term administration of aqueous extract from dried calyces of HS in rats (Orisakwe *et al*., 2003; Hussaini *et al*., 2004).

It is interesting that long term administration of HSE enhances MDA level in normal rats. This because of the HSE may act as a prooxidative substance by generating free radicals which consequently amplify renal lipid peroxidation. It has been reported earlier that some constituents of HS calyces exert prooxidative effect. Using the fluorescent probe H_2DCF-DA in human promyelocytic leukemia cells, Hou *et al*. (2005) reported that delphinidin-3-sambubioside (25-125 μM) isolated from the HS dried calyces caused a dose- and time-dependent elevation of intracellular ROS. Other HS constituents such as polyphenolic compound and flavonoids have been shown to increase MDA formation in human promyelocytic leukemia cells (Sergediene *et al*., 1999) and generate $HO^{\cdot}$ by Cu^{2+}-dependent reaction (Said Ahmad *et al*., 1992; Cao *et al*., 1997), respectively. Moreover, vitamin C which has been found to be one of the HS constituents could generate free radical by Cu^{2+}-dependent reaction (Cao and Cutler, 1993), stimulate the methionine oxidation and induce apoptotic cell death in human myelogenous leukemic cell lines (Sakagami and Satoh, 1997), cause DNA damage mediated by oxygen radicals in DNA isolated from lymphocytes (Podmore *et al*., 1998) and produce $O_2^{\cdot -}$ in hepatic microsomes (Paolini *et al*., 1999).

It is concluded that the short term administration of HS water extract (250 mg/kg) 24 hr and 10 min before 7.5 mg/kg cisplatin injection exerts a renal protective effect against the alterations in glomerular filtration, renal plasma flow and BUN level. This protective effect may be attributed to its antioxidative action. There could be more than one mechanism responsible for cisplatin-induced ARF since an impaired excretory function (urine flow rate and FE_{Na}) and body weight loss could not be attenuated by the dose and duration of HSE used in this study. In long term

administration of HSE, the improvement of cisplatin-induced renal damage and renal lipid peroxidation was not significantly difference. This might have resulted from dose and schedule of treatment. Before applying HSE oral administration in patient with cisplatin-induced ARF, the dose, duration and the mechanism(s) of protection of HSE should be further clearified.