Acute and Subchronic Treatment of *Hibiscus sabdariffa* Linn. Extract on Renal Function and Lipid Peroxidation in Cisplatin-Induced Acute Renal Failure Rats

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Abstract

Acute renal failure (ARF) and an increased renal lipid peroxidation have been found after cisplatin treatment which may be due to an increase in free radical. *Hibiscus sabdariffa* Linn. (HS) extract has been shown to possess antioxidant effect both *in vitro* and *in vivo*, but never been shown to have renal protective effect in ARF rat caused by cisplatin. This study investigated the protective effect of HS water extract (HSE) in ARF rat caused by cisplatin using renal clearance and renal lipid peroxidation study. The experiments were designed as acute and subchronic treatment. Cisplatin (7.5 mg/kg, i.p.) was used to induce ARF. In acute treatment, HSE (250 mg/kg) was orally administered twice; one day and 10 minutes before cisplatin injection. In subchronic treatment, HSE was given daily for seven days before and three days after cisplatin injection. It was found that the acute treatment with HSE was able to attenuate cisplatin-induced ARF by improving both renal plasma flow and glomerular filtration rate and reducing renal malondialdehyde level. The renoprotective effect of HSE may be due to its free radical scavenging property. The toxicity from long term treatment of HSE should be aware of.

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Cisplatin, *cis*-diaminedichloroplatinum (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 malignancies. The most common adverse effect of cisplatin is ARF (including > 50% reduction in glomerular filtration rate (GFR) and increase in blood urea nitrogen, BUN). One of the underlying mechanisms of cisplatin-induced ARF is the induction of oxidative stress status by 1) an increase in generation of reactive oxygen and nitrogen species[1-3] and 2) depletion of antioxidant defense system(s),[4-6] which result in renal tissue lipid peroxidation.[7-11] Several antioxidants such as vitamin C and E,[10,12] and plant extract[11] have been reported in playing a role against cisplatin-induced ARF. However, *Hibiscus sabdariffa* Linn. or Roselle which was previously reported to possess antioxidant activities both *in vitro*[13-15] and *in vivo*[16-18] has never been shown to protect renal damage caused by cisplatin. In folk medicine, *Hibiscus sabdariffa* (HS) has been used to lower blood pressure, and to treat liver diseases and fever[19] and these properties may be related to its antioxidative effect. The antioxidative effect of HS may depend on the bioavailability of its chemical constituents, anthocyanins and proto-catechuic acid.[20-22] This study aimed to investigate the acute and subchronic oral administration of HS calyces aqueous extract in cisplatin-induced renal dysfunction. Its renal protective role and anti-lipid peroxidation on renal tissues was also examined.

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Materials and Methods

Plant materials and preparation of the extracts

Fresh calyces of *Hibiscus sabdariffa* Linn. (Malvaceae) were obtained from Amphur Jana, Songkhla province, Thailand in April 2004. The plant specimen (No. SKP 1090819) was deposited in the herbarium of the Faculty of Pharmaceutical Sciences, Prince of Songkla University, HatYai, Songkhla, Thailand. The collected calyces were dried at 50°C and then extracted using water. In brief, the water extraction was carried out by boiling the calyces of HS (5 kg) in water (30 L) for 15 min. The water extract was filtered through nylon cloth and then dried using vacuum dry at 40°C for 8-10 hr. The yield (calculated on the dried extract) was 4.6% of the fresh plant weight. Dried HSE was then packed in tight containers and kept in a desiccator at room temperature. HS calyces were accepted by standard of RAISE (Rural and Agricultural Incomes with a Sustainable Environment). It showed standard values as follow: total ash 7.81% (RAISE standard < 10%),

moisture 6.2% (RAISE standard < 12%) and acid insoluble ash 0.12% (RAISE standard < 1.5%). Determination of quantitative active compound of HS water extract by HPLC found that it was composed of quercetin 0.433 mg/g and delphinidin 3-sambubioside 3.74 mg/g.

Animals

Male Wistar rats (body weight, bw, 200-250g) were obtained from Southern Laboratory Animal Facility, (Prince of Songkla University, Songkhla, Thailand). Rats were housed under controlled conditions (temperature 23-24°C; humidity 50-55%; lighting 06.00–18.00 h), fed a normal laboratory diet and allowed free access to tap water. The research was conducted according to the guidelines for experimental animals suggested by The National Research Council of Thailand (2007). All experiments were approved by the Prince of Songkla University Animal Ethics Committee (MOE 0521.11/101).

Experimental design

The experiments were divided into 2 parts. Part I was designed to investigate the dose-response of cisplatin on renal functions and renal lipid peroxidation. The minimal dose that significantly induced ARF will be chosen for Part II study. In Part I, rats were injected intraperitoneally (i.p.) with cisplatin at the doses of 4.5, 6, 7.5 and 9 mg/kg and 0.9% NaCl was used as vehicle control. Three days after the injection, renal functions (using clearance study) and renal lipid peroxidation (using MDA measurement) were assessed.

Part II study was designed to investigate the protective effect of HSE on renal functions and renal lipid peroxidation in cisplatin-induced ARF rats. HSE treatments were designed as acute and subchronic treatment. In acute treatment study, oral administration of HSE at the dose of 250 mg/kg were performed twice, 24 hr and 10 min prior to either 7.5 mg/kg cisplatin or vehicle injection intraperitoneally and distilled water (DW) was used as vehicle solvent of HSE. In subchronic treatment study, rats were given the similar dose of HSE daily, seven days before cisplatin injection and continuing until the day of experiment. Three days after cisplatin injection, all rats were investigated with similar protocol for renal function assessment and renal MDA concentration.

Preparation of kidney homogenate

On the day of experiment, animals were anaesthetized with pentobarbitone sodium (Sigma, St. Louis, MO, USA) i.p. at the dose of 60 mg/kg. A cannula (PE-100) was inserted into the abdominal aorta beneath the left renal artery and used to retrogradely perfuse both kidneys simultaneously with 50 ml of an ice-cold isotonic buffer (pH 7.4) containing (in mM) 130 NaCl, 5 $NaHCO_3$, 1.6 Na_2HPO_4, 0.4 NaH_2PO_4, 1.3 $CaCl_2$, 5 KCl, 1 $MgSO_4$, 10 CH_3COONa, 10 HEPES, 3 glucose and 2 glycine. When the kidneys were blood cleared, they were removed, decapsulated and chopped into small pieces and homogenized in an ice-cold 1.15% KCl (4 ml/g tissue) solution using hand homogenizers. After that, the homogenated samples were sonicated for one hour.

Measurement of lipid peroxidation

Lipid peroxidation was determined by measuring the concentration of MDA in the kidney homogenates modified from method of Ohkawa *et al.*[23] Kidney homogenates were mixed with 0.2 ml of 8.1% sodium dodecyl sulfate, 1.5 ml of 20% acetic acid (pH adjusted to 3.5 with NaOH) and 1.5 ml of a 0.8% aqueous solution of thiobarbituric acid. The volume of resultant solution was made up to 4.0 ml with distilled water (DW) and heated for 60 min at 95°C. After cooling in ice bath, 1.0 ml of DW and 5.0 ml of n-butanol solution were added before shaking the sample vigorously. After centrifugation (4,000 rpm, 10 min), absorbance at 532 nm of the organic layer was measured with the spectrophotometer (model Spectro SC, Labomed, Culver City, California, USA). MDA content was assayed in the form of thiobarbituric acid reacting substances (TBARs) by comparing the absorption to the standard curve of MDA equivalents generated by acid catalyzed hydrolysis of 1,1,3,3-tetramethoxypropane. The values of MDA were expressed as nmol/mg protein.

Determination of protein content in renal tissue homogenate

Protein content of the renal tissue homogenate was determined by the micro-biuret method,[24] using albumin from bovine serum as a standard.

Experimental protocol for measurement of urinary excretion of electrolytes

On the day of experiments, rats were anesthetized with 60 mg/kg pentobarbitone sodium intraperitoneally (additional dose was given when necessary) and placed on thermostatically controlled heated table to maintain the body temperature at 37°C. A tracheostomy was performed and right carotid artery was cannulated for blood sampling and arterial blood pressure recording (Grass Polygraph model 7DAG; Grass Instrument Co., Quincy, MA, USA) throughout the experiment. The right jugular vein was then cannulated and infused with 0.9% NaCl solution containing 0.5% inulin and 0.5% para-aminohippuric acid (PAH) at the rate of 1.6 ml/hr/100 g bw, throughout the experiment. Urine samples were collected in preweighed tubes through a cannula placed in the bladder via suprapubic midline incision. A one-hour equilibration period was allowed in order to obtain the steady state of plasma inulin and PAH concentration before clearance measurement was undertaken.

After the equilibration period, all rats were subjected to an identical protocol in which four consecutive 30-minute urine collections were made. An arterial blood sample (400 μl) was taken. A small

amount of this blood was used to measure hematocrit while the remainder was centrifuged and the plasma collected and stored frozen for determination of the concentration of clearance markers and electrolytes. The blood cells were resuspended in 200 µl isotonic saline and returned slowly to the animal via the jugular vein catheter. At the end of the experiment both kidneys were removed, blotted and weighed. Renal clearance of either PAH, inulin, Na^+ or K^+ was calculated according to the clearance equation: $C_x = (U_x \times \dot{V})/P_x$, where C_x is clearance of either PAH, inulin, Na^+ or K^+; U_x and P_x are concentrations of PAH, inulin, Na^+ and K^+ in urine and plasma, respectively; $\dot{V}$ is urine flow rate. Fractional excretion of Na^+ (FE_{Na}) and K^+ (FE_K) were calculated according to the equation: $FE_x = (C_x/C_{in}) \times 100$, where FE_x is fractional excretion of Na^+ or K^+; C_x is clearance of Na^+ or K^+; C_{in} is clearance of inulin.

The experiment protocol was designed with four groups of rats including vehicle control (DW), HSE, C+DW and C+HSE groups in either acute or subchronic treatment (n = 11-26 each). In the vehicle control groups, distilled water was given by gavage. HSE dissolved in DW was given at the dose of 250 mg/kg in both HSE and C+HSE group. Cisplatin (C) (7.5 mg/kg) was given intraperitoneally in C+DW and C+HSE groups and 0.9% NaCl was injected as vehicle.

Blood and urine sample analysis

Urine output was determined gravimetrically assuming a density of 1 g/ml. Hematocrit was measured by microcapillary method. Sodium and potassium concentrations in plasma and urine samples were measured using an electrolyte analyser (AVL, ISE Analyser, Model 988-31988-4, Graz, Austria). Inulin and PAH concentrations were estimated by a spectrophotometric method.[25,26] Blood urea nitrogen (BUN) was estimated by enzymatic method using urease enzyme kit.

Statistical analysis

All data are presented as mean ± SEM. Analysis of variance (ANOVA) was employed to analyze the data. Multiple comparisons were performed using Student-Newman-Keuls post hoc test to determine the difference between the mean values. A P value < 0.05 was considered significantly different.

Results

Effects of cisplatin on renal function and renal lipid peroxidation (A dose-response study)

The Part I experiment examined the effects of cisplatin (4.5, 6, 7.5 and 9 mg/kg) on renal function and renal lipid peroxidation. As shown in Figure 1, cisplatin at the dose of 7.5 mg/kg is the minimal dose that induced ARF, as indicated by a reduction in C_{in} (61%) and an increase in BUN (4 folds). So in Part II of the experiment, cisplatin at the dose of 7.5 mg/kg was used to induce ARF in rats. Besides, elevation in urine flow rate ($\dot{V}$), FE_{Na}, Hct, renal MDA level, kidney weight/body weight (kw/bw) ratio and reduction in C_{PAH} and body weight were found whereas FE_K, MABP and HR were maintained after 7.5 mg/kg cisplatin injection.

Effects of acute and subchronic treatment with HSE on body weight change, kw/bw ratio, renal functions and renal MDA level in cisplatin-induced ARF

During the three days after 7.5 mg/kg cisplatin injection, diarrhea, reduction of food ingestion and water intake were observed. The significant reduction in body weight was found as shown in Table 1. Both

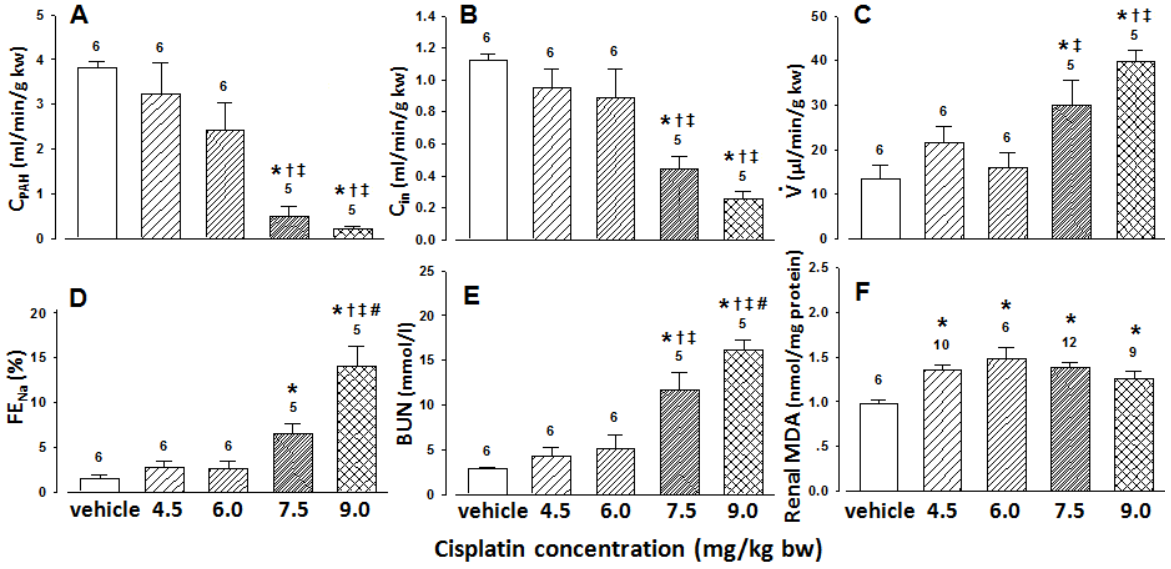

Figure 1 Effects of cisplatin on A) PAH clearance (C_{PAH}), B) inulin clearance (C_{in}), C) urine flow rate ($\dot{V}$), D) fractional excretion of sodium (FE_{Na}), E) blood urea nitrogen (BUN) and F) renal malondialdehyde (MDA) in rats. Data are mean ± SEM; number above each bar indicates animal count; *, †, ‡ and # indicate $P < 0.05$ compared to vehicle and cisplatin-treated groups at 4.5, 6 and 7.5 mg/kg, respectively (one-way ANOVA with multiple comparison using Student-Newman Keuls post hoc test).

Table 1 Effects of cisplatin on body weight change, mean arterial blood pressure (MABP) and on heart rate (HR), hematocrit (Hct), plasma concentration of sodium and potassium (P_{Na} and P_K) during clearance study.

		Cisplatin concentration (mg/kg)			
	vehicle (n=6)	4.5 (n=6)	6.0 (n=6)	7.5 (n=5)	9.0 (n=5)
Pre-study body weight (g)	223.4 ± 5.2	239.7 ± 2.8	236.7 ± 6.1	238.9 ± 4.1	235.7 ± 4.6
Post-study body weight (g)	229.4 ± 4.2	241.8 ± 7.6	225.3 ± 4.7	208.8 ± 5.2*†‡	201.6 ± 3.5*†‡
Body weight change (g)	6.0 ± 3.4	2.2 ± 9.3	-11.4 ± 6.2	-30.1 ± 2.9*†‡	-34.1 ± 2.5*†‡
kw/bw (%)	0.64 ± 0.02	0.64 ± 0.02	0.70 ± 0.05	0.85 ± 0.04*†‡	0.85 ± 0.03*†‡
MABP (mmHg)	126 ± 9	114 ± 3	128 ± 11	106 ± 9	106 ± 3
HR (beat/min)	432 ± 18	422 ± 17	417 ± 21	391 ± 13	362 ± 9
Hct (%)	41.8 ± 0.9	42.2 ± 1.1	43.3 ± 0.7	49.6 ± 1.0*†‡	53.0 ± 1.9*†‡
P_{Na} (mmol/l)	137.3 ± 1.5	142.0 ± 0.8	137.6 ± 1.5	139.1 ± 2.5	127.9 ± 1.5*†‡#
P_K (mmol/l)	3.79 ± 0.12	3.63 ± 0.09	3.50 ± 0.16	3.23 ± 0.18	3.02 ± 0.17*†

Post-study body weight and blood samples were collected on day 3 after cisplatin injection. Data are mean ± SEM.
*, †, ‡ and # $P < 0.05$ compared to vehicle and cisplatin treated group at the doses of 4.5, 6 and 7.5 mg/kg, respectively (one-way ANOVA with multiple comparison using Student-Newman Keuls post hoc test).

acute and subchronic treatment did not improve this body weight reduction. The bloating of the stomach in cisplatin treatment was observed at the end of clearance study but this was not found in vehicle group. The increased kw/bw ratio caused by cisplatin was improved by acute treatment with HSE but not by subchronic treatment.

The clearance studies of acute treatment showed the reduction of C_{in} and C_{PAH} in cisplatin-induced ARF (0.44 ± 0.08 and 0.51 ± 0.20 ml/min/g kw when compared to vehicle control 1.13 ± 0.04 and 3.82 ± 0.13 ml/min/g kw, respectively) was improved to 0.80 ± 0.17 and 2.16 ± 0.74 ml/min/g kw, respectively as shown in Figure 2B and 2A (left panels). In contrast, the subchronic treatment did not improve the reduction of these two values as shown in Figure 2B and 2A (right panels). Both acute and subchronic treatment did not significantly alter mean arterial blood pressure (MABP), heart rate (HR) and hematocrit (Hct) when compared to their respective controls (data not shown).

After cisplatin injection, urine flow rate ($\dot{V}$) significantly increased by 0.9-1.2 folds as shown in Figure 2C and fractional sodium excretion (FE_{Na}) increased by 3.0-3.3 folds as shown in Figure 3B. Both acute and subchronic treatment with HSE did not bring these values down to their respective control values. As shown in Figure 3C, a significant decrease in potassium excretion rate ($U_K\dot{V}$) (0.23 ± 0.04 from vehicle control 0.56 ± 0.06 mmol/min/g kw) after cisplatin injection was prevented by acute treatment but not by subchronic treatment with HSE.

Blood urea nitrogen (BUN) after cisplatin injection increased significantly by 3-4 folds whereas plasma sodium (P_{Na}) and plasma potassium (P_K) remained unaltered, as shown in Table 1. Both acute and subchronic treatment with HSE showed a statistically significant decrement in BUN level (from 11.7 ± 1.8 and 14.2 ± 1.5 to 7.0 ± 2.4 and 10.0 ± 2.1 mmol/l, respectively). Subchronic treatment with HSE in cisplatin-induced ARF rats significantly decreased P_{Na} from 138.2 ± 1.8 to 131.7 ± 1.8 mmol/l.

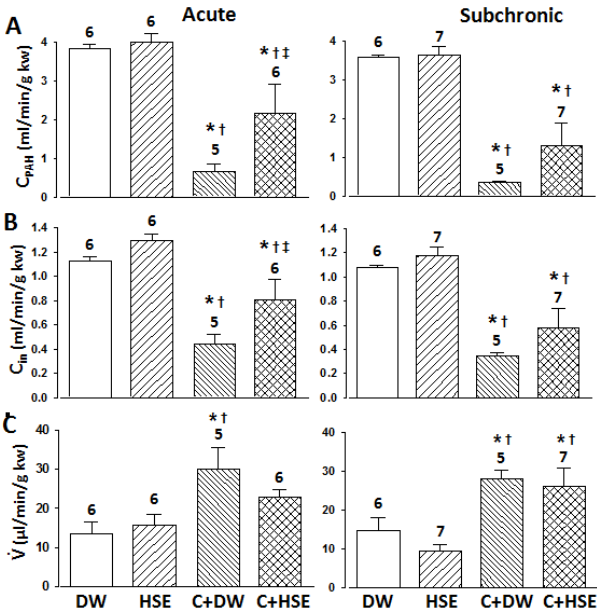

Figure 2 Effects of acute and subchronic treatments with *Hibiscus sabdariffa* Linn. extract on A) PAH clearance, B) inulin clearance and C) urine flow rate in 7.5 mg/kg cisplatin-induced ARF rats. DW = distilled water, HSE = *Hibiscus sabdariffa* Linn. water extract 250 mg/kg, C+DW = cisplatin 7.5 mg/kg + distilled water, C+HSE = cisplatin 7.5 mg/kg + *Hibiscus sabdariffa* Linn. water extract 250 mg/kg, kw = kidney weight. Data are mean ± SEM; number above each bar indicates animal count. *, † and ‡ $P < 0.05$ compared to DW, HSE and C+DW groups, respectively (one-way ANOVA with multiple comparison using Student-Newman Keuls post hoc test).

As shown in Figure 4, renal MDA level increased significantly from 0.97 ± 0.05 to 1.38 ± 0.06 nmol/mg protein after cisplatin injection. Acute treatment with HSE subsided this increased MDA level to 0.91 ± 0.08 nmol/mg protein, as shown in Figure 4 (left panel). Subchronic treatment with HSE significantly increased MDA level from 1.14 ± 0.06 in vehicle control rats to 1.55 ± 0.04 nmol/mg protein as shown in Figure 4 (right panel). This treatment did not further increase MDA level in cisplatin-induced ARF (1.45 ± 0.09 compared to 1.44 ± 0.12 nmol/mg protein of cisplatin alone).

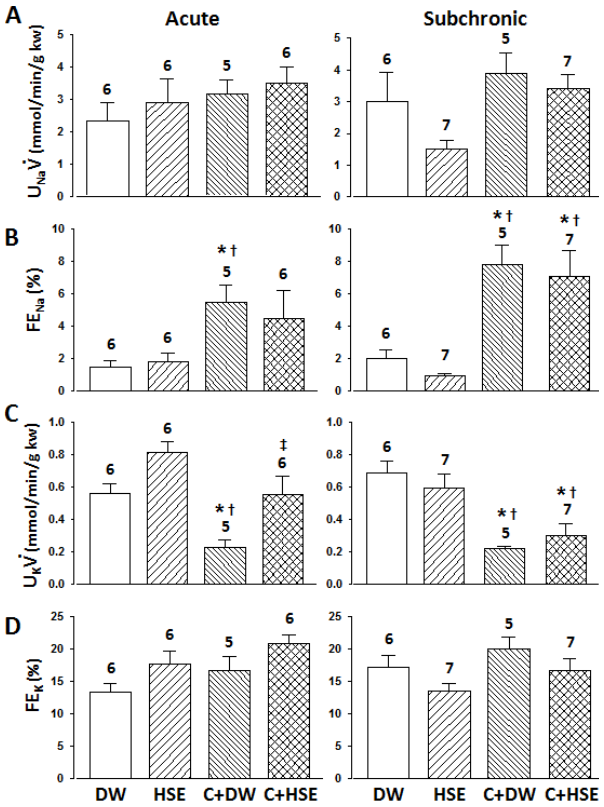

Figure 3 Effects of acute and subchronic treatment with *Hibiscus sabdariffa* Linn. extract on A) sodium excretion rate, B) fractional sodium excretion, C) potassium excretion rate and D) fractional potassium excretion in 7.5 mg/kg cisplatin-induced ARF rats. DW = distilled water, HSE = *Hibiscus sabdariffa* Linn. water extract 250 mg/kg, C+DW = cisplatin 7.5 mg/kg + distilled water, C+HSE = cisplatin 7.5 mg/kg + *Hibiscus sabdariffa* Linn. water extract 250 mg/kg, kw = kidney weight. Data are mean ± SEM; number above each bar indicates animal count. *, † and ‡ $P < 0.05$ compared to DW, HSE and C+DW groups, respectively (one-way ANOVA with multiple comparison using Student-Newman Keuls post hoc test).

Discussion

As shown in Figure 1, three days after the i.p. bolus injection of cisplatin at the two higher doses (7.5 and 9 mg/kg) there were a significant reduction in C_{in} or glomerular filtration rate (GFR) by 61% and 78% and C_{PAH} or effective renal plasma flow (RPF) by 87% and 94%, respectively, despite MABP was maintained. However, cisplatin at the two lower doses (4.5 and 6 mg/kg) did not result in any significant changes in renal function parameters as compared to vehicle control. This indicated that cisplatin at the dose of 7.5 mg/kg is the minimal dose that induce ARF in rats within 3 days after injection, and thus used to induce ARF in rat in following experiments.

Administration of cisplatin (7.5 mg/kg) in this study resulted in a significant reduction in GFR and RPF. The reduction of GFR and RPF after cisplatin (5-9 mg/kg) injection in rats was also reported by other investigators.[6,7,27,28] This impaired glomerular function may be due to the reduction in renal blood flow caused by renal vasoconstriction.[29] However, it is also likely that the decrease in PAH clearance may be due to the impairment of tubular PAH secretion caused by cisplatin.[9,30,31] A 3-4 folds increase in BUN found after cisplatin injection in this study was similar to those reported earlier[4,32,33] This significant increase suggested an impaired glomerular function and perhaps an enhancement of tissue breakdown caused by cisplatin leading to the generation of ammonia and then urea. A significant increase in urine flow rate by 1.2 folds and FE_{Na} by 3.3 folds caused by cisplatin injection were observed during a significant reduction in GFR. The findings suggested an impairment of renal tubular reabsorptive capacity and renal concentrating mechanism similar to those previously reported.[30,34-38] Moreover, a significant weight loss and increase in hematocrit value were observed three days after cisplatin injection. These may be due to the gastrointestinal toxicity of cisplatin leading the reduction of food and water intake as reported earlier[39] and diarrhea was also observed. A significant increase in kidney weight/body weight ratio observed after cisplatin injection may resulted from fluid accumulation in dilated renal tubule as reported earlier.[30]

In this study, administration of cisplatin caused a significant increase in renal MDA level. The increased lipid peroxidation may be due to 1) an elevation of reactive oxygen and nitrogen species,[1-3] or 2) depletion of both enzymatic and non-enzymatic antioxidant defense system.[4-6,40] However, the acute treatment with HSE in cisplatin treated rat attenuate the elevation of MDA level confirming its possible roles as an antioxidative substance. The chemical constituents of HSE responsible for the reduction of renal MDA level in cisplatin treated rats are likely to be quercetin and/or delphinidine 3-sambubioside (anthocyanin compounds) which have been reported to possess antioxidative properties.[22,41] The acute treatment with HSE in these cisplatin treated rats was able to improve the decreased GFR (from 49 to 71% of control value) and RPF (from 13 to 57% of control value) and attenuate the increase in BUN levels (from

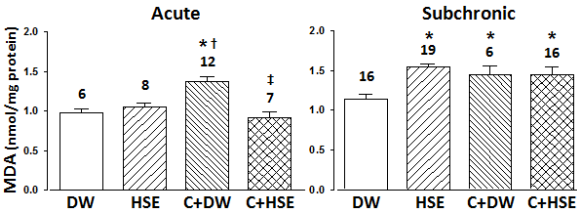

Figure 4 Effects of acute and subchronic treatment with *Hibiscus sabdariffa* Linn. extract on renal MDA level in 7.5 mg/kg cisplatin-induced ARF rats. DW = distilled water, HSE = *Hibiscus sabdariffa* Linn. water extract 250 mg/kg, C+DW = cisplatin 7.5 mg/kg + distilled water, C+HSE = cisplatin 7.5 mg/kg + *Hibiscus sabdariffa* Linn. water extract 250 mg/kg. Data are mean ± SEM; number above each bar indicates animal count. *, † and ‡ are $P < 0.05$ compared to DW, HSE and C+DW groups, respectively (one-way ANOVA with multiple comparison using Student-Newman Keuls post hoc test).

3 to 1.4 folds of control value). One of the likely mechanisms in improving this renal damage may be due to the antioxidative property of HSE as discussed earlier. A nonsignificant improved in urine concentrating ability and Na^+ reabsorptive capacity may be due to the insufficient dose and duration of HSE treatment and/or an irreversible binding of cisplatin to the renal tubular transporter(s). However, the dose of HSE in this study is sufficient to returned MDA level back to control value despite the damage in glomerular and tubular function. It is suggested that free radical generated during cisplatin-induced renal toxicity is not the direct cause of renal failure. An interference of cisplatin with other normal cellular functions and an induction of cell death by cisplatin may be the principle causes of ARF.

The subchronic treatment with HSE in this study did not seem to improve the ARF caused by cisplatin. However, the decrease in BUN level (from 4 to 2.6 folds of control value) was also observed. This BUN improvement may be due to the decrease in body tissue damages while the impaired renal function remained. It is noted that the hyponatremia was seen after subchronic administration of HSE in cisplatin treated rat only. This may be due to the natriuretic effect of HSE which was previously observed in patients with mild to moderate hypertension[42] and in rats.[43,44] It is interesting that this subchronic administration of HSE in normal rats enhanced renal MDA level. Thus, the multiple doses of HSE may act as a prooxidative substance by generating free radicals which consequently amplifying renal lipid peroxidation. The prooxidative effect of some constituents of HS calyces, such as delphinidin-3-sambubioside,[45] polyphenolic compound,[46] flavonoids[41,47] and vitamin C,[48-50] have been reported elsewhere.

It is concluded that the acute 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 GFR, RPF 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 tubular reabsorptive function (assessed by $\dot{V}$ and FE_{Na}) and body weight loss could not be attenuated by the dose and duration of HSE used in this study. In subchronic administration of HSE, the improvement of cisplatin-induced renal damage and renal lipid peroxidation was not significantly observed which might have resulted from dose and schedule of treatment. The toxicity of long-term HSE administration should be further studied.

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Conflict of Interest

None to declare.

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