APPENDIX A

Method for the determination of inulin concentration (Davidson *et al*., 1963)

Preparation of anthrone reagent

1. Add 100 ml of H_2SO_4 slowly to 26 ml of ice-cooled DW in a flask immersed in an ice-bath. The mixture was allowed to cool.
2. Add 147 mg of anthron.
3. Stir until the mixture completely dissolved.

Precipitation of plasma protein

1. Mix 30 μl of plasma with 1.8 ml of 3% trichloroacetic acid (CCl_3COOH).
2. Mix well and stand for 10 min, shaking again at least once.
3. Centrifuge at 4000 rpm for 20 min to precipitated protein.

Procedure

1. Add 0.5 ml of DW (blank), fructose standard solution, plasma filtrate and diluted urine into cold test tubes, which the bottom of tubes are completed immersed in ice-bath. All determinations were done is duplicate.
2. Add 3.0 ml of anthrone reagent, mix well. Cool in ice-bath before its place in water bath.
3. Place in water bath at 38°C for 50 min.
4. The concentration of inulin is determined directly from standard curve for range of 2 to 6.8 mg% of fructose.

APPENDIX B

Method for the determination of para-aminohippuric acid concentration

(Smith *et al.*, 1945)

Solutions

1. 0.1% sodium nitrite ($NaNO_2$)

   Add 0.1 g of $NaNO_2$ into DW and dilute to make 100 ml solution.

2. 0.5% ammonium sulfamate ($H_6N_2O_3S$)

   Add 0.5 g of $H_6N_2O_3S$ into DW and dilute to make 100 ml solution.

3. 0.1% N-(1-naphthyl)-ethylenediamine dihydrochloride ($C_{12}H_{16}Cl_2N_2$)

   Add 0.1 g of $C_{12}H_{16}Cl_2N_2$ into DW and dilute to make 100 ml solution.

4. 3.2% trichloroacetic acid (CCl_3COOH)

   Add 3.2 g of CCl_3COOH into distilled water and dilute to make 100 ml solution.

5. 0.2N hydrochloric acid (HCl)

   Dilute 1.67 ml of HCl with DW, make up to 100 ml solution.

Procedure

1. Add 50 μl of PAH standard solution, plasma and diluted urine into 1100 μl of 3.2% CCl_3COOH. Mix well and after about 10 min, centrifuge plasma for 2 min at 4000 rpm to precipitated protein. All determinations were done is duplicate.

2. Pipette 1.0 ml of 3.2% CCl_3COOH (blank) and solution from (1) into test tubes.

3. Add 0.2 ml of 0.2 N HCl and 0.1 ml of 0.1% $NaNO_2$. Mix thoroughly, let stand not less than 3 min or more than 5 min after $NaNO_2$.
4. Add 0.1 ml of 0.5% $H_6N_2O_3S$. Mix thoroughly, let stand not less than 3 min or more than 5 min later.
5. Add 0.1 ml of 0.1% $C_{12}H_{16}Cl_2N_2$, mix well.
6. Let stand at room temperature for 15-60 min.
7. Measure the developed color by the spectrophotometer at 540 nm.
8. The concentration of PAH is determined directly from standard curve for the range of 1-10 mg% of PAH.

APPENDIX C

Method for the determination of malondialdehyde content in renal tissue homogenate (Modified from Ohkawa *et al*., 1979)

Solutions

1. 8.1% sodium dodecyl sulfate ($C_{12}H_{25}NaO_4S$)

   Add 8.1 g of $C_{12}H_{25}NaO_4S$ into DW and dilute to make 100 ml solution.

2. 20% acetic acid

   Dilute 20 ml acetic acid with DW and make up to 100 ml solution.

3. 0.8% thiobarbituric acid (TBA)

   Add 0.8 g of TBA into DW, heat and stir until the solution completely dissolved. Make up to 100 ml solution.

4. 1.5 N sodium hydroxide (NaOH)

   Add 6.122 g of NaOH into DW and dilute to make 100 ml solution.

5. n-butanol

Procedure

1. Add 0.2 ml of DW, 1,1,3,3-tetramethoxypropane (MDA standard solution) and tissue homogenate solution into the test tubes for blank, standard and sample, respectively. All determinations were done is triplicate.
2. Add 0.2 ml of 8.1% $C_{12}H_{25}NaO_4S$ and 1.5 ml of 20% acetic acid, mix thoroughly.
3. Adjust the pH of mixture to 3.5 with 1.5 N NaOH.
4. Add 1.5 ml of 0.8% TBA and make up to 4.0 ml with DW, mix well.

5. Heat for 60 min in 95°C water bath.
6. Cool in ice-bath.
7. Add 1.0 ml of DW and 5.0 ml of n-butanol, shake vigorously.
8. Centrifuge at 4000 rpm for 10 min.
9. Read absorbance of the organic layer (upper layer) at 532 nm.
10. The concentration of MDA is determined directly from standard curve of 1,1,3,3-tetramethoxypropane for the range of 0.2 to $1x10^{-4}$ M.

APPENDIX D

Method for the determination of protein content in renal tissue homogenate (Itzhaki and Gill, 1964)

Preparation of biuret reagent (0.21% $CuSO_4 \cdot 5H_2O$ in 30% NaOH)

1. Add 0.525 g of $CuSO_4 \cdot 5H_2O$ into DW and dilute to make 50 ml solution.
2. 75 g of NaOH is added to DW and diluted to make 200 ml solution.

   The solution was allowed to cool at room temperature.
3. Mix 50 ml of $CuSO_4 \cdot 5H_2O$ solution with 200 ml of NaOH solution.

Procedure

1. Dilute tissue homogenate into 200 times with DW, mix thoroughly.
2. Add 1 ml of DW, protein standard solution and tissue homogenate solution into test tubes for blank, standard and unknown, respectively.

   All determinations were done is triplicate.
3. Add to all tubes with 1 ml of biuret reagent, mix well.
4. Read absorbance at 310 nm after 10 min.
5. The concentration of protein is determined directly from a standard curve of bovine serum albumin for the range of 50-650 μg.

APPENDIX E

Effects of cisplatin on mean arterial blood pressure (MABP), urine flow rate ($\dot{V}$), clearance of inulin (C_{in}), clearance of para-aminohippuric acid (C_{PAH}), sodium excretion rate ($U_{Na}\dot{V}$), fractional excretion of sodium (FE_{Na}), potassium excretion rate ($U_K\dot{V}$), fractional excretion of potassium (FE_K), plasma concentration of inulin and para-aminohippuric acid (P_{in} and P_{PAH}) and blood urea nitrogen (BUN) in rats.

	vehicle (n=6)	**Cisplatin concentration (mg/kg bw)**			
		4.5 (n=6)	**6.0 (n=6)**	**7.5 (n=5)**	**9.0 (n=5)**
MABP (mmHg)	126 ± 9	114 ± 3	128 ± 11	106 ± 9	106 ± 3
$\dot{V}$ **(µl/min/g kw)**	13.58 ± 2.85	21.55 ± 3.54	15.88 ± 3.40	30.06 ± 5.40*‡	39.69 ± 2.58*†‡
C_{in} **(ml/min/g kw)**	1.13 ± 0.04	0.95 ± 0.12	0.89 ± 0.19	0.44 ± 0.08*†‡	0.25 ± 0.05*†‡
C_{PAH} **(ml/min/g kw)**	3.82 ± 0.13	3.24 ± 0.68	2.41 ± 0.62	0.51 ± 0.20*†‡	0.22 ± 0.05*†‡
$U_{Na}\dot{V}$ **(mmol/min/g kw)**	2.32 ± 0.58	3.48 ± 0.53	2.27 ± 0.47	3.15 ± 0.43	4.01 ± 0.35
FE_{Na} **(%)**	1.49 ± 0.38	2.82 ± 0.58	2.60 ± 0.82	6.48 ± 1.18*	14.07 ± 2.23$^{*†‡\#}$
$U_K\dot{V}$ **(mmol/min/g kw)**	0.56 ± 0.06	0.66 ± 0.09	0.50 ± 0.10	0.23 ± 0.04*†‡	0.16 ± 0.02*†‡
FE_K **(%)**	14.46 ± 1.35	19.32 ± 0.83	18.97 ± 2.73	16.71 ± 2.16	22.40 ± 2.28
P_{in} **(mg%)**	21.27 ± 0.77	28.87 ± 3.20	31.13 ± 7.68	38.40 ± 5.14	60.48 ± 6.48$^{*†‡\#}$
P_{PAH} **(mg%)**	6.00 ± 0.22	9.81 ± 2.48	14.82 ± 6.30	27.20 ± 4.43*†‡	38.82 ± 3.51*†‡
BUN (mmol/l)	2.9 ± 0.2	4.2 ± 1.0	5.2 ± 1.6	11.7 ± 1.8*†‡	16.1 ± 1.1$^{*†‡\#}$

Animals were tested on the third day after cisplatin injection. Data are mean ± S.E.M.

*, †, ‡ 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).

APPENDIX F

Effects of *Hibiscus sabdariffa* Linn. extract on mean arterial blood pressure (MABP), urine flow rate ($\dot{V}$),clearance of inulin (C_{in}), clearance of para-aminohippuric acid (C_{PAH}) sodium excretion rate ($U_{Na}\dot{V}$), fractional excretion of sodium (FE_{Na}), potassium excretion rate ($U_K\dot{V}$), fractional excretion of potassium (FE_K), plasma concentration of inulin and para-aminohippuric acid (P_{in} and P_{PAH}) and blood urea nitrogen (BUN) in cisplatin-induced ARF rats.

	Short term treatment of HSE				**Long term treatment of HSE**			
	DW (n=6)	**HSE (n=6)**	**C+DW (n=5)**	**C+HSE (n=6)**	**DW (n=6)**	**HSE (n=7)**	**C+DW (n=5)**	**C+HSE (n=7)**
MABP (mmHg)	126 ± 9	125 ± 4	106 ± 9	126 ± 4	112 ± 7	114 ± 6	93 ± 2	100 ± 5
$\dot{V}$ (µl/min/g kw)	13.58 ± 2.85	15.72 ± 2.72	30.06 ± 5.40*†	22.85 ± 1.99	14.80 ± 3.26	9.52 ± 1.71	28.13 ± 2.31*†	26.03 ± 4.90*†
C_{in} (ml/min/g kw)	1.13 ± 0.04	1.30 ± 0.05	0.44 ± 0.08*†	0.80 ± 0.17*†‡	1.08 ± 0.02	1.18 ± 0.07	0.34 ± 0.03*†	0.58 ± 0.16*†
C_{PAH} (ml/min/g kw)	3.82 ± 0.13	4.00 ± 0.22	0.51 ± 0.20*†	2.16 ± 0.74*†‡	3.56 ± 0.80	3.63 ± 0.21	0.34 ± 0.05*†	1.29 ± 0.60*†
$U_{Na}\dot{V}$ (mmol/min/g kw)	2.32 ± 0.58	2.90 ± 0.72	3.15 ± 0.43	3.49 ± 0.50	3.00 ± 0.94	1.51 ± 0.27	3.88 ± 0.68	3.40 ± 0.46
FE_{Na} (%)	1.49 ± 0.38	1.77 ± 0.54	6.48 ± 1.18*†	4.47 ± 1.77	1.97 ± 0.59	0.90 ± 0.16	7.85 ± 1.16*†	7.09 ± 1.62*†
$U_K\dot{V}$ (mmol/min/g kw)	0.56 ± 0.06	0.81 ± 0.07	0.23 ± 0.04*†	0.55 ± 0.12‡	0.69 ± 0.07	0.60 ± 0.09	0.22 ± 0.01*†	0.30 ± 0.07*†
FE_K (%)	14.46 ± 1.35	17.74 ± 1.98	16.71 ± 2.16	20.94 ± 1.35	16.66 ± 1.63	13.52 ± 1.21	19.16 ± 1.80	16.64 ± 1.86
P_{in} (mg%)	21.27 ± 0.77	22.52 ± 0.56	38.40 ± 5.14*†	35.96 ± 6.61*†	24.61 ± 0.97	22.75 ± 0.80	50.97 ± 3.09*†	41.70 ± 5.95*†
P_{PAH} (mg%)	6.00 ± 0.22	7.26 ± 0.20	27.20 ± 4.43*†	22.34 ± 8.41	7.21 ± 0.23	6.95 ± 0.49	37.45 ± 3.26*†	25.94 ± 5.69*†‡
BUN (mmol/l)	2.9 ± 0.2	2.5 ± 0.1	11.7 ± 1.8*†	7.0 ± 2.4‡	2.8 ± 0.2	3.1 ± 0.1	14.2 ± 1.5*†	10.0 ± 2.1*†‡

Animals were tested on the third day after cisplatin injection. 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 ± S.E.M.

*, † and ‡ $P < 0.05$ compared with DW, HSE and C+DW groups, respectively (one-way ANOVA with multiple comparison using Student-Newman Keuls post hoc test).

APPENDIX G

Table 1 Effect of cisplatin on renal MDA level in rats.

	vehicle (n=6)	Cisplatin concentration (mg/kg)			
		4.5 (n=10)	6.0 (n=6)	7.5 (n=12)	9.0 (n=9)
MDA (nmol)	6.29 ± 0.45	7.67 ± 0.33	7.58 ± 0.68	8.32 ± 0.30	7.36 ± 0.34
Renal protein (mg)	6.45 ± 0.15	5.69 ± 0.16	5.15 ± 0.11	6.09 ± 0.15	5.95 ± 0.17
MDA (nmol/mg protein)	0.97 ± 0.05	1.35 ± 0.06*	1.47 ± 0.14*	1.38 ± 0.06*	1.25 ± 0.08*

Animals were tested on the third day after cisplatin injection.

Data are mean ± S.E.M. * $P < 0.05$ compared to vehicle group (one-way ANOVA with multiple comparison using Student-Newman Keuls post hoc test).

Table 2 Effect of *Hibiscus sabdariffa* Linn. extract on renal MDA level in cisplatin-induced ARF rats.

	Short term treatment of HSE				Long term treatment of HSE			
	DW (n=6)	HSE (n=8)	C+DW (n=12)	C+HSE (n=7)	DW (n=16)	HSE (n=19)	C+DW (n=6)	C+HSE (n=16)
MDA (nmol)	6.29 ± 0.45	7.02 ± 0.32	8.32 ± 0.30	5.51 ± 0.40	7.36 ± 0.34	7.58 ± 0.68	8.32 ± 0.30	7.36 ± 0.34
Renal protein (mg)	6.45 ± 0.15	6.64 ± 0.13	6.09 ± 0.15	6.07 ± 0.15	5.95 ± 0.17	5.15 ± 0.11	6.09 ± 0.15	5.95 ± 0.17
MDA (nmol/mg protein)	0.97 ± 0.05	1.06 ± 0.04	1.38 ± 0.06*†	0.91 ± 0.08‡	1.14 ± 0.06	1.55 ± 0.04*	1.44 ± 0.12*	1.45 ± 0.09*

Animals were tested on the third day after cisplatin injection. 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 ± S.E.M. *, † and ‡ $P < 0.05$ compared with DW, HSE and C+DW groups, respectively (one-way ANOVA with multiple comparison using Student-Newman Keuls post hoc test).