CHAPTER 3

RESULTS

## 1. Effects of cisplatin on renal functions and renal lipid peroxidation

1.1 Systemic effects of cisplatin

The administration of all doses of cisplatin demonstrated no significant changes in either mean arterial blood pressure (MABP) as shown in Figure 3.1a or in heart rate (Table 3.1). Hematocrit values of rats treated with cisplatin (4.5 and 6 mg/kg) were unaltered, while the higher doses (7.5 and 9 mg/kg) were 49.6 ± 1.0 and $53.0 \pm 1.9\%$, respectively significantly increased when compared to vehicle group ($41.8 \pm 0.9\%$) as shown in Table 3.1.

1.2 Effects of cisplatin on clearances of inulin and para-aminohippuric acid

As shown in Figure 3.2, the administration of low doses of cisplatin (4.5 and 6 mg/kg) did not significantly alter either clearance of inulin (C_{in}) or clearance of para-aminohippuric acid (C_{PAH}). However, the two high doses of cisplatin (7.5 and 9 mg/kg) significantly decreased C_{in} to 0.44 ± 0.08 and to 0.25 ± 0.05 ml/min/g kidney weight (kw), respectively when compared to vehicle control (1.13 ± 0.04 ml/min/g kw). C_{PAH} was decreased to 0.51 ± 0.20 and 0.22 ± 0.05 ml/min/g kw, respectively when compared to vehicle control (3.82 ± 0.13 ml/min/g kw).
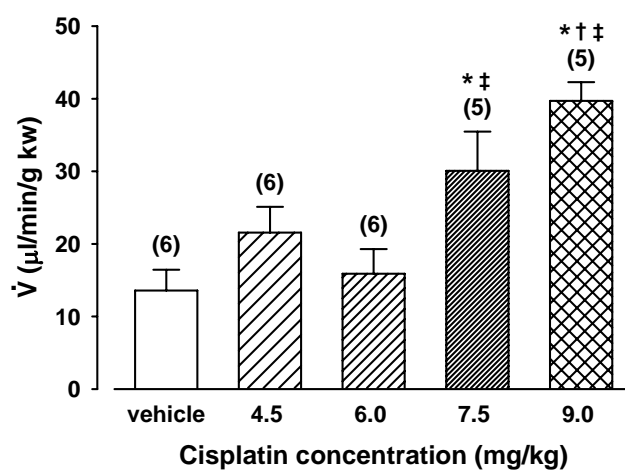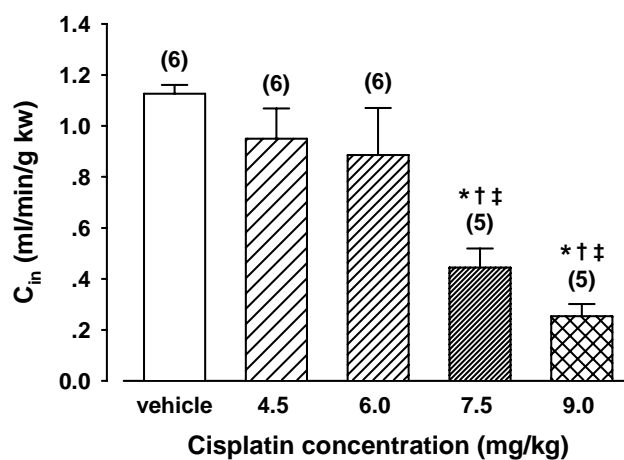
(a)
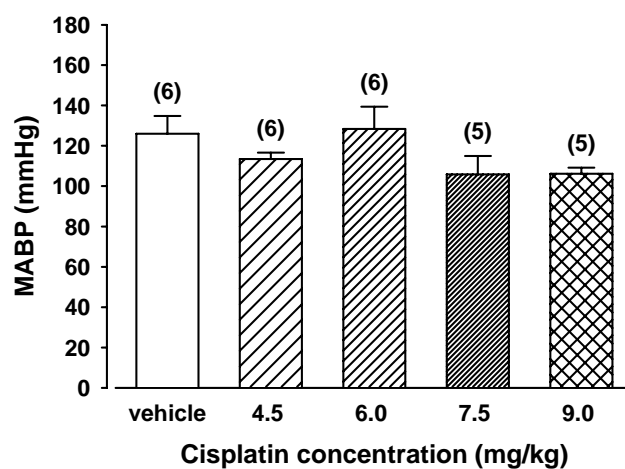
(b)

Figure 3.1 Effects of cisplatin on (a) mean arterial blood pressure (MABP) and (b) urine flow rate ($\dot{V}$) in rats.

Data are mean ± S.E.M., parentheses indicate number of animal.

*, † and ‡ P < 0.05 compared to vehicle and cisplatin treated group at the doses of 4.5 and

6 mg/kg, respectively (one-way ANOVA with multiple comparison using Student-Newman

Keuls post hoc test).

(a)

(b)

Figure 3.2 Effects of cisplatin on (a) clearance of inulin (C_{in}) and (b) clearance of para-aminohippuric

acid (C_{PAH}) in rats.

Data are mean ± S.E.M., parentheses indicate number of animal.

*, † and ‡ $P < 0.05$ compared to vehicle and cisplatin treated group at the doses of 4.5 and

6 mg/kg, respectively (one-way ANOVA with multiple comparison using
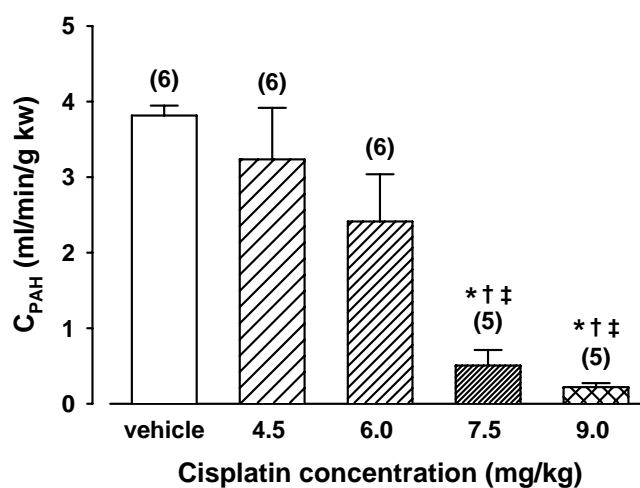
Student-Newman Keuls post hoc test).

1.3 Effects of cisplatin on the urine flow rate and urinary excretion of sodium

    and potassium

As shown in Figure 3.1b, urine flow rate ($\dot{V}$) of rats treated with 7.5 and 9 mg/kg cisplatin were 30.06 ± 5.40 and 39.69 ± 2.58 μl/min/g kw, respectively which increased significantly when compared to vehicle control (13.58 ± 2.85 μl/min/g kw).

Sodium excretion rate ($U_{Na}\dot{V}$) of all cisplatin-treated groups was not significantly changed when compared to vehicle as shown in Figure 3.3a. However, fractional excretion of sodium (FE_{Na}) of rats treated with 7.5 and 9 mg/kg cisplatin increased significantly to 6.48 ± 1.18 and $14.07 \pm 2.23\%$, respectively when compared to the vehicle group ($1.49 \pm 0.38\%$).

Potassium excretion rate ($U_K\dot{V}$) was unaltered with the two lower doses of cisplatin (4.5 and 6 mg/kg). However, $U_K\dot{V}$ decreased with the two higher doses (7.5 and 9 mg/kg) of cisplatin (0.23 ± 0.04 and 0.16 ± 0.02 mmol/min/g kw, respectively) when compared to the vehicle (0.56 ± 0.06 mmol/min/g kw). However, fractional excretion of potassium (FE_K) of all cisplatin-treated groups was unaltered when compared to vehicle group.

(a)
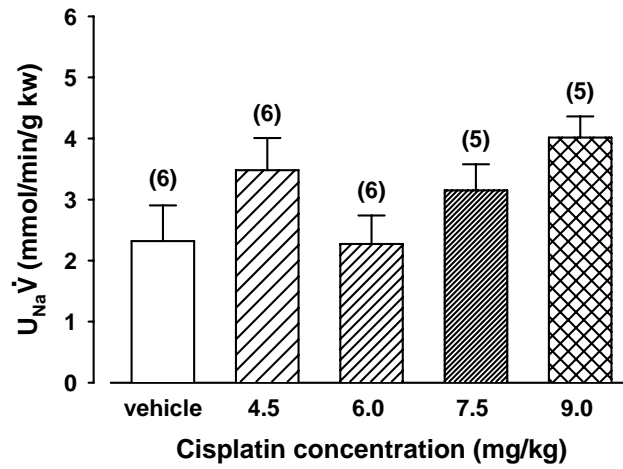
(b)

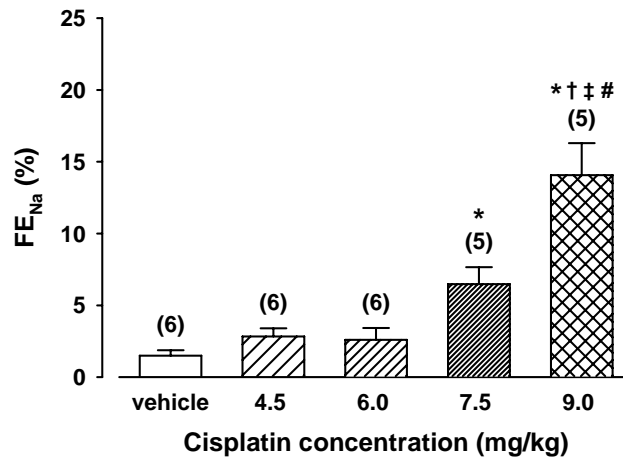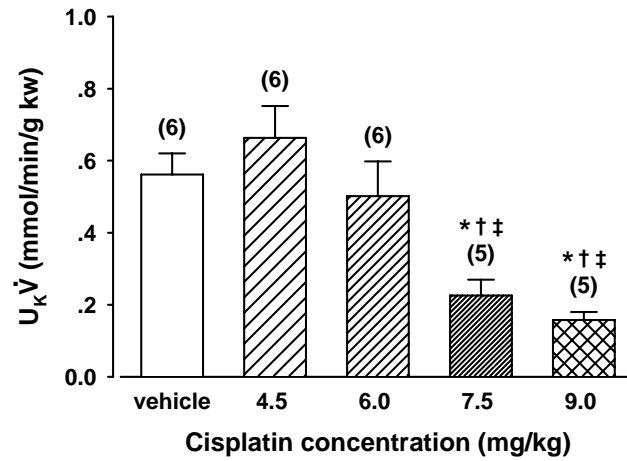**Figure 3.3** Effects of cisplatin on (a) sodium excretion rate ($U_{Na}\dot{V}$) and (b) fractional excretion of

sodium (FE_{Na}) in rats.

Data are mean ± S.E.M., parentheses indicate number of animal.

*, †, ‡ 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).

(a)

(b)
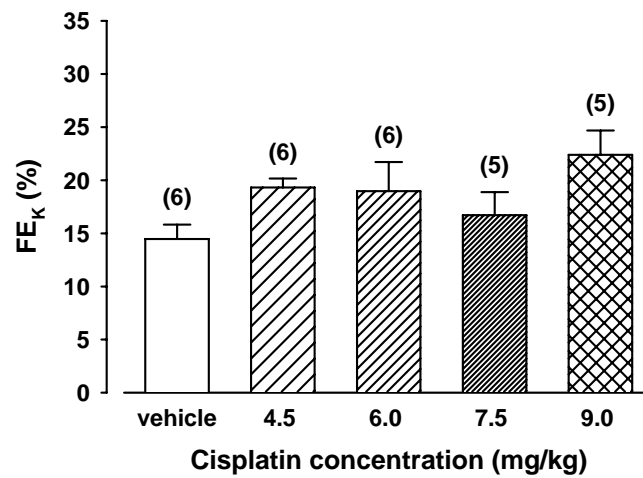
Figure 3.4 Effects of cisplatin on (a) potassium excretion rate ($U_K\dot{V}$) and (b) fractional excretion of

potassium (FE_K) in rats.

Data are mean ± S.E.M., parentheses indicate number of animal.

*, † and ‡ $P < 0.05$ compared to vehicle and cisplatin treated group at the doses of 4.5 and

6 mg/kg, respectively (one-way ANOVA with multiple comparison using

Student-Newman Keuls post hoc test).

1.4 Effects of cisplatin on plasma constituents

Figure 3.5 shows the effect of cisplatin on BUN. Two lower doses of cisplatin (4.5 and 6 mg/kg) did not change BUN levels. However, there were significantly increased in BUN with 7.5 and 9 mg/kg cisplatin (11.7 ± 1.8 and 16.1 ± 1.1 mmol/l, respectively) when compared to vehicle control (2.9 ± 0.2 mmol/l).

As shown in Table 3.1, plasma concentration of sodium in the highest dose of cisplatin (9 mg/kg) was significantly decrease to 127.9 ± 1.5 mmol/l when compared to vehicle (137.3 ± 1.5 mmol/l). A significant reduction in plasma potassium concentration was observed in rats treated with 9 mg/kg cisplatin (3.02 ± 0.17 mmol/l) when compared to the vehicle (3.79 ± 0.12 mmol/l).

Figure 3.5 Effect of cisplatin on blood urea nitrogen (BUN) in rats.

Data are mean $\pm$ S.E.M., parentheses indicate number of animal.

*, †, ‡ 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).
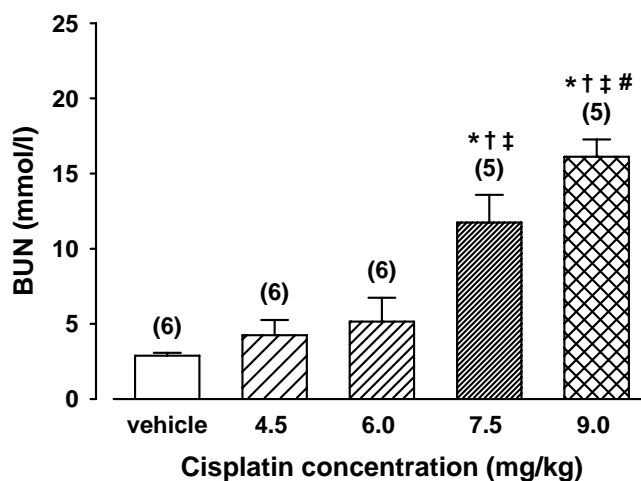
1.5 Effect of cisplatin on body weight

Table 3.1 shows the effect of cisplain on body weight (before and 3 days after cisplatin injection). No significant differences in the pre-study body weight were observed among all study groups. However, the significant reduction in post-study body weight was observed in 7.5 and 9 mg/kg cisplatin-treated groups (208.8 $\pm$ 5.2 and 201.6 $\pm$ 3.5 g, respectively). Injection of the two lower doses of cisplatin (4.5 and 6 mg/kg) did not affect body weight. However, cisplatin at the doses of 7.5 and 9 mg/kg significantly decreased body weight (-30.1 $\pm$ 2.9 and -34.1 $\pm$ 2.5 g, respectively) when compared to the vehicle and cisplatin at the doses of 4.5 and 6 mg/kg.

During the 3 days after cisplatin injection, diarrhea, reduction of food ingestion and water intake were observed in 7.5 and 9 mg/kg cisplatin-treated groups while these finding were less in 4.5 and 6 mg/kg cisplatin-treated groups. The bloating of the stomach in 7.5 and 9 mg/kg cisplatin treatment was observed at the end of clearance study but this was not found in vehicle group.

1.6 Effect of cisplatin on renal lipid peroxidation

Cisplatin at the doses of 4.5, 6, 7.5 and 9 mg/kg increased concentrations of renal MDA to 1.35 $\pm$ 0.06, 1.47 $\pm$ 0.14, 1.38 $\pm$ 0.06 and 1.25 $\pm$ 0.08 nmol/mg protein, respectively when compared to vehicle (0.97 $\pm$ 0.05 nmol/mg protein) as shown in Figure 3.6.

Table 3.1 Effects of cisplatin on body weight, heart rate (HR), hematocrit (Hct) and plasma

concentration of sodium and potassium (P_{Na} and P_K) during clearance study.

	vehicle (n=6)	Cisplatin concentration (mg/kg)			
		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	$208.8 \pm 5.2^{*\dagger\ddagger}$	$201.6 \pm 3.5^{*\dagger\ddagger}$
Body weight change (g)	6.0 ± 3.4	2.2 ± 9.3	-11.4 ± 6.2	$-30.1 \pm 2.9^{*\dagger\ddagger}$	$-34.1 \pm 2.5^{*\dagger\ddagger}$
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	$49.6 \pm 1.0^{*\dagger\ddagger}$	$53.0 \pm 1.9^{*\dagger\ddagger}$
P_{Na} (mmol/l)	137.3 ± 1.5	142.0 ± 0.8	137.6 ± 1.5	139.1 ± 2.5	$127.9 \pm 1.5^{*\dagger\ddagger\#}$
P_K (mmol/l)	3.79 ± 0.12	3.63 ± 0.09	3.50 ± 0.16	3.23 ± 0.18	$3.02 \pm 0.17^{*\dagger}$

Post-study body weight and blood samples were collected on day 3 after cisplatin injection.

Data are mean $\pm$ S.E.M. $*$, $\dagger$, $\ddagger$ 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

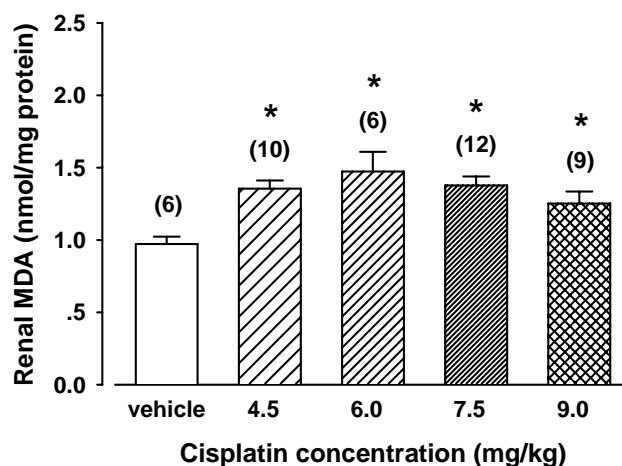Student-Newman Keuls post hoc test)

Figure 3.6 Effect of cisplatin on renal malondialdehyde (MDA) level in rats.

Data are mean $\pm$ S.E.M., parentheses indicate number of animal.

$*$ $P < 0.05$ compared to vehicle group (one-way ANOVA with multiple comparison using

Student-Newman Keuls post hoc test).

2. Effects of *Hibiscus sabdariffa* Linn. on cisplatin-induced acute renal failure rats

From part 1 of the experiment, cisplatin at the dose of 7.5 mg/kg is the minimal dose that induced ARF, indicated by the reduction in C_{in} (61%) and increase in BUN (4 folds). So in part 2 of the experiment, cisplatin at the dose of 7.5 mg/kg was used to induce ARF in rats.

2.1 Effects of short and long term treatments of *Hibiscus sabdariffa* Linn. on body weight, mean arterial blood pressure, heart rate, hematocrit and clearances of inulin and para-aminohippuric acid in cisplatin-induced ARF rats

As shown in Figure 3.7a and b and Table 3.2, short and long term treatments of HSE in either normal or in cisplatin-induced ARF rat did not significantly alter MABP, body weight change, HR and Hct when compared to their respective controls.

Both short and long term treatments of HSE did not cause any significant differences either C_{in} or C_{PAH} in normal rats as shown in Figure 3.8. Decrease in C_{in} and C_{PAH} in cisplatin-induced ARF rats (0.44 ± 0.08 and 0.51 ± 0.20 ml/min g kw, respectively) were not protected with the long term treatment of HSE. On the other hand, short term treatment of HSE showed a statistically significant increase either C_{in} or C_{PAH} (0.80 ± 0.17 and 2.16 ± 0.74 ml/min/g kw, respectively) in cisplatin-induced ARF rats.

2.2 Effects of short and long term treatments of *Hibiscus sabdariffa* Linn. on the urine flow rate and urinary excretion of sodium and potassium in cisplatin-induced ARF rats

Short and long term treatments of HSE in either normal or in cisplatin-induced ARF rats did not significantly alter $\dot{V}$ (Figure 3.7c and d), $U_{Na}\dot{V}$, FE_{Na} (Figure 3.9) and FE_K (Figure 3.10c and d) when compared to their respective controls. As shown in Figure 3.10a and b, decrease in $U_K\dot{V}$ in cisplatin-induced ARF rats was prevented by short term treatment of HSE (0.55 ± 0.12 mmol/min/g kw) but not by long term treatment of HSE (0.30 ± 0.07 mmol/min/g kw)

(a)

Short term treatment

(b)

Long term treatment

(c)

Short term treatment

(d)
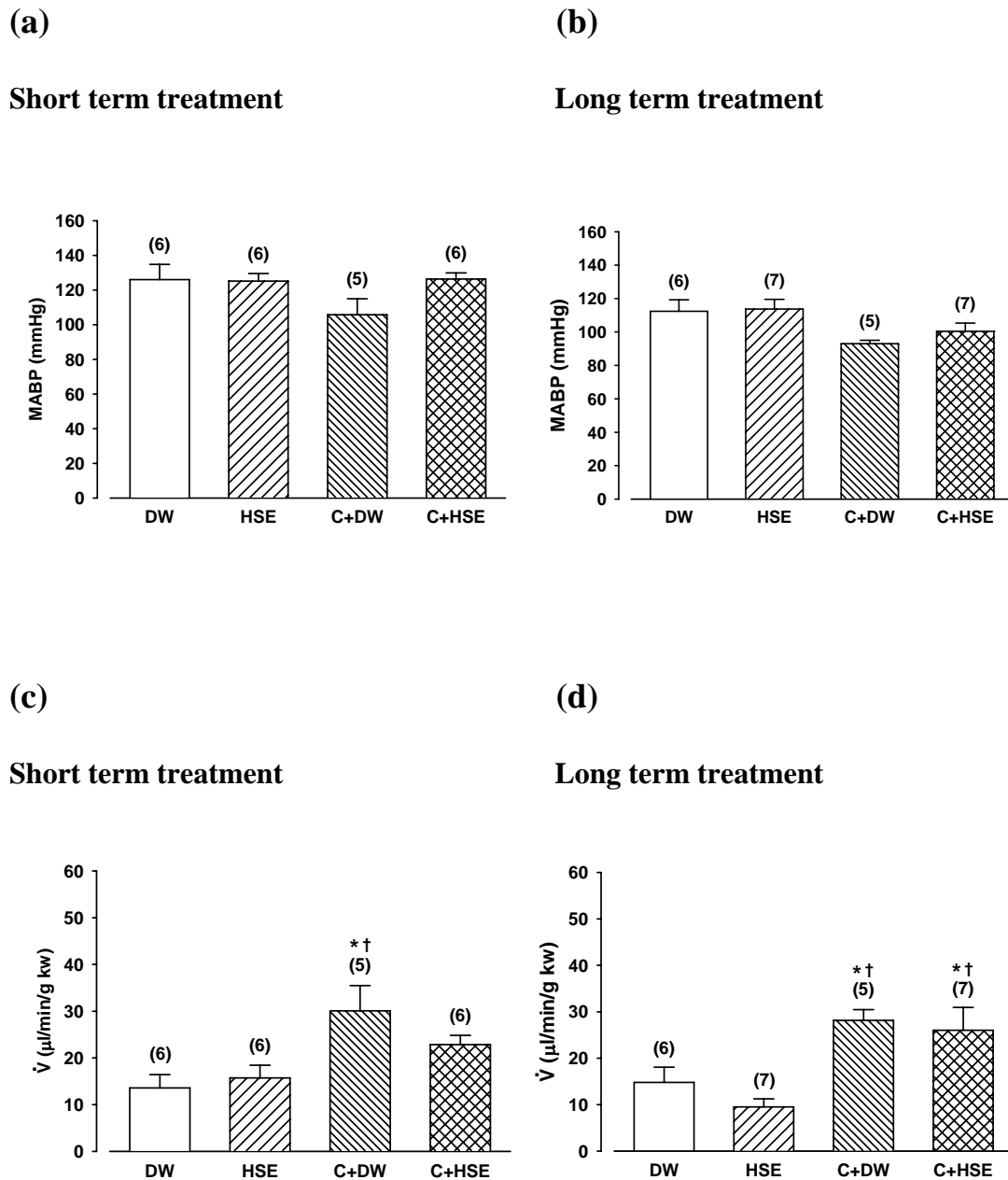
Long term treatment

Figure 3.7 Effects of short and long term treatments of *Hibiscus sabdariffa* Linn. extract on (a and b) mean arterial blood pressure (MABP) and (c and d) urine flow rate ($\dot{V}$) 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 ± S.E.M., parentheses indicate number of animals.

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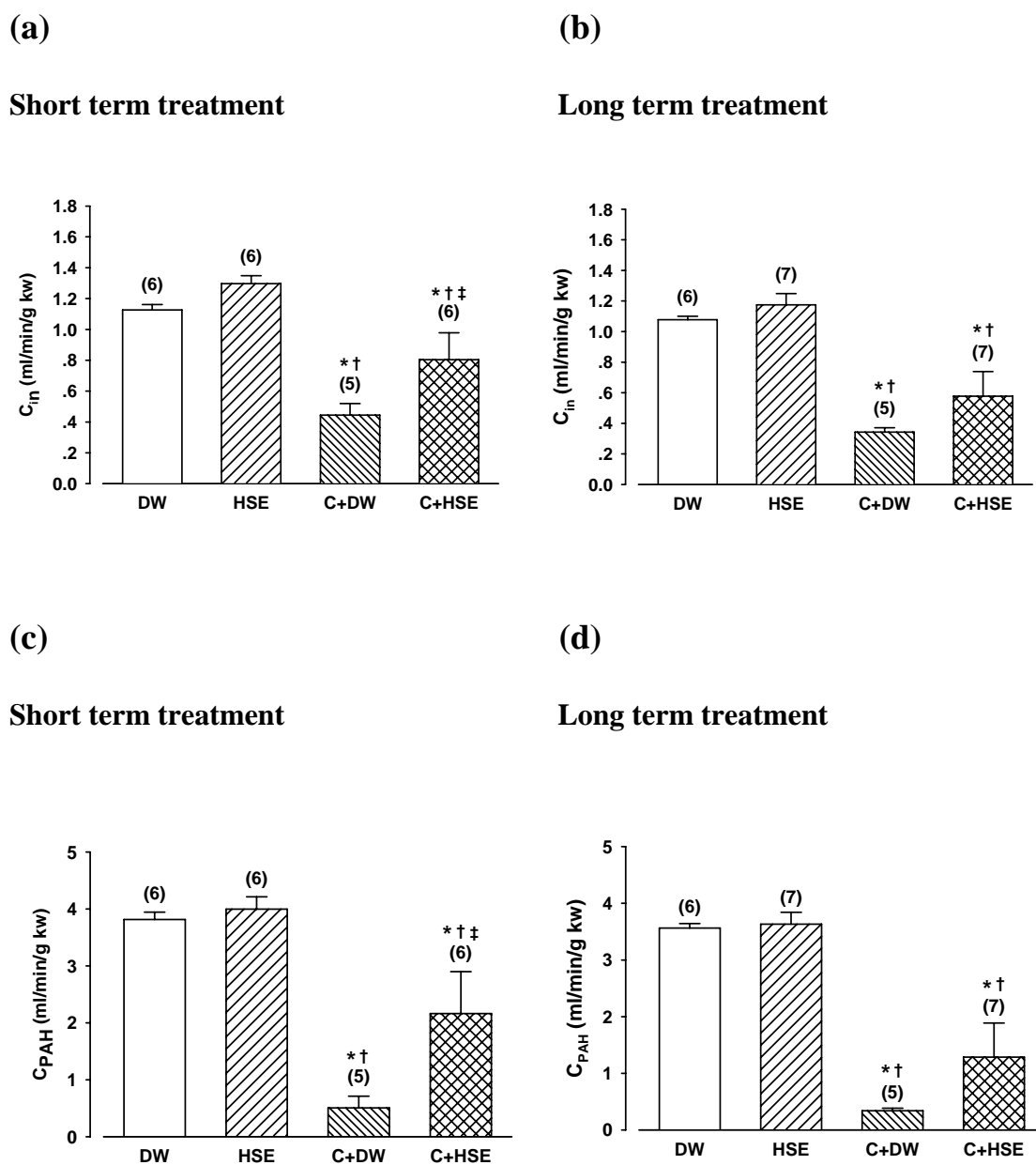
(a)

Short term treatment

(b)

Long term treatment

(c)

Short term treatment

(d)

Long term treatment

Figure 3.8 Effects of short and long term treatment of *Hibiscus sabdariffa* Linn. extract on (a and b) clearance of inulin (C_{in}) and (c and d) clearance of para-aminohippuric acid (C_{PAH}) 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 ± S.E.M., parentheses indicate number of animals.

*, † 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).

(a)

Short term treatment

(b)

Long term treatment

(c)

Short term treatment

(d)
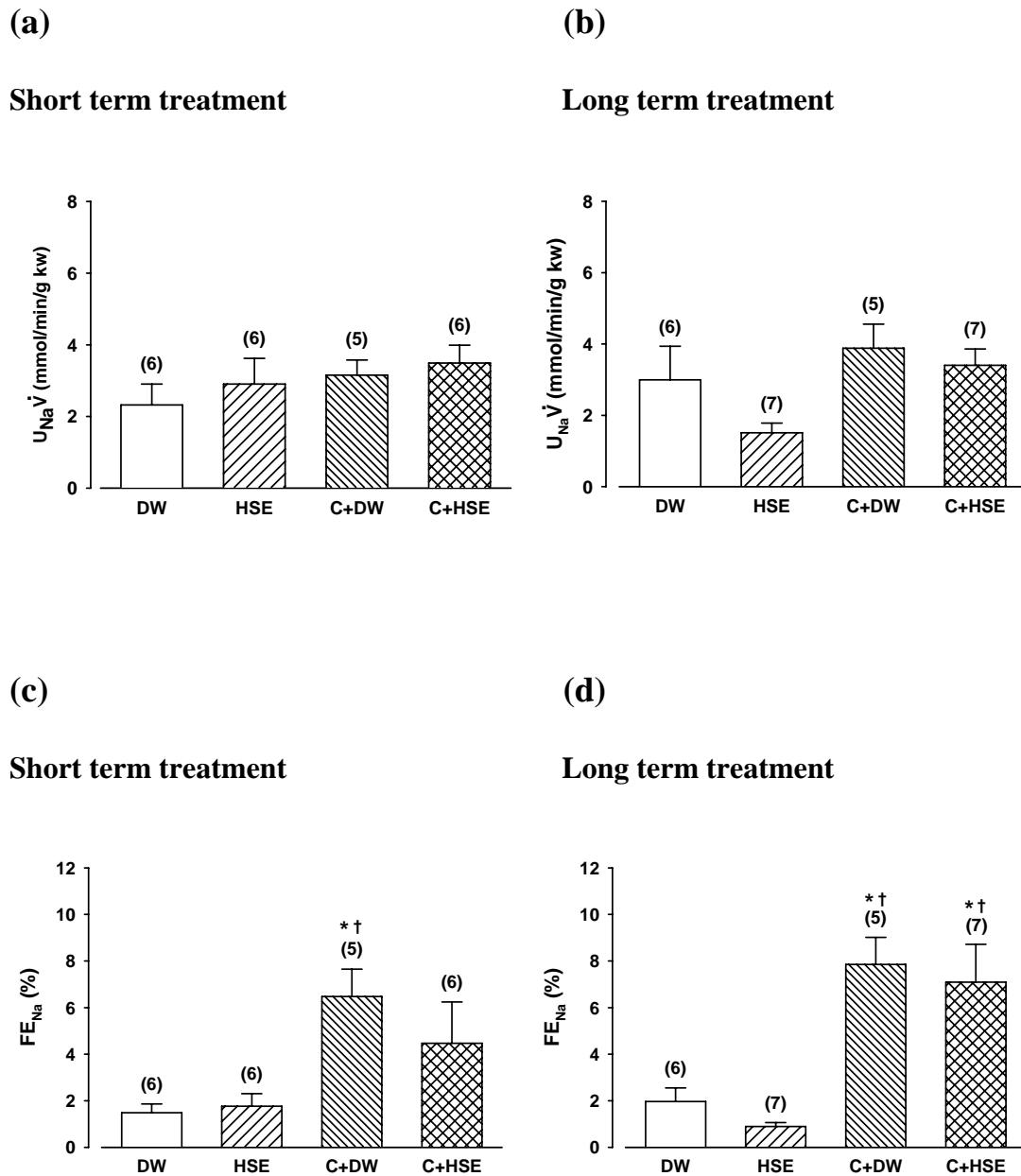
Long term treatment

Figure 3.9 Effects of short and long term treatment of *Hibiscus sabdariffa* Linn. extract on (a and b) sodium excretion rate ($U_{Na}\dot{V}$) and (c and d) fractional excretion of sodium (FE_{Na}) 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 ± S.E.M., parentheses indicate number of animals.

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

(a)

Short term treatment

(b)

Long term treatment

(c)

Short term treatment

(d)
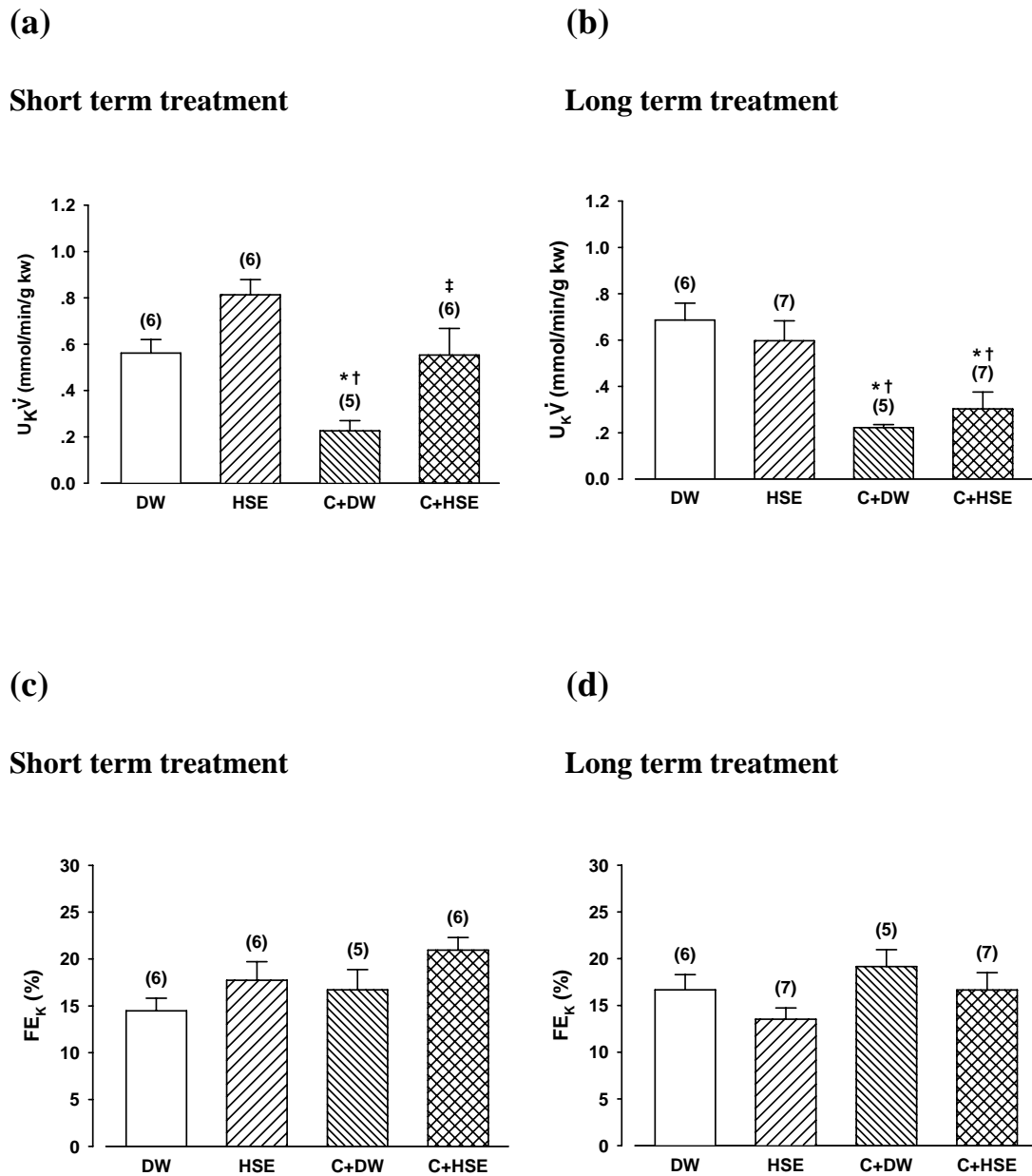
Long term treatment

Figure 3.10 Effects of short and long term treatment of *Hibiscus sabdariffa* Linn. extract on (a and b) potassium excretion rate ($U_K \dot{V}$) and (c and d) fractional excretion of potassium (FE_K) 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.

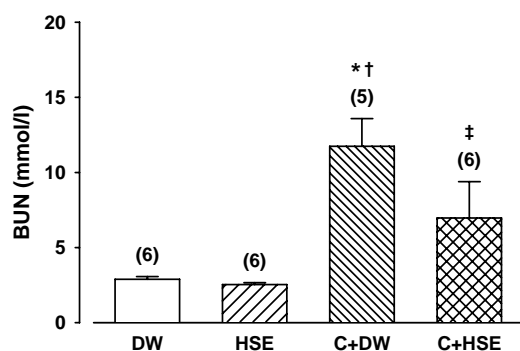Data are mean ± S.E.M., parentheses indicate number of animals.

*, † 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).

2.3 Effects of short and long term treatments of *Hibiscus sabdariffa* Linn. on plasma constituents in cisplatin-induced ARF rats

Both short and long term treatments of HSE showed a statistically significant decrease in BUN (7.0 ± 2.4 and 10.0 ± 2.1 mmol/l, respectively) in cisplatin-induced ARF rats as shown in Figure 3.11.

As shown in Table 3.2, short and long term treatments of HSE in either normal or in cisplatin-induced ARF rats did not significantly alter plasma concentration of potassium when compared to their respective controls. Short term treatment of HSE did not significantly alter plasma concentration of sodium in either normal or in cisplatin-induced ARF rats. Long term treatment of HSE significantly decreased plasma concentration of sodium to 131.7 ± 1.8 mmol/l in cisplatin-induced ARF rats.

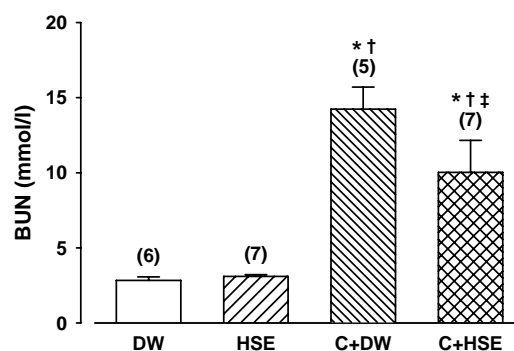**Short term treatment**   **Long term treatment**

Figure 3.11 Effect of short and long term treatment of *Hibiscus sabdariffa* Linn. extract on blood urea nitrogen (BUN) 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 ± S.E.M., parentheses indicate number of animals.

*, † 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).

2.4 Effect of short and long term treatments of *Hibiscus sabdariffa* Linn. on renal lipid peroxidation in cisplatin-induced ARF rats

As shown in Figure 3.12, short term treatment of HSE in cisplatin-induced ARF rats significantly decreased MDA level to 0.91 ± 0.08 nmol/mg protein when compared to cisplatin-induced ARF group (1.38 ± 0.06 nmol/mg protein). Long term treatment of HSE in normal rats significantly increase MDA level (1.55 ± 0.04 nmol/mg protein) when compared to vehicle control group (1.14 ± 0.06 nmol/mg protein). Cisplatin-induced increased MDA which was unaltered by long term treatment of HSE (1.45 ± 0.09 and 1.44 ± 0.12 nmol/mg protein, respectively).

Short term treatment   **Long term treatment**
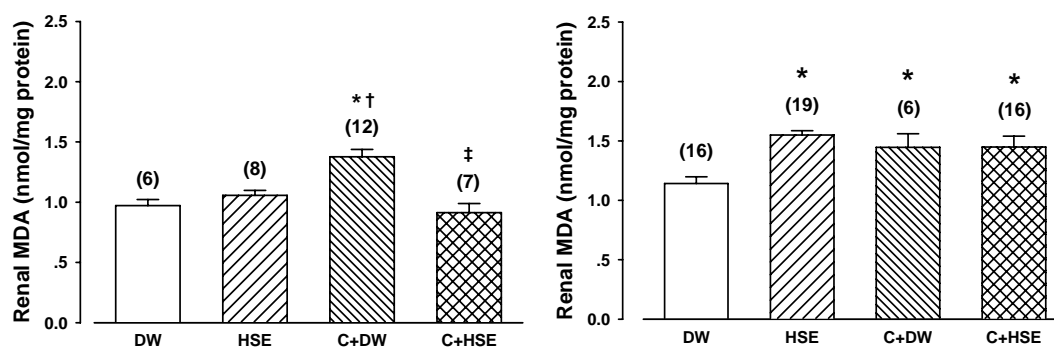

Figure 3.12   Effect of short and long term treatment of *Hibiscus sabdariffa* Linn. extract on renal malondialdehyde (MDA) level in 7.5 mg/kg cisplatin-induced ARF rats.

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 ± S.E.M., parentheses indicate number of animals.

*, † 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).

Table 3.2 Effects of short and long term treatments of *Hibiscus sabdariffa* Linn. extract on body weight, heart rate (HR), hematocrit (Hct) and plasma concentration of sodium and potassium (P_{Na} and P_K) 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)
Pre-study body weight (g)	223.4 ± 5.2	227.3 ± 5.1	238.9 ± 4.1	240.5 ± 2.1	234.3 ± 4.7	231.2 ± 5.3	218.2 ± 6.1	222.3 ± 5.1
Body weight at the injection day (g)	223.4 ± 5.2	230.8 ± 4.0	238.9 ± 4.1	246.8 ± 4.1	269.0 ± 4.0	239.6 ± 7.5	258.6 ± 9.0	248.3 ± 7.6
Post-study body weight (g)	229.4 ± 4.2	241.0 ± 8.3	208.8 ± 5.2	233.7 ± 9.8	278.7 ± 2.5	244.4 ± 6.0	225.2 ± 4.6	228.4 ± 11.1
Body weight change after injection (g)	6.0 ± 3.4	10.2 ± 6.5	$-30.1 \pm 2.9^{*\dagger}$	$-13.2 \pm 7.9^{*\dagger}$	9.7 ± 2.3	4.9 ± 1.9	$-33.4 \pm 6.0^{*\dagger}$	$-19.9 \pm 7.5^{*\dagger}$
HR (beat/min)	432 ± 18	460 ± 22	391 ± 13	407 ± 17	424 ± 17	429 ± 11	382 ± 7	398 ± 11
Hct (%)	41.8 ± 0.9	41.2 ± 0.7	$49.6 \pm 1.0^{*\dagger}$	$48.5 \pm 2.4^{*\dagger}$	43.3 ± 1.0	44.0 ± 1.7	48.6 ± 1.1	$50.0 \pm 1.8^{*\dagger}$
P_{Na} (mmol/l)	137.3 ± 1.5	138.0 ± 1.0	139.1 ± 2.5	135.6 ± 2.0	143.5 ± 1.4	139.7 ± 0.8	138.2 ± 1.8	$131.7 \pm 1.8^{*\dagger\ddagger}$
P_K (mmol/l)	3.79 ± 0.12	3.64 ± 0.16	3.23 ± 0.18	3.44 ± 0.22	3.78 ± 0.10	3.71 ± 0.10	3.35 ± 0.10	3.52 ± 0.21

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.

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