

## CHAPTER IV

### RESULT

#### Effects of Cobra Venom on General Circulation.

The results of changes in general circulation in dogs given normal saline solution alone (group I), crude cobra venom without pretreatment of various drugs (group II), crude cobra venom with pretreatment of intravenous infusion of verapamil (group III), cardiotoxic fraction of cobra venom (group IV), and cardiotoxic fraction with pretreatment of intravenous infusion of verapamil (group V) are shown in Table 1 - Table 4 and Figure 1 - Figure 7.

Group I : Control animal.

As shown in Table 1, the results are expressed on mean $\pm$ SEM. After intravenous saline infusion, all parameters did not indicate the significant changes throughout the experimental period.

Group II : Effects of crude cobra venom injection.

Animal pretreated with intravenous infusion of normal saline solution before crude cobra venom injection caused no changes in any of the measurements made. Envenomation in the first 15 minutes caused significant decrease in systolic blood pressure ( $P_s$ ) by average from  $157.5\pm 12$  to  $55\pm 8.4$  mmHg, diastolic blood pressure ( $P_d$ ) from  $92.5\pm 4.33$  to  $30\pm 5$  mmHg and mean arterial blood pressure from  $114.2\pm 6.6$  to  $38.3\pm 5.8$  mmHg. After that, the decrease of systolic and diastolic blood pressure was not significant and gradually increased to control level (Figure 1, panel a.). The significant decrease in pulse pressure was noted in 15, 30 and 45 minutes after envenomation from  $65\pm 8.7$  to  $25\pm 5.4$ ,  $33.8\pm 7.2$  and  $43.8\pm 8.3$  mmHg respectively.

There was no significant change in heart rate and respiratory rate. No significant change in packed cell volume was observed, except in the period of 45 minutes after venom injection, it fell from control value of  $32.6 \pm 3.9\%$  to  $28.9 \pm 4.2\%$  ( $P < 0.05$ ) (Table 3). At 60 minutes after envenomation, cardiac output decreased significantly from  $1.7 \pm 0.1$  L/min to  $1.3 \pm 0.2$  L/min (Table 4). In the same period, stroke volume decreased significantly from  $14.1 \pm 1.3$  ml/beat to  $9.8 \pm 0.9$  ml/beat (Table 4). As shown in Figure 5, total peripheral resistance slightly decreased at the first period of experiment. After 60 minutes of envenomation, it increased significantly from control value  $5.1 \pm 0.3 \times 10^3$  dynes - sec/cm<sup>5</sup> to  $7.8 \pm 0.7 \times 10^3$  dynes - sec/cm<sup>5</sup> ( $P < 0.05$ ) (Table 4).

Group III : Animal pretreated with verapamil before crude cobra venom injection.

Intravenous infusion of verapamil alone produced slight decrease in MAP from  $124.9 \pm 8.1$  mmHg to  $116.4 \pm 6.7$  mmHg but there was no significant change (Table 2). Heart rate decreased significantly from  $162 \pm 5$  beats/time to  $152 \pm 7$  beats/time ( $6.4 \pm 2.1\%$ ) (Figure 4). Cobra venom caused significant decrease in mean arterial pressure along the experiment period (Table 2). There were no significant change of respiratory rate and packed cell volume (Table 3). As shown in Figure 5 (panel a), packed cell volume increased slightly by approximately  $6.3 \pm 5.6\%$  at the second period of the experiment (Figure 5). At 15 minutes after envenomation, cardiac output and stroke volume decreased significantly from  $1.7 \pm 0.2$  L/min to  $1.4 \pm 0.2$  L/min and from  $11.2 \pm 0.9$  ml/beat to  $9.4 \pm 1.0$  ml/beat respectively (Table 4). Total peripheral resistance slightly increased in the experimental period (Table 4 and Figure 5).

Group IV : Effects of cardiotoxic fraction of cobra venom injection.

Animal pretreated with intravenous infusion of normal saline solution before cardiotoxic fraction of injection caused no significant changes in any of the measurements made. As shown in Figure 2 (panel a), the significant decreases in systolic and diastolic blood pressure were noted after cardiotoxic fraction administration. Cardiotoxic fraction injection caused a marked decrease in mean



arterial pressure from the control value of  $112 \pm 9.8$  mmHg to  $58.7 \pm 20.3$  mmHg and  $67.7 \pm 16.9$  mmHg at 15 minutes and 30 minutes after envenomation (Table 2). Pulse pressure slightly decreased from the control value to  $42.4 \pm 14.9\%$ ,  $44.3 \pm 16.7\%$  and  $16.1 \pm 6.8\%$  at 15, 30 and 60 minutes after cardiotoxic fraction administration, respectively (Figure 3). There was no significant alteration in heart rate during the experimental period (Table 2). Respiratory rate significantly increased at 30 minutes, 45 minutes and 60 minutes after cardiotoxic fraction administration as shown in Table 3. Packed cell volume slightly increased to  $38 \pm 14.9\%$  of the control value in the period of 30 minutes after cardiotoxic fraction administration (Figure 5). Cardiac output decreased to  $27 \pm 13.7\%$  without significant change from the control value whereas stroke volume significantly decreased to  $22.3 \pm 5.8\%$  of the control value (Figure 6). There was also no significant alteration in total peripheral resistance during the experimental period (Table 4).

Group V : Animal pretreated with verapamil before cardiotoxic fraction of cobra venom injection.

Intravenous infusion of verapamil alone produced slightly decreased of mean arterial blood pressure from  $103.8 \pm 13.7$  mmHg to  $98.3 \pm 13.2$  mmHg but there was no significant change (Table 2). After 15 minutes of cardiotoxic fraction administration, only systolic blood pressure significantly increased from pretreatment value of  $150 \pm 20.7$  mmHg to  $156.3 \pm 19.7$  mmHg (Figure 2, panel b). In the first period of the experiment, mean arterial blood pressure slightly decreased from control value and there was gradually increased (Figure 3). No significantly change in pulse pressure, heart rate, respiratory rate and packed cell volume was observed (Table 2 and Table 3). Cardiac output significantly decreased from the control value of  $1.9 \pm 0.2$  L/min to  $1.6 \pm 0.2$  L/min and  $1.5 \pm 0.3$  L/min in 30 minutes and 60 minutes after cardiotoxic fraction administration, respectively (Table 4). Stroke volume showed no significant decrease from the control value of  $15.2 \pm 1.5$  ml/beat to  $12.9 \pm 1.5$  ml/beat and  $13 \pm 2.0$  ml/beat in 30 minutes and 60 minutes after cardiotoxic fraction administration, respectively. Total peripheral resistance slightly increased (Table 4).

**Table 1.** Changes in mean arterial blood pressure, pulse pressure, heart rate, respiratory rate, packed cell volume, cardiac output, stroke volume and total peripheral resistance in control dogs (n=6).

Parameters	NSS Intravenous Infusion		
	Control	30 min	60 min
MAP (mmHg)	105.3 ± 8.7	103.3 ± 8.2 NS	110.3 ± 7.5 NS
PP (mmHg)	65.8 ± 10.3	60.0 ± 5.6 NS	58.3 ± 7.3 NS
HR (beats/min)	140 ± 7	133 ± 9 NS	129 ± 13 NS
RR (breaths/min)	22 ± 5	20 ± 2 NS	18 ± 2 NS
PCV (%)	27.8 ± 3.3	26.7 ± 3.7 NS	26.6 ± 3.6 NS
CO (L/min)	1.4 ± 0.1	1.2 ± 0.2 NS	1.6 ± 0.3 NS
SV (ml/beat)	9.7 ± 1.0	9.1 ± 1.0 NS	12.1 ± 1.6 NS
TPR (10 <sup>3</sup> dynes-sec/cm <sup>5</sup> )	6.6 ± 1.2	7.1 ± 1.0 NS	6.4 ± 1.3 NS

Values are mean ± SEM. Abbreviations: MAP, mean arterial blood pressure; PP, pulse pressure; HR, heart rate; RR, respiratory rate; PCV, packed cell volume; CO, cardiac output; SV, stroke volume; TPR, total peripheral resistance.

Significant difference values using paired t-test are indicated by \* P < 0.05 different from control values, NS = not significant.



Table 2. Changes in mean arterial blood pressure, pulse pressure and heart rate in response to crude cobra venom and/ or its cardiotoxic fraction administration in four groups.

	Control	Venom administration				
		15 min	30 min	45 min	60 min	
<b>MAP (mmHg)</b>						
II. NSS + CV (n=4)	114.2 ± 6.6	115.8 ± 7.7 NS	38.3 ± 5.8 †	93.8 ± 9.4 NS	113.6 ± 6.7 NS	119.2 ± 8.9 NS
III. Ver + CV (n=6)	124.9 ± 8.1	116.4 ± 6.7 NS	45.3 ± 8.5 †	92.5 ± 10.4 †	101.1 ± 8.4 *	114.2 ± 8.5 *
IV. NSS + CTX (n=5)	112.0 ± 9.8	110.7 ± 8.1 NS	58.7 ± 20.3 †	67.7 ± 16.9 †	78.0 ± 16.1 NS	81.6 ± 15.3 NS
V. Ver + CTX (n=4)	103.8 ± 13.7	98.3 ± 13.2 NS	101.3 ± 12.1 NS	103.3 ± 12.1 NS	107.9 ± 8.4 NS	107.1 ± 10.4 NS
<b>PP (mmHg)</b>						
II. NSS + CV (n=4)	65.0 ± 8.7	66.3 ± 8.5 NS	25.0 ± 5.4 †	33.8 ± 7.2 †	43.8 ± 8.3 †	53.8 ± 11.4 NS
III. Ver + CV (n=6)	60.0 ± 7.6	64.2 ± 10.2 NS	30.8 ± 6.5 †	32.5 ± 5.1 †	53.3 ± 7.6 NS	60.0 ± 11.5 NS
IV. NSS + CTX (n=5)	57.0 ± 6.4	50.0 ± 7.7 NS	32.0 ± 9.7 NS	32.0 ± 11.5 NS	39.0 ± 10.0 NS	46.6 ± 4.5 NS
V. Ver + CTX (n=4)	67.5 ± 4.8	77.5 ± 12.0 NS	82.5 ± 12.0 NS	81.3 ± 11.1 NS	80.0 ± 16.7 NS	81.3 ± 11.1 NS
<b>HR (beats/min)</b>						
II. NSS + CV (n=4)	119 ± 9	123 ± 8 NS	123 ± 19 NS	121 ± 16 NS	130 ± 16 NS	124 ± 20 NS
III. Ver + CV (n=6)	162 ± 5	152 ± 7 *	124 ± 7 †	142 ± 10 *	156 ± 11 NS	153 ± 11 NS
IV. NSS + CTX (n=5)	126 ± 22	134 ± 21 *	123 ± 10 NS	117 ± 13 NS	126 ± 15 NS	137 ± 14 NS
V. Ver + CTX (n=4)	137 ± 7	126 ± 4 NS	126 ± 3 NS	124 ± 6 NS	121 ± 10 NS	120 ± 10 NS

Values are mean ± SEM. Abbreviations: CV, crude cobra venom; CTX, cardiotoxic fraction; Ver, verapamil; MAP, mean arterial blood pressure; PP, pulse pressure; HR, heart rate.

Significant difference values using paired t-test are indicated by \* P<0.05 different from control values, † P<0.05 different from pretreatment values and NS = not significant.

Table 3. Changes in respiratory rate, and packed cell volume in response to crude cobra venom and/ or its cardiotoxic fraction administration in four groups.

	Control	Venom administration				
		Pretreatment	15 min	30 min	45 min	60 min
RR (breaths/min)						
II. NSS + CV (n=4)	14 ± 5	14 ± 5 NS	15 ± 3 NS	13 ± 3 NS	18 ± 5 NS	16 ± 4 NS
III. Ver + CV (n=6)	15 ± 3	18 ± 2 NS	21 ± 2 NS	22 ± 2 †	29 ± 4 †	27 ± 3 †
IV. NSS + CTX (n=5)	12 ± 1	13 ± 2 *	20 ± 6 NS	20 ± 2 †	21 ± 1 †	20 ± 2 †
V. Ver + CTX (n=4)	14 ± 4	15 ± 3 NS	16 ± 2 NS	16 ± 3 NS	15 ± 3 NS	14 ± 2 NS
PCV (%)						
II. NSS + CV (n=4)	32.6 ± 3.9	31.0 ± 4.1 NS	31.1 ± 4.7 NS	31.6 ± 4.4 NS	28.9 ± 4.2 *	28.8 ± 4.7 NS
III. Ver + CV (n=6)	26.6 ± 1.8	27.1 ± 1.7 NS	27.0 ± 2.4 NS	28.3 ± 2.3 NS	26.8 ± 1.9 NS	27.3 ± 2.1 NS
IV. NSS + CTX (n=5)	29.6 ± 1.5	29.9 ± 1.4 NS	38.4 ± 7.2 NS	41.4 ± 6.2 NS	39.4 ± 5.7 NS	38.4 ± 5.5 NS
V. Ver + CTX (n=4)	26.3 ± 3.7	28.3 ± 4.4 NS	28.3 ± 4.4 NS	28.3 ± 4.4 NS	28.3 ± 4.4 NS	28.3 ± 4.4 NS

Values are mean ± SEM. Abbreviations: CV, crude cobra venom; CTX, cardiotoxic fraction; Ver, versipamli; RR, respiratory rate; PCV, packed cell volume.

Significant difference values using paired t-test are indicated by \* P<0.05 different from control values, † P<0.05 different from pretreatment values and NS = not significant.

**Table 4.** Changes in cardiac output, stroke volume, and total peripheral resistance in response to crude cobra venom and/ or its cardiotoxic fraction administration in four groups.

		Venom administration	
		Pretreatment	30 min      60 min
<b>CO (L/min)</b>			
II. NSS + CV (n=4)	1.7 ± 0.1	1.7 ± 0.6 NS	1.3 ± 0.2 †
III. Ver + CV (n=6)	1.7 ± 0.2	1.4 ± 0.2 †	1.5 ± 0.3 NS
IV. NSS + CTX (n=5)	1.2 ± 0.2	0.9 ± 0.3 NS	1.2 ± 0.2 NS
V. Ver + CTX (n=4)	1.9 ± 0.2	1.6 ± 0.2 †	1.5 ± 0.3 †
<b>SV (ml/beat)</b>			
II. NSS + CV (n=4)	14.1 ± 1.3	14.8 ± 6.1 NS	9.8 ± 0.9 †
III. Ver + CV (n=6)	11.2 ± 0.9	9.4 ± 1.0 †	9.8 ± 1.3 NS
IV. NSS + CTX (n=5)	9.7 ± 1.9	7.4 ± 1.6 NS	8.2 ± 0.8 NS
V. Ver + CTX (n=4)	15.2 ± 1.5	12.9 ± 1.5 NS	13.0 ± 2.0 †
<b>TPR (10<sup>3</sup> dynes-sec/cm<sup>5</sup>)</b>			
II. NSS + CV (n=4)	5.1 ± 0.3	4.1 ± 0.9 NS	7.8 ± 0.7 †
III. Ver + CV (n=6)	5.6 ± 0.4	6.1 ± 1.3 NS	6.8 ± 1.1 NS
IV. NSS + CTX (n=5)	8.7 ± 2.3	7.0 ± 1.6 NS	6.7 ± 1.1 NS
V. Ver + CTX (n=4)	4.2 ± 0.6	5.4 ± 1.0 NS	5.9 ± 0.9 NS

Values are mean ± SEM. Abbreviations: CV, crude cobra venom; CTX, cardiotoxic fraction; Ver, Verapamil; CO, cardiac output; SV, stroke volume; TPR, total peripheral resistance.

Significant difference values using paired t-test are indicated by † P<0.05 different from pretreatment values and NS = not significant.



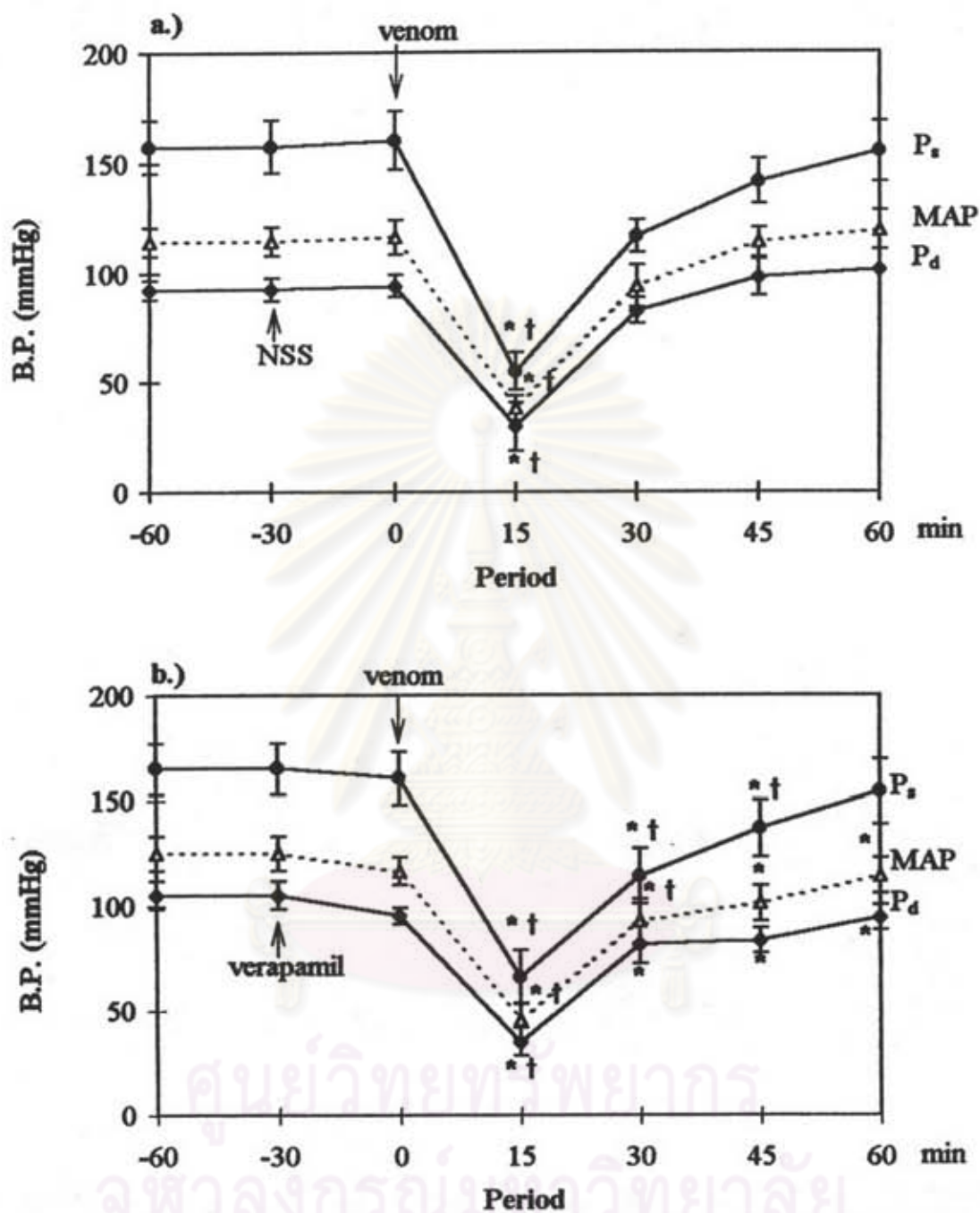


Figure 1. Change in blood pressure in response to crude cobra venom (CV) alone (panel a.) and pretreated intravenous infusion with verapamil (ver) (panel b.) Significant differences compared to the control value using paired t-test are indicated by \*  $P < 0.05$  and †  $P < 0.05$  compared to the pretreatment value of each group.



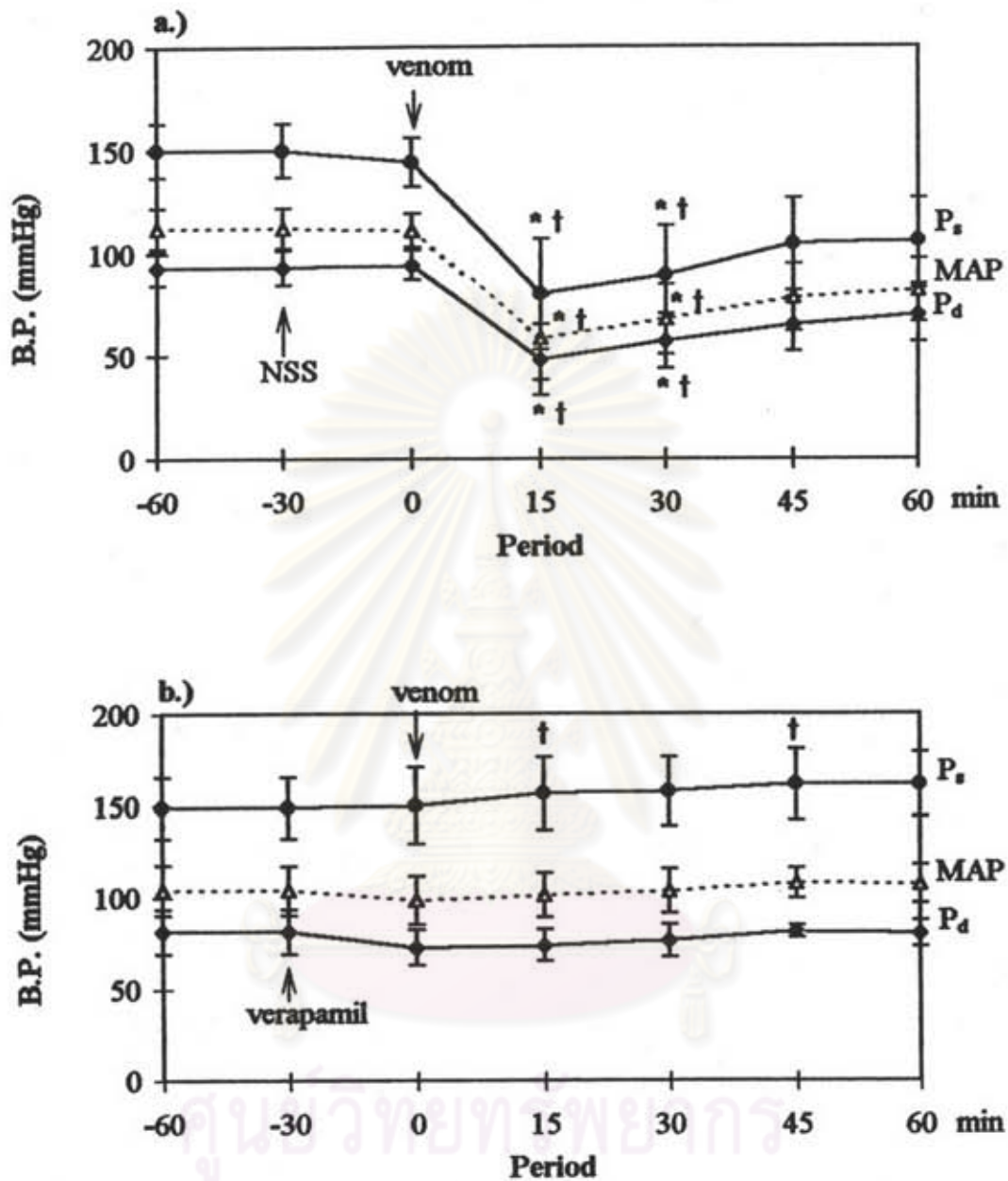


Figure 2. Change in blood pressure in response to cardiotoxic fraction (CTX) alone (panel a.) and pretreated intravenous infusion with verapamil (Ver) (panel b.) Significant differences compared to the control value using paired t-test are indicated by \*  $P < 0.05$  and †  $P < 0.05$  compared to the pretreatment value of each group.

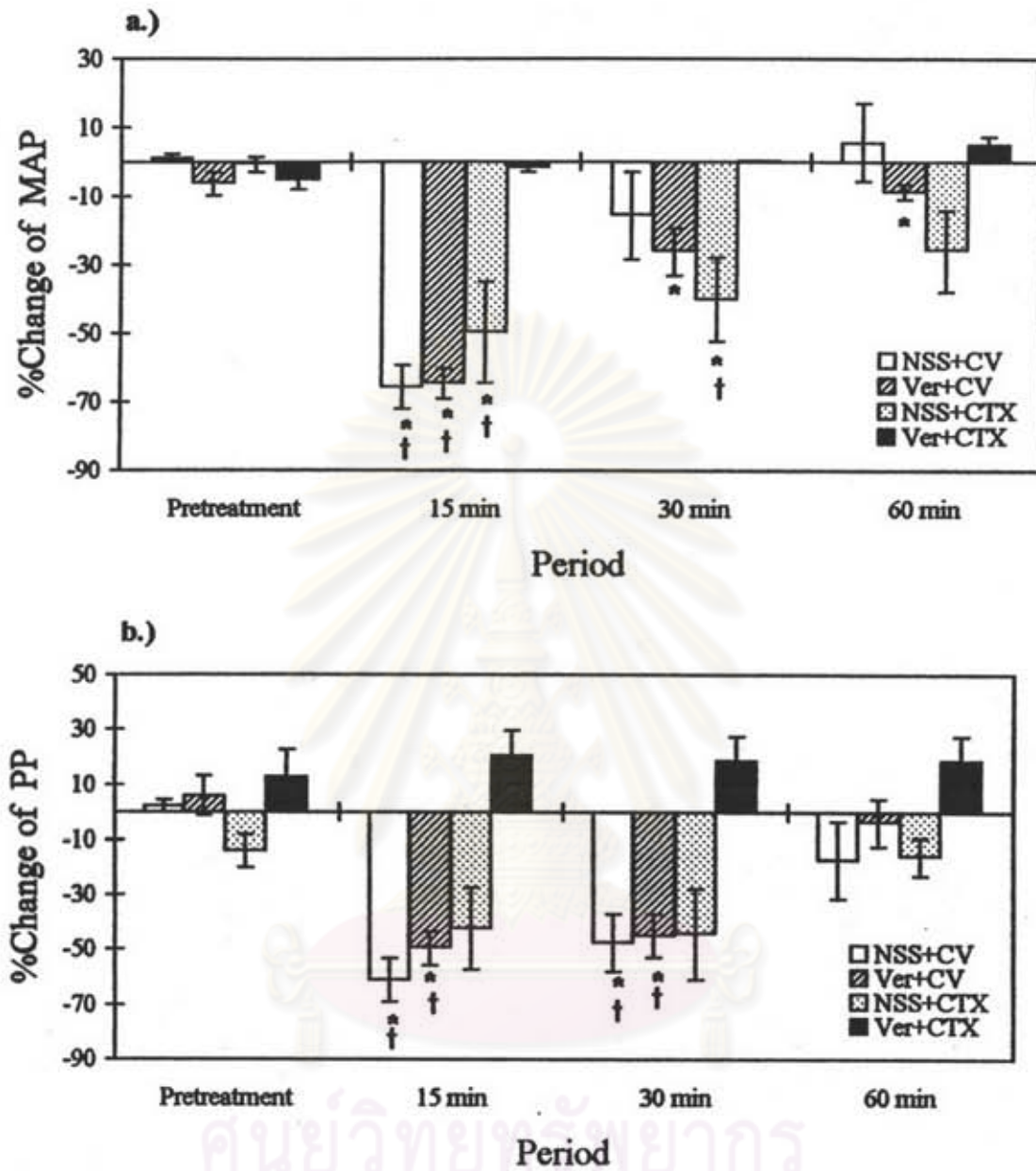


Figure 3. Percentage changes in mean arterial blood pressure (MAP) (a.) and pulse pressure (PP) (b.) in response to crude cobra venom (CV) and / or its cardiotoxic fraction (CTX) and pretreated intravenous infusion with normal saline solution (NSS) and/or verapamil (Ver).

Significant difference values using paired t-test are indicated by \*  $P < 0.05$  compared to the control value and †  $P < 0.05$  compared to the pretreatment value of each group.

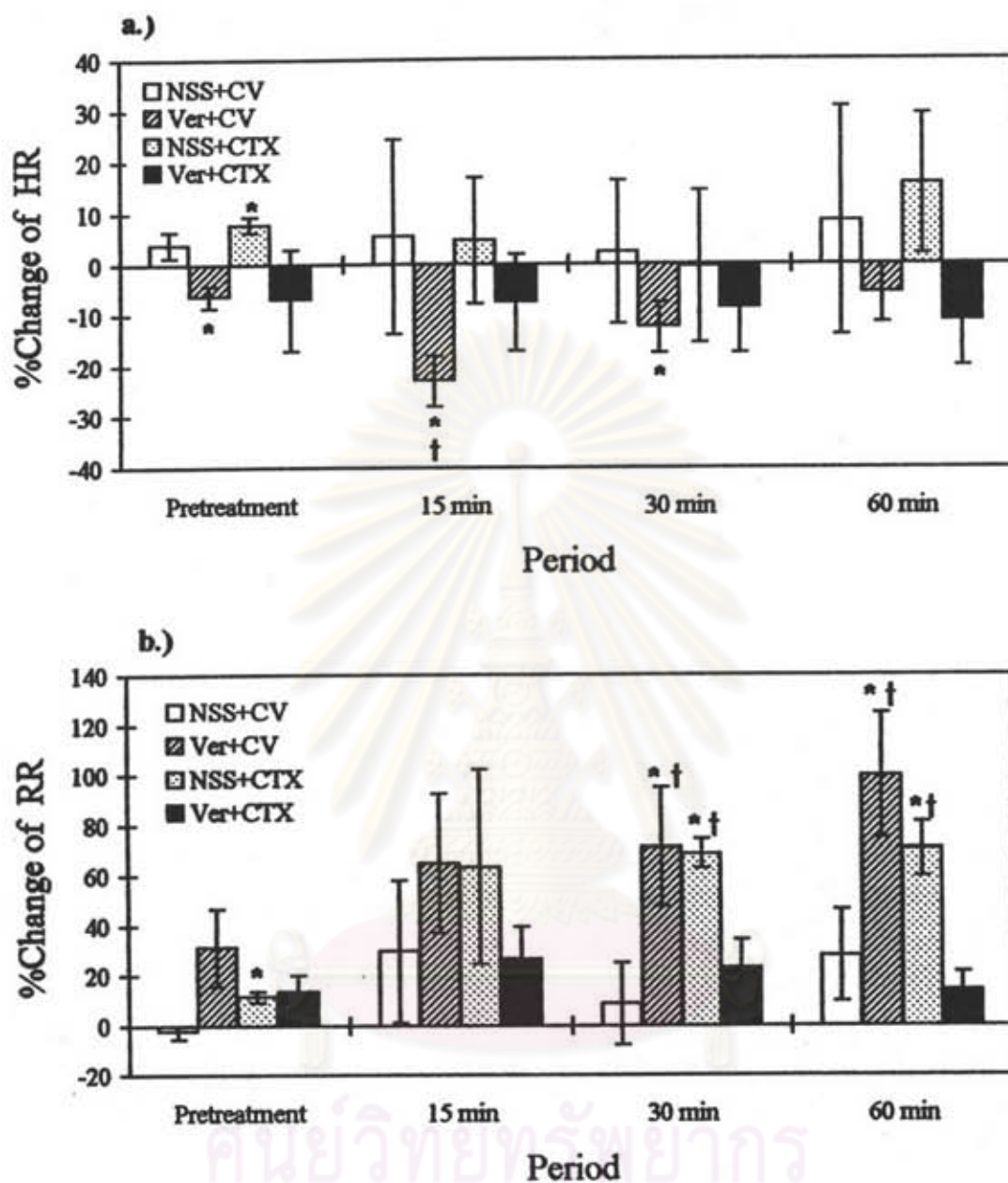


Figure 4. Percentage changes in heart rate (HR) (a.) and respiratory rate (RR) (b.) in response to crude cobra venom ( CV ) and / or its cardiotoxic fraction (CTX) and pretreated intravenous infusion with normal saline solution (NSS) and/or verapamil (Ver).

Significant difference values using paired t-test are indicated by \*  $P < 0.05$  compared to the control value and †  $P < 0.05$  compared to the pretreatment value of each group.



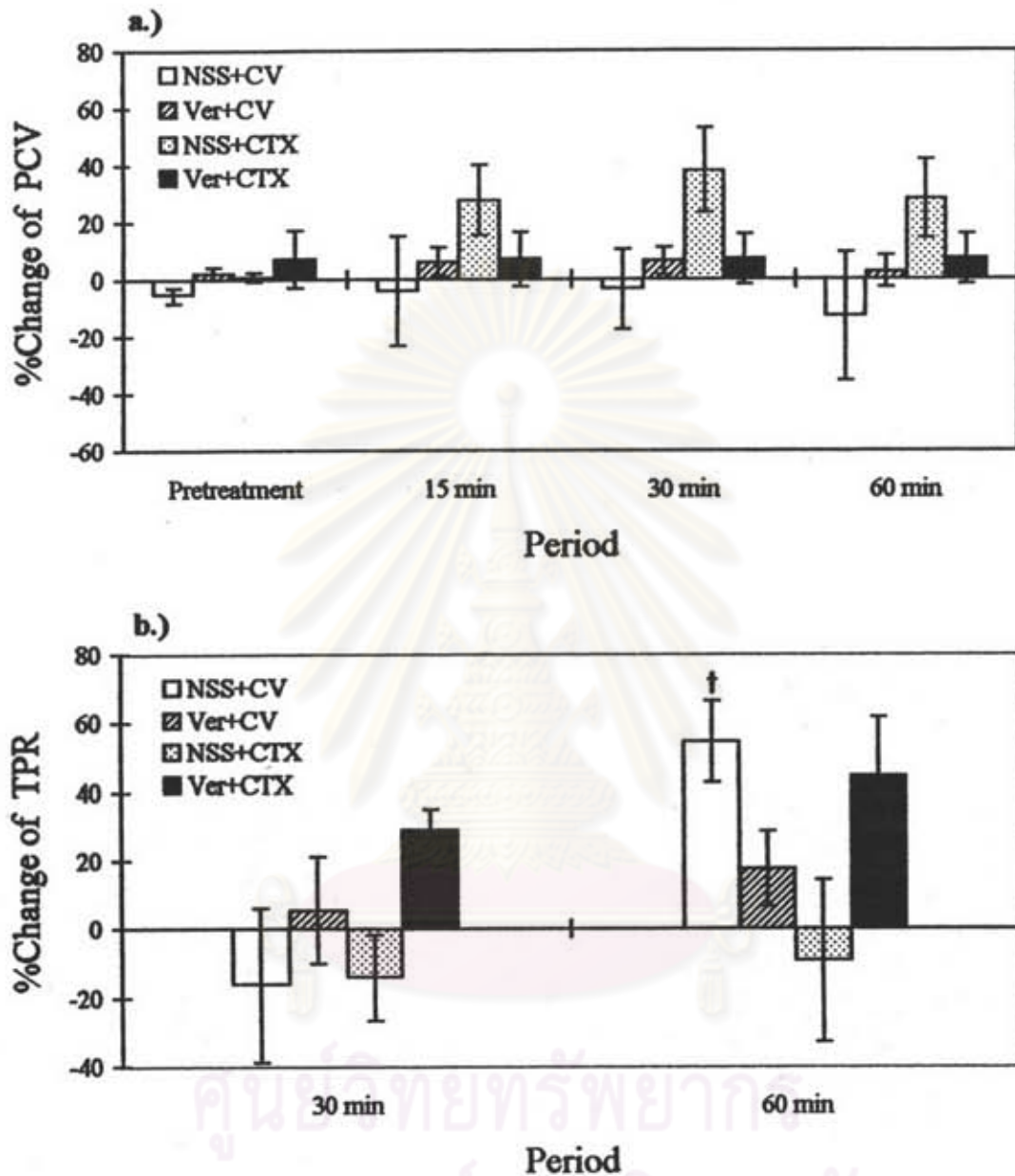


Figure 5. Percentage changes in packed cell volume (PCV) (a.) and total peripheral resistance (TPR) (b.) in response to crude cobra venom ( CV ) and / or its cardiotoxic fraction ( CTX ) and pretreated intravenous infusion with normal saline solution ( NSS ) and/or verapamil ( Ver ).

Significant difference values using paired t-test are indicated by \*  $P < 0.05$  compared to the control value and †  $P < 0.05$  compared to the pretreatment value of each group.

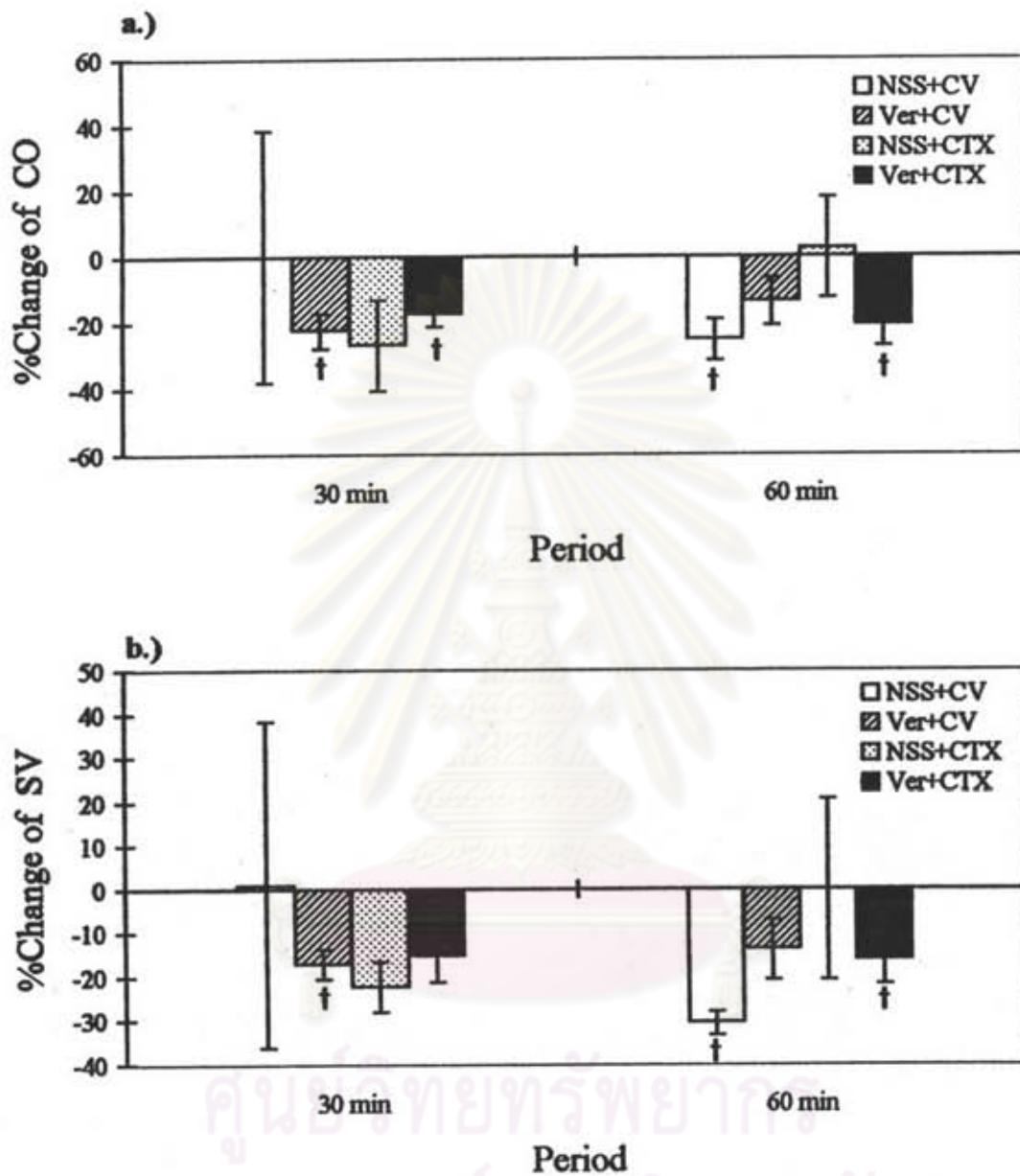
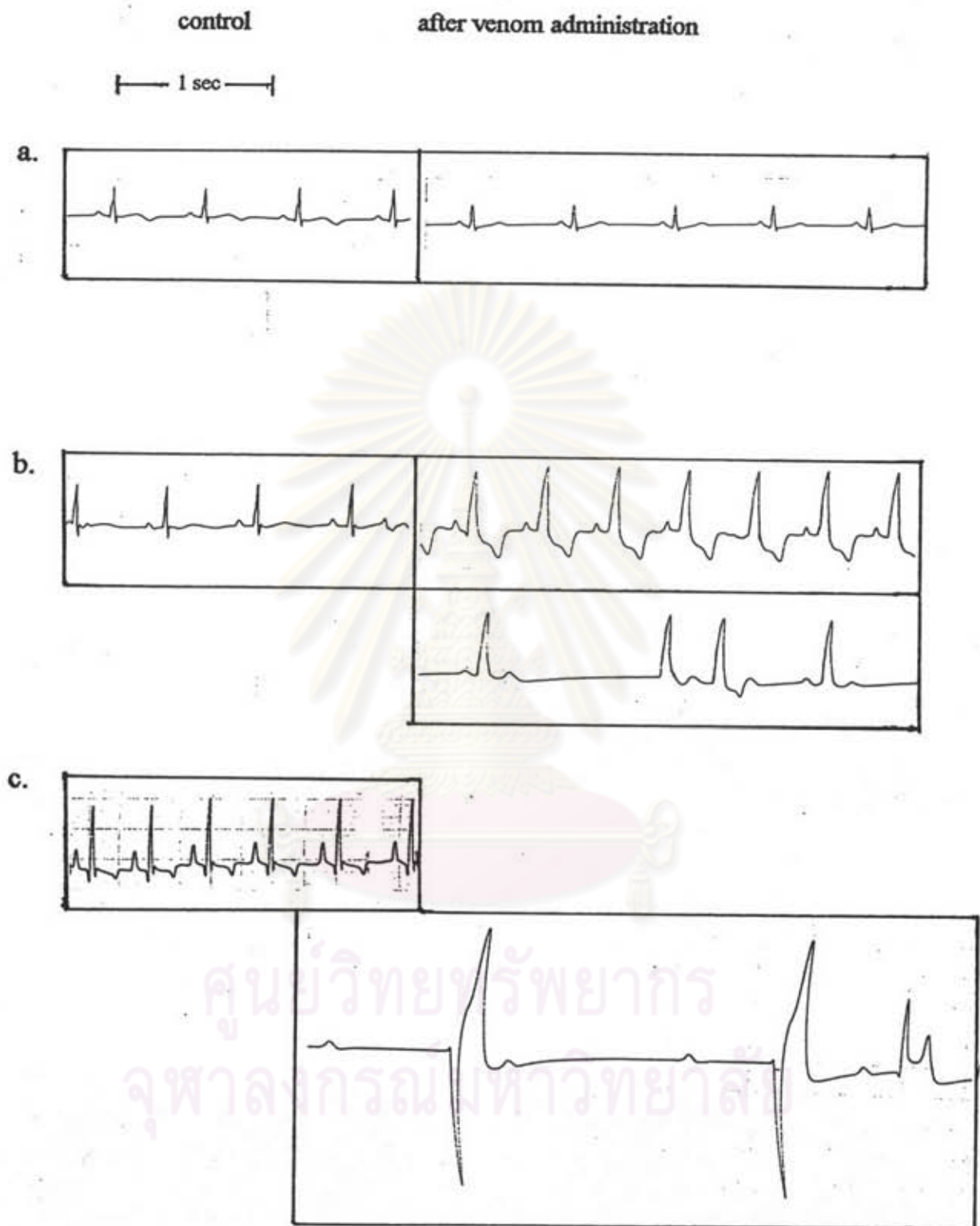


Figure 6. Percentage changes in cardiac output (CO) (a.) and stroke volume (SV) (b.) in response to crude cobra venom (CV) and / or its cardiotoxic fraction (CTX) and pretreated intravenous infusion with normal saline solution (NSS) and/or verapamil (Ver).

Significant difference values using paired t-test are indicated by \*  $P < 0.05$  compared to the control value and †  $P < 0.05$  compared to the pretreatment value of each group.



**Figure 7. Electrocardiogram of dogs after cobra venom administration**



### Effects of Cobra Venom on the Heart.

The results of changes in electrocardiogram in dogs given cobra venom are shown in Figure 7.

Cobra venom produced ST segment depression (panel b., upper), ectopic beats (panel b., lower), ventricular premature contraction and third degree A-V block (panel c.). Cardiotoxic fraction of cobra venom produced similar results but T wave inversion was the first changes that occurring within a minute or two of the injection (panel a.).

### Effects of Cobra Venom on Renal Hemodynamics.

The results of changes in renal hemodynamics in dogs given cobra venom are shown in Table 5 - Table 7 and Figure 8 - Figure 10.

Group I : Control animal.

As shown in Table 5, the results are expressed on mean $\pm$ SEM. After intravenous saline infusion, all parameters did not indicate the significant changes throughout the experimental period.

Group II : Effects of crude cobra venom injection.

Animal pretreated with intravenous infusion of normal saline solution before crude cobra venom injection caused no changes in any of the measurements made (Table 6 and Table 7). Renal vascular resistance (RVR) insignificantly increased to  $108.3\pm 112.8\%$  of the control value after 30 minutes of envenomation (Figure 10), there was insignificant decrease in effective renal plasma flow (ERPF) to  $48.5\pm 19.6\%$  of control value and effective renal blood flow (ERBF) to  $49.6\pm 18.5\%$  of control value (Figure 9) which resulted in a significant decrease in glomerular filtration rate (GFR) to  $53.2\pm 14.9\%$  of control value ( $P<0.05$ ) and urine flow rate (V) to  $54.9\pm 15.9\%$  of control value ( $P<0.05$ ), (Figure 8).

**Group III** : Animal pretreated with verapamil before crude cobra venom injection.

Intravenous infusion of verapamil alone caused no changes in any of the measurements made (Table 6 and Table 7). Renal vascular resistance (RVR) showed insignificant increase in stepwise  $19.2 \pm 66.2\%$  at 15 minutes,  $234.2 \pm 239.7\%$  at 30 minutes and  $708.3 \pm 690\%$  at 60 minutes of the control value whereas filtration fraction (FF) significantly increased by approximately  $34.5 \pm 11.4\%$  of control value at 60 minutes after envenomation (Figure 10). In the first 15 minutes of envenomation, there were significant decreases in effective renal plasma flow (ERPF) and effective renal blood flow (ERBF) by approximately  $40.9 \pm 14.7\%$  and  $39.3 \pm 14.7\%$  of the control value ( $P < 0.05$ ) respectively. Glomerular filtration rate (GFR) and urine flow rate (V) decreased  $39.6 \pm 17\%$  and  $40.6 \pm 21\%$  of the control value, respectively (Figure 8 and Figure 9).

**Group IV** : Effects of cardiotoxic fraction of cobra venom injection.

Animal pretreated with intravenous infusion of normal saline solution caused no changes in any of the measurements made except for filtration fraction that significantly decreased to  $11.1 \pm 2.9\%$  of the control value (Table 6 and Table 7). Renal vascular resistance (RVR) increased but not significant after envenomation, whereas filtration fraction significantly decreased to  $8.9 \pm 6.3\%$  of the control value. After that filtration fraction was fell to  $8.6 \pm 17.2\%$  of the control value and slightly raised to the control value at 30 minutes and 60 minutes after envenomation (Figure 10). Effective renal plasma flow (ERPF) significantly decreased by approximately  $68.9 \pm 22.5\%$ ,  $71.7 \pm 171\%$  and  $61.5 \pm 17.7\%$  of the control value at 15 minutes, 30 minutes and 60 minutes respectively after envenomation. Effective renal blood flow, glomerular filtration rate and urine flow rate showed no significant decreases throughout the experimental period (Figure 8 and Figure 9).

**Group V** : Animal pretreated with verapamil before cardiotoxic fraction of cobra venom injection.

Intravenous infusion of verapamil caused no changes in any of the measurements made (Table 6 and Table 7). Renal vascular (RVR) slightly increased throughout the experiment. Filtration fraction gradually increased and in the last period it increased to  $32.1 \pm 15.9\%$  of the control value (Figure 10). Effective renal plasma flow and effective renal blood flow slightly increased throughout the experimental period (Figure 9). In the first 15 minutes after envenomation, glomerular filtration rate decreased and then it increased to the control level. Urine flow rate showed no significant increase throughout the experiment (Figure 8).



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**Table 5.** Changes in urine flow rate, glomerular filtration rate, effective renal plasma flow, effective renal blood flow, filtration fraction, renal vascular resistance in control dogs (n=6).

	NSS intravenous infusion		
	Control	30 min	60 min
V ( $\mu$ l/min/kg. bw.)	47.7 $\pm$ 14.1	56.8 $\pm$ 16.3 NS	51.1 $\pm$ 14.4 NS
GFR (ml/min/kg. bw.)	1.4 $\pm$ 0.2	1.7 $\pm$ 0.2 NS	1.5 $\pm$ 0.1 NS
ERPF (ml/min/kg. bw.)	4.9 $\pm$ 0.6	5.5 $\pm$ 0.6 NS	4.6 $\pm$ 0.5 NS
ERBF (ml/min/kg. bw.)	6.8 $\pm$ 0.8	7.5 $\pm$ 0.8 NS	6.2 $\pm$ 0.6 NS
FF (%)	29.6 $\pm$ 2.6	30.5 $\pm$ 2.9 NS	32.5 $\pm$ 1.6 NS
RVR ( $10^3$ dynes-sec/cm <sup>5</sup> )	1.3 $\pm$ 0.2	1.1 $\pm$ 0.1 NS	1.5 $\pm$ 0.1 NS

Values are mean  $\pm$  SEM. Abbreviations: V, urine flow rate; GFR, glomerular filtration rate; ERPF, effective renal plasma flow; ERBF, effective renal blood flow; FF, filtration fraction; RVR, renal vascular resistance. Significant difference values using paired t-test are indicated by \* P<0.05 different from control values and NS = not significant.

**Table 6.** Changes in urine flow rate, glomerular filtration rate, effective renal plasma flow and effective renal blood flow in response to crude cobra venom and/ or its cardiotoxic fraction administration in four groups.

	Control	Venom administration				
		Pretreatment	15 min	30 min	45 min	60 min
<b>V (<math>\mu\text{l}/\text{min}/\text{kg}\cdot\text{bw}</math>)</b>						
II. NSS + CV (n=4)	31.5 $\pm$ 9.9	39.5 $\pm$ 18.0 NS	13.3 $\pm$ 4.7 NS	24.0 $\pm$ 13.0 NS	26.5 $\pm$ 11.4 NS	25.3 $\pm$ 9.4 NS
III. Ver + CV (n=6)	43.2 $\pm$ 14.6	53.0 $\pm$ 17.7 NS	19.5 $\pm$ 5.3 *†	39.8 $\pm$ 16.2 NS	42.2 $\pm$ 17.9 NS	38.1 $\pm$ 13.9 NS
IV. NSS + CTX (n=5)	25.4 $\pm$ 7.5	27.8 $\pm$ 6.9 NS	8.0 $\pm$ 3.3 NS	6.1 $\pm$ 4.1 NS	17.3 $\pm$ 8.2 NS	28.0 $\pm$ 16.7 NS
V. Ver + CTX (n=4)	14.0 $\pm$ 6.0	22.0 $\pm$ 14.4 NS	30.8 $\pm$ 24.1 NS	32.5 $\pm$ 24.2 NS	24.3 $\pm$ 12.8 NS	23.0 $\pm$ 10.0 NS
<b>GFR (<math>\text{ml}/\text{min}/\text{kg}\cdot\text{bw}</math>)</b>						
II. NSS + CV (n=4)	1.5 $\pm$ 0.1	1.6 $\pm$ 0.1 NS	0.7 $\pm$ 0.3 *	1.2 $\pm$ 0.4 NS	1.1 $\pm$ 0.2 NS	1.4 $\pm$ 0.1 NS
III. Ver + CV (n=6)	1.5 $\pm$ 0.2	1.4 $\pm$ 0.2 NS	0.9 $\pm$ 0.3 NS	0.9 $\pm$ 0.2 NS	1.1 $\pm$ 0.3 NS	1.4 $\pm$ 0.4 NS
IV. NSS + CTX (n=5)	1.9 $\pm$ 0.1	1.8 $\pm$ 0.2 NS	0.6 $\pm$ 0.4 *	0.5 $\pm$ 0.4 *	0.8 $\pm$ 0.4 NS	0.8 $\pm$ 0.4 NS
V. Ver + CTX (n=4)	1.6 $\pm$ 0.2	1.8 $\pm$ 0.4 NS	1.2 $\pm$ 0.2 NS	1.7 $\pm$ 0.2 NS	1.8 $\pm$ 0.3 NS	1.9 $\pm$ 0.2 NS
<b>ERPF (<math>\text{ml}/\text{min}/\text{kg}\cdot\text{bw}</math>)</b>						
II. NSS + CV (n=4)	5.7 $\pm$ 0.7	6.5 $\pm$ 1.1 NS	3.0 $\pm$ 1.3 NS	4.1 $\pm$ 1.2 NS	3.7 $\pm$ 0.6 *	4.7 $\pm$ 0.9 NS
III. Ver + CV (n=6)	5.9 $\pm$ 0.6	5.1 $\pm$ 0.4 NS	3.4 $\pm$ 0.9 NS	3.7 $\pm$ 0.7 NS	3.5 $\pm$ 0.9 NS	3.7 $\pm$ 0.9 NS
IV. NSS + CTX (n=5)	6.1 $\pm$ 0.6	6.3 $\pm$ 0.7 NS	1.9 $\pm$ 1.4 *	1.6 $\pm$ 1.1 *	2.4 $\pm$ 1.0 *	2.1 $\pm$ 1.0 *
V. Ver + CTX (n=4)	8.2 $\pm$ 0.2	8.8 $\pm$ 1.7 NS	6.4 $\pm$ 1.0 NS	7.5 $\pm$ 0.5 NS	8.2 $\pm$ 1.7 NS	7.6 $\pm$ 0.9 NS
<b>ERBF (<math>\text{ml}/\text{min}/\text{kg}\cdot\text{bw}</math>)</b>						
II. NSS + CV (n=4)	8.6 $\pm$ 1.2	9.5 $\pm$ 1.4 NS	4.3 $\pm$ 1.9 NS	6.3 $\pm$ 1.9 NS	5.4 $\pm$ 1.0 *	6.6 $\pm$ 1.1 NS
III. Ver + CV (n=6)	8.0 $\pm$ 0.8	7.0 $\pm$ 0.6 NS	4.8 $\pm$ 1.3 NS	5.3 $\pm$ 1.0 NS	4.8 $\pm$ 1.2 NS	5.2 $\pm$ 1.3 NS
IV. NSS + CTX (n=5)	8.5 $\pm$ 1.1	8.5 $\pm$ 1.2 NS	3.3 $\pm$ 2.4 NS	2.8 $\pm$ 1.8 NS	4.3 $\pm$ 1.7 NS	3.7 $\pm$ 1.6 NS
V. Ver + CTX (n=4)	11.2 $\pm$ 0.8	12.7 $\pm$ 2.8 NS	9.1 $\pm$ 1.6 NS	10.5 $\pm$ 0.7 NS	11.2 $\pm$ 2.0 NS	10.6 $\pm$ 1.4 NS

Values are mean  $\pm$  SEM. Abbreviations: CV, crude cobra venom; CTX, cardiotoxic fraction; Ver, verapamil; V, urine flow rate; GFR, glomerular filtration rate; ERPF, effective renal plasma flow; ERBF, effective renal blood flow. Significant difference values using paired t-test are indicated by \* P<0.05 different from control values, † P<0.05 different from pretreatment values and NS = not significant.

**Table 7. Changes in filtration fraction and renal vascular resistance in response to crude cobra venom and/ or its cardiotoxic fraction administration in four groups.**

	Control	Venom administration								
		Pretreatment	15 min	30 min	45 min	60 min				
<b>FF (%)</b>										
II. NSS + CV (n=4)	26.4 ± 2.0	26.5 ± 3.0 NS	26.4 ± 1.4 NS	28.6 ± 3.0 NS	31.2 ± 5.2 NS	31.2 ± 5.0 NS				
III. Ver + CV (n=6)	26.7 ± 2.9	27.2 ± 2.6 NS	25.9 ± 1.9 NS	24.5 ± 3.9 NS	29.7 ± 2.9 NS	35.1 ± 4.4 †				
IV. NSS + CTX (n=5)	32.0 ± 1.8	28.3 ± 1.4 *	34.6 ± 2.2 †	28.8 ± 5.0 NS	31.4 ± 3.7 NS	33.0 ± 4.8 NS				
V. Ver + CTX (n=4)	19.4 ± 2.3	19.5 ± 1.2 NS	18.5 ± 1.6 NS	22.3 ± 1.6 †	22.9 ± 1.4 NS	24.6 ± 0.6 †				
<b>RVR (10<sup>3</sup> dynes-sec/cm<sup>5</sup>)</b>										
II. NSS + CV (n=4)	1.1 ± 0.2	1.0 ± 0.2 NS	1.0 ± 0.3 NS	2.4 ± 1.3 NS	1.9 ± 0.4 NS	1.6 ± 0.4 NS				
III. Ver + CV (n=6)	1.3 ± 0.2	1.4 ± 0.1 NS	1.3 ± 0.5 NS	3.1 ± 1.8 NS	6.2 ± 5.0 NS	7.0 ± 5.9 NS				
IV. NSS + CTX (n=5)	1.1 ± 0.1	1.1 ± 0.2 NS	3.3 ± 1.1 NS	10.1 ± 6.3 NS	5.1 ± 3.7 NS	6.4 ± 4.5 NS				
V. Ver + CTX (n=4)	0.7 ± 0.1	0.7 ± 0.1 NS	0.9 ± 0.1 NS	0.8 ± 0.1 NS	0.9 ± 0.2 NS	0.9 ± 0.2 NS				

Values are mean ± SEM. Abbreviations: CV, crude cobra venom; CTX, cardiotoxic fraction; Ver, verapamil; FF, filtration fraction; RVR, renal vascular resistance.

Significant difference values using paired t-test are indicated by \* P<0.05 different from control values and by † P<0.05 different from pretreatment values.



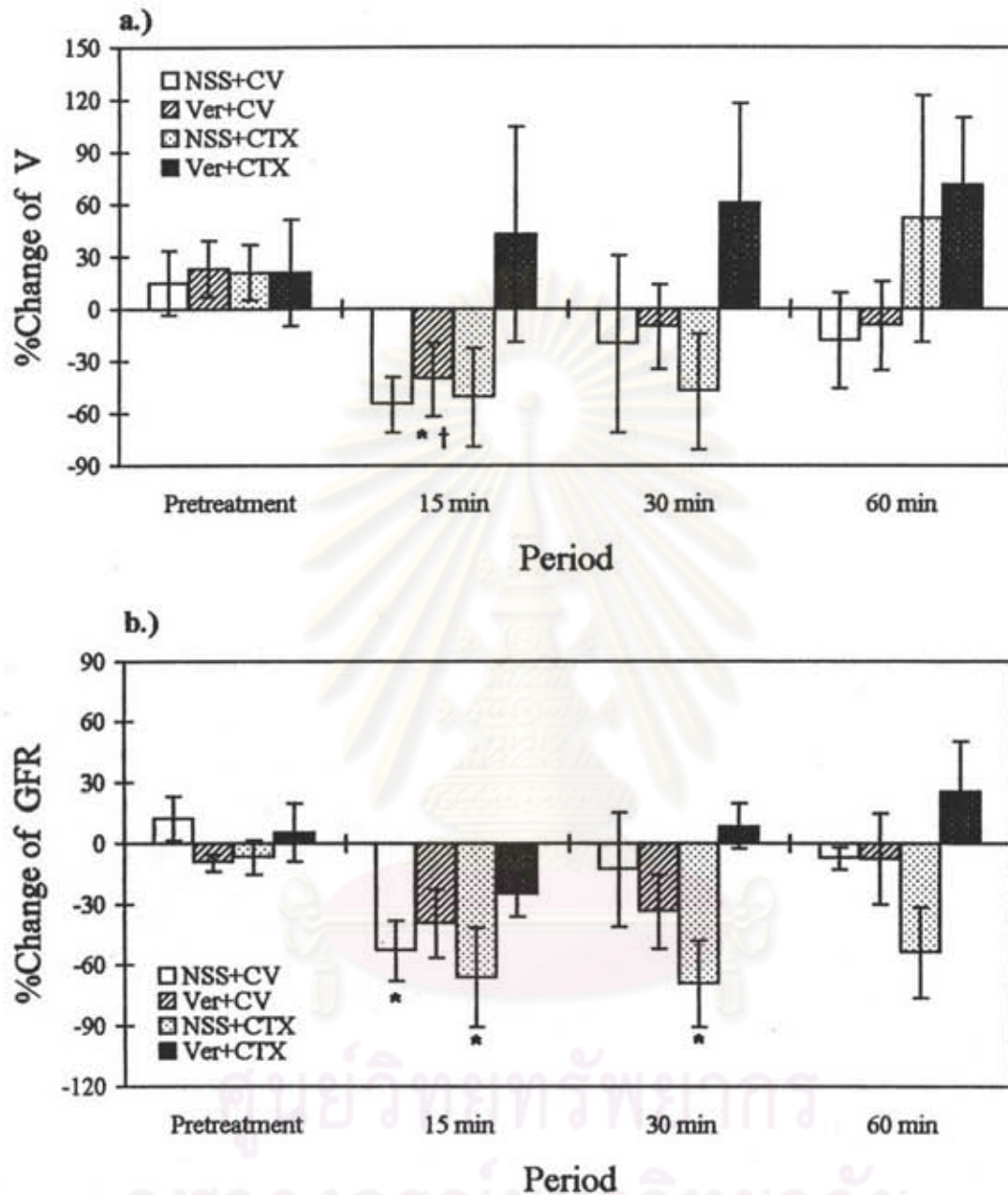


Figure 8. Percentage changes in urine flow rate (V) (a.) and glomerular filtration rate (GFR) (b.) in response to crude cobra venom (CV) and / or its cardiotoxic fraction (CTX) and pretreated intravenous infusion with normal saline solution (NSS) and/or verapamil (Ver).

Significant difference values using paired t-test are indicated by \*  $P < 0.05$  compared to the control value and †  $P < 0.05$  compared to the pretreatment value of each group.

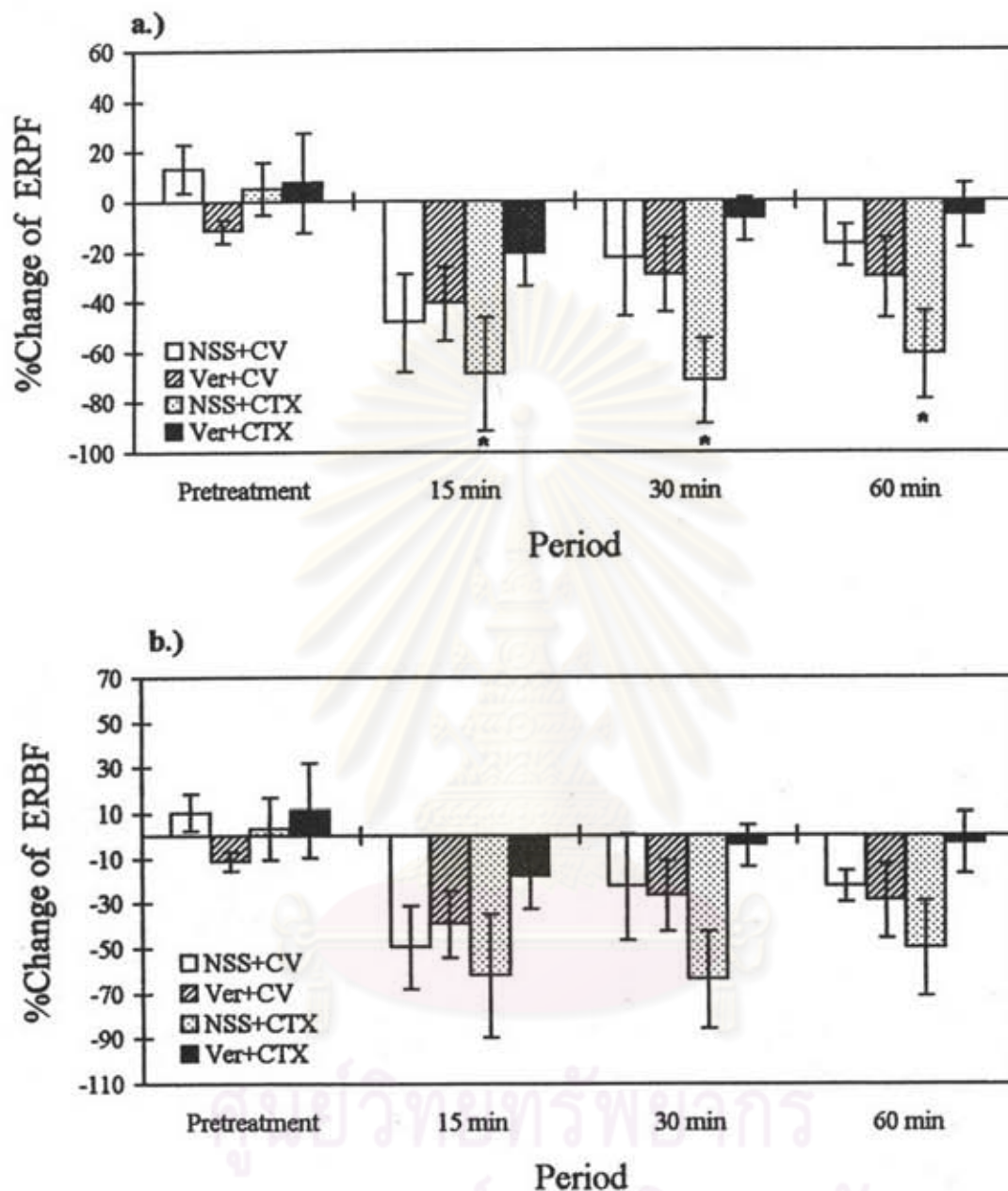


Figure 9. Percentage changes in effective renal plasma flow (ERPF) (a.) and effective renal blood flow (ERBF) (b.) in response to crude cobra venom (CV) and / or its cardiotoxic fraction (CTX) and pretreated intravenous infusion with normal saline solution (NSS) and/or verapamil (Ver).

Significant difference values using paired t-test are indicated by \*  $P < 0.05$  compared to the control value and †  $P < 0.05$  compared to the pretreatment value of each group.

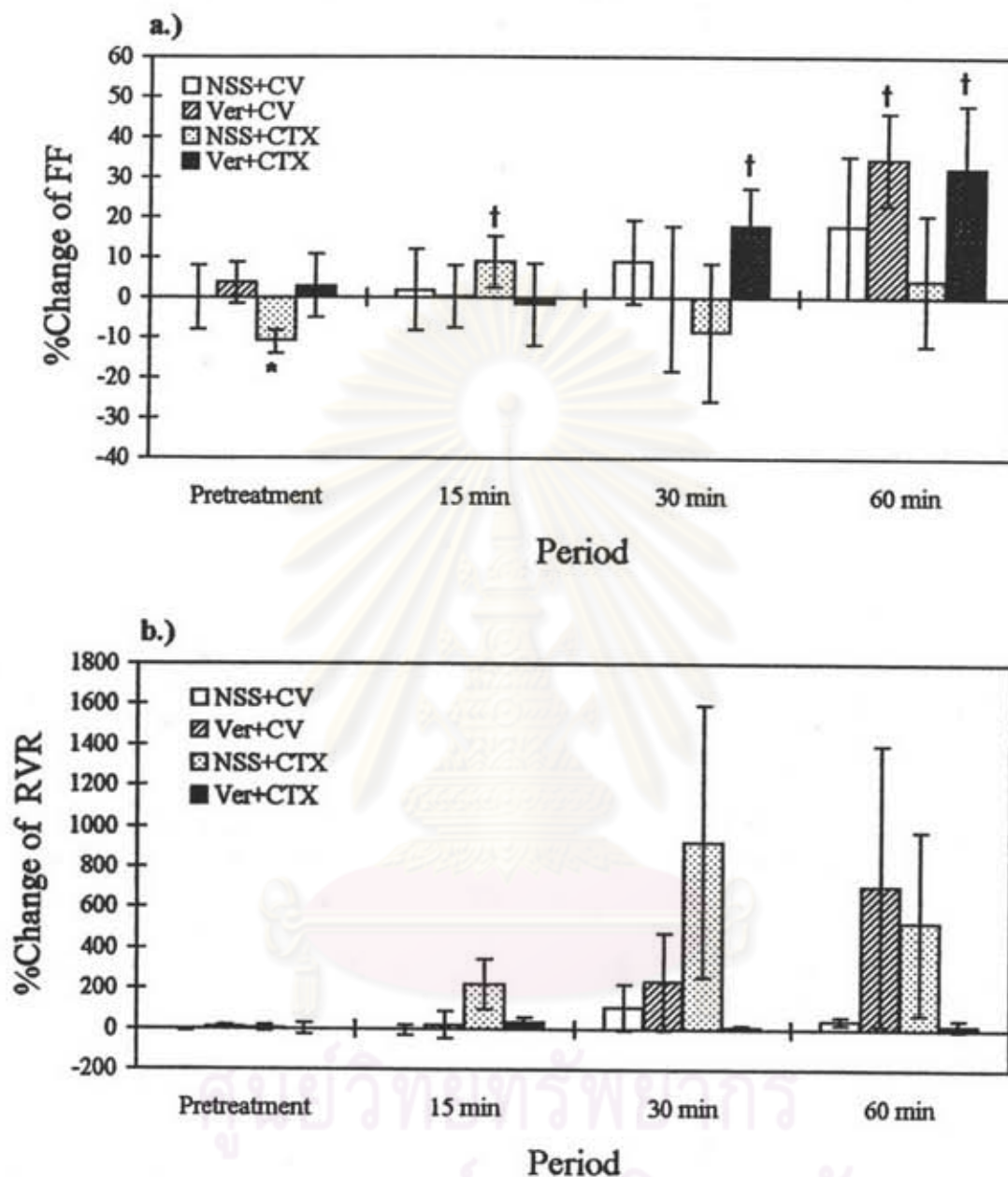


Figure 10. Percentage changes in filtration fraction (FF) (a.) and renal vascular resistance (RVR) (b.) in response to crude cobra venom (CV) and / or its cardiotoxic fraction (CTX) and pretreated intravenous infusion with normal saline solution (NSS) and/or verapamil (Ver).

Significant difference values using paired t-test are indicated by \*  $P < 0.05$  compared to the control value and †  $P < 0.05$  compared to the pretreatment value of each group.



### Effect of Cobra Venom on Urinary Electrolytes Excretion.

The results of changes in urinary electrolytes excretion in dogs given cobra venom are shown in Table 8 - Table 11 and Figure 11 - Figure 16.

Group I : Control animal.

As shown in Table 8 the results are expressed on mean $\pm$ SEM. After intravenous saline infusion, all parameters did not indicate the significant changes throughout the experimental period.

Group II : Effects of crude cobra venom injection.

Animal pretreated with intravenous saline infusion before crude cobra venom injection caused no changes in any of the measurements made (Table 9, Table 10 and Table 11). After 15 minutes of crude cobra venom administration, there were no differences in plasma concentration of sodium, potassium and chloride from the control values. Filtered load of sodium ( $GFR \times P_{Na}$ ) and urinary excretion of sodium ( $U_{Na}V$ ) significantly fell by approximately 56% of the control value whereas fractional excretion of sodium ( $FE_{Na}$ ) slightly decreased in comparison to the control value (Figure 11 and Figure 12). Filtered load of potassium ( $GFR \times P_K$ ) and urinary excretion of potassium ( $U_KV$ ) fell to 41% of the control value, while fractional excretion of potassium ( $FE_K$ ) slightly increased to 16% of the control value (Figure 13 and Figure 14). Filtered load of chloride ( $GFR \times P_{Cl}$ ) and urinary excretion of chloride ( $U_{Cl}V$ ), significantly fell to 52% of the control value, no significance change in fractional excretion of chloride ( $FE_{Cl}$ ) was observed (Figure 15 and Figure 16).

Group III : Animal pretreated with verapamil before crude cobra venom injection.

Intravenous infusion of verapamil alone caused no changes in any of the measurements made (Table 9, Table 10 and Table 11). After 15 minutes of crude cobra venom administration, there were no differences in plasma concentration of sodium, potassium and chloride from the control value. Filtered load of sodium slightly

decreased to  $38.8 \pm 17.0\%$  of the control value whereas urinary excretion of sodium decreased at the first 15 minutes and then it gradually increased. Fractional excretion of sodium increased to  $62 \pm 67.2\%$  but there was no significant of the control value (Figure 11 and Figure 12). Filtered load of potassium and urinary excretion of potassium insignificantly fell approximately 40% of the control value, whereas fractional excretion of potassium slightly increased (Figure 13 and Figure 14). Filtered load of chloride insignificantly fell to  $39.2 \pm 16.4\%$  whereas urinary excretion of chloride slightly increased to  $10.1 \pm 53.9\%$  of the control value. Fractional excretion of chloride significantly at the period of 30 minutes after envenomation ( $P < 0.05$ ) (Figure 15 and Figure 16).

Group IV : Effects of cardiotoxic fraction of cobra venom injection.

Animal pretreated with intravenous infusion of normal saline infusion caused no changes in any of the measurements made (Table 9, Table 10 and Table 11). Cardiotoxic fraction of cobra venom produced a significant raise in plasma sodium concentration ( $P < 0.05$ ) in the period of 60 minutes after envenomation, whereas filtered load of sodium fell to  $53.3 \pm 22.6\%$  of the control value. Fractional excretion of sodium insignificantly fell to  $148.5 \pm 83.5\%$  of the control value (Figure 11 and Figure 12). Plasma potassium concentration increased throughout the experimental period and significantly increase ( $P < 0.05$ ) at 30 minutes after envenomation, whereas filtered load of potassium and urinary excretion of potassium insignificantly decreased approximately 64% and 55% of the control value respectively. Fractional excretion of potassium insignificantly increased to  $25.4 \pm 32.5\%$  of the control value (Figure 13 and Figure 14). There were no significant differences in plasma concentration of chloride. Filtered load of chloride significantly and urinary excretion of chloride insignificantly fell to  $61.8 \pm 4.2\%$  of the control value after 30 minutes of envenomation. Fractional excretion of chloride showed no significantly fell to  $61.8 \pm 24.2\%$  of the control value (Figure 15 and Figure 16).



Group V : Animal pretreated with verapamil before cardiotoxic fraction of cobra venom injection.

Intravenous infusion of verapamil caused no changes in any of the measurements made (Table 9, Table 10 and Table 11). After cardiotoxic fraction of cobra venom administration, there were no significant differences in plasma concentration of sodium, potassium and chloride from the control value (Figure 11, Figure 13 and Figure 15). Filtered load of sodium slightly decreased and gradually increased whereas urinary excretion of sodium increased. Fractional excretion of sodium insignificantly increased throughout the experimental period (Figure 11 and Figure 12). Filtered load of potassium slightly decreased at 15 minutes and gradually increased at 30 and 60 minutes whereas urinary excretion of potassium slightly increased. Fractional excretion of potassium and filtered load of chloride slightly increased and decreased nearly the control value. Urinary excretion of chloride insignificantly increased therefore fractional excretion of chloride insignificantly increased throughout the experimental period (Figure 13, Figure, 14, Figure 15 and Figure 16).



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Table 8. Changes in electrolyte excretion in control dogs (n=6).

	NSS intravenous infusion		
	Control	NSS intravenous infusion	
		30 min	60 min
$P_{Na}$ (mEq/L)	136.2 ± 4.5	132.7 ± 3.5 NS	135.8 ± 4.6 NS
$P_K$ (mEq/L)	3.0 ± 0.1	3.2 ± 0.1 NS	3.2 ± 0.3 NS
$P_{Cl}$ (mEq/L)	120.7 ± 2.9	121.0 ± 2.9 NS	120.5 ± 3.4 NS
$GFR \times P_{Na}$ ( $\mu$ Eq/min/kg.bw.)	192.5 ± 23.0	223.6 ± 32.9 NS	199.6 ± 20.0 NS
$GFR \times P_K$ ( $\mu$ Eq/min/kg.bw.)	4.3 ± 0.6	5.4 ± 0.9 NS	4.8 ± 0.6 NS
$GFR \times P_{Cl}$ ( $\mu$ Eq/min/kg.bw.)	170.2 ± 19.7	200.4 ± 25.4 NS	175.4 ± 13.6 NS
$U_{Na}V$ ( $\mu$ Eq/min/kg.bw.)	1.8 ± 0.5	1.6 ± 0.4 NS	1.7 ± 0.4 NS
$U_KV$ ( $\mu$ Eq/min/kg.bw.)	0.9 ± 0.2	0.9 ± 0.1 NS	0.8 ± 0.1 NS
$U_{Cl}V$ ( $\mu$ Eq/min/kg.bw.)	1.9 ± 0.6	1.9 ± 0.5 NS	1.6 ± 0.4 NS
$FE_{Na}$ (%)	0.9 ± 0.2	0.7 ± 0.2 NS	0.8 ± 0.2 NS
$FE_K$ (%)	21.2 ± 1.9	17.0 ± 2.1 NS	18.0 ± 1.8 NS
$FE_{Cl}$ (%)	1.1 ± 0.3	0.9 ± 0.3 NS	0.9 ± 0.2 NS

Values are mean ± SEM. Abbreviations:  $P_{Na}$ , plasma sodium concentration;  $P_K$ , plasma potassium concentration;  $P_{Cl}$ , plasma chloride concentration;  $GFR \times P_{Na}$ , filtered load of sodium;  $GFR \times P_K$ , filtered load of potassium;  $GFR \times P_{Cl}$ , filtered load of chloride;  $U_{Na}V$ , urinary sodium excretion;  $U_KV$ , urinary potassium excretion;  $U_{Cl}V$ , urinary chloride excretion;  $FE_{Na}$ , fractional excretion of sodium;  $FE_K$ , fractional excretion of potassium;  $FE_{Cl}$ , fractional excretion of chloride.

Significant difference values using paired t-test are indicated by \*  $P < 0.05$  different from control values and NS = not significant.

**Table 9.** Changes in sodium excretion in response to crude cobra venom and/ or its cardiotoxic fraction administration in four groups.

	Control	Venom administration				
		Pretreatment	15 min	30 min	45 min	60 min
<b>P<sub>Na</sub> (mEq/L)</b>						
II. NSS + CV (n=4)	135.5 ± 0.9	137.5 ± 1.3 NS	134.5 ± 1.5 NS	136.5 ± 1.0 NS	136.8 ± 1.6 NS	136.5 ± 1.3 NS
III. Ver + CV (n=6)	136.8 ± 3.0	138.7 ± 1.3 NS	139.0 ± 1.1 NS	140.8 ± 1.4 NS	144.7 ± 5.2 NS	139.8 ± 2.8 NS
IV. NSS + CTX (n=5)	142.0 ± 1.9	144.4 ± 2.8 NS	138.8 ± 3.5 NS	142.8 ± 1.9 NS	143.8 ± 2.3 NS	145.4 ± 1.7 *
V. Ver + CTX (n=4)	141.5 ± 3.7	141.3 ± 0.9 NS	143.8 ± 0.9 NS	144.5 ± 2.2 NS	141.5 ± 2.2 NS	142.3 ± 1.4 NS
<b>GFRxP<sub>Na</sub> (μEq/min/kg.bw.)</b>						
II. NSS + CV (n=4)	200.4 ± 20.2	224.6 ± 17.0 NS	100.2 ± 41.7 *	168.3 ± 49.9 NS	154.7 ± 26.1 NS	184.8 ± 16.0 NS
III. Ver + CV (n=6)	210.4 ± 30.5	190.1 ± 22.4 NS	130.4 ± 40.0 NS	127.8 ± 33.0 NS	157.9 ± 41.9 NS	195.4 ± 56.7 NS
IV. NSS + CTX (n=5)	274.3 ± 21.7	261.6 ± 34.0 NS	86.2 ± 61.9 *	76.3 ± 53.2 *	119.2 ± 52.3 NS	113.9 ± 54.9 NS
V. Ver + CTX (n=4)	223.1 ± 23.6	249.6 ± 54.9 NS	173.5 ± 33.8 NS	245.4 ± 34.0 NS	259.2 ± 47.6 NS	265.7 ± 34.1 NS
<b>U<sub>Na</sub>V (μEq/min/kg.bw.)</b>						
II. NSS + CV (n=4)	4.8 ± 1.2	5.8 ± 2.1 NS	1.9 ± 0.7 NS	3.4 ± 1.8 NS	3.5 ± 1.9 NS	3.3 ± 1.6 NS
III. Ver + CV (n=6)	2.1 ± 0.9	2.4 ± 0.7 NS	1.4 ± 0.5 NS	2.6 ± 0.8 NS	3.0 ± 1.5 NS	3.0 ± 1.3 NS
IV. NSS + CTX (n=5)	4.7 ± 1.2	5.2 ± 1.4 NS	1.4 ± 0.5 NS	0.9 ± 0.6 NS	1.9 ± 0.9 NS	3.0 ± 1.6 NS
V. Ver + CTX (n=4)	2.6 ± 1.8	3.8 ± 3.0 NS	5.5 ± 4.7 NS	6.1 ± 5.2 NS	4.2 ± 3.0 NS	4.3 ± 2.6 NS
<b>FE<sub>Na</sub> (%)</b>						
II. NSS + CV (n=4)	2.5 ± 0.8	2.5 ± 0.8 NS	2.7 ± 1.4 NS	2.1 ± 0.7 NS	1.9 ± 0.8 NS	1.7 ± 0.7 NS
III. Ver + CV (n=6)	1.0 ± 0.3	1.3 ± 0.4 NS	1.1 ± 0.3 NS	2.6 ± 1.5 NS	1.5 ± 0.5 NS	1.2 ± 0.3 *
IV. NSS + CTX (n=5)	1.6 ± 0.3	1.9 ± 0.3 NS	3.1 ± 0.7 *†	2.1 ± 0.7 NS	2.0 ± 0.9 NS	4.1 ± 1.8 NS
V. Ver + CTX (n=4)	1.0 ± 0.7	1.2 ± 0.9 NS	2.8 ± 2.4 NS	1.9 ± 1.5 NS	1.6 ± 1.0 NS	1.4 ± 0.8 NS

Values are mean ± SEM. Abbreviations: CV, crude cobra venom; CTX, cardiotoxic fraction; Ver, verapamil; P<sub>Na</sub>, plasma sodium concentration; GFRxP<sub>Na</sub>, filtered load of sodium; U<sub>Na</sub>V, urinary sodium excretion; FE<sub>Na</sub>, fractional excretion of sodium. Significant difference values using paired t-test are indicated by \* P<0.05 different from control values, † P<0.05 different from pretreatment values and NS = not significant.



**Table 10.** Changes in potassium excretion in response to crude cobra venom and/ or its cardiotoxic fraction administration in four groups.

	Control	Venom administration				
		Pretreatment	15 min	30 min	45 min	60 min
<b>P<sub>x</sub> (mEq/L)</b>						
II. NSS + CV (n=4)	3.3 ± 0.1	3.3 ± 0.1 NS	3.7 ± 0.2 *†	3.2 ± 0.1 NS	3.2 ± 0.3 NS	3.3 ± 0.1 NS
III. Ver + CV (n=6)	3.8 ± 0.1	3.8 ± 0.2 NS	3.9 ± 0.1 NS	3.6 ± 0.1 NS	3.6 ± 0.2 NS	3.4 ± 0.2 †
IV. NSS + CTX (n=5)	3.5 ± 0.3	3.7 ± 0.4 NS	4.8 ± 0.5 *	4.6 ± 0.4 *†	4.1 ± 0.3 *	3.8 ± 0.3 NS
V. Ver + CTX (n=4)	3.1 ± 0.3	3.2 ± 0.4 NS	3.1 ± 0.3 NS	3.2 ± 0.3 NS	3.2 ± 0.3 NS	3.3 ± 0.3 NS
<b>GFRxP<sub>x</sub> (μEq/min/kg.bw.)</b>						
II. NSS + CV (n=4)	4.9 ± 0.6	5.4 ± 0.4 NS	2.7 ± 1.1 *	3.9 ± 1.2 NS	3.7 ± 0.8 NS	4.4 ± 0.5 NS
III. Ver + CV (n=6)	5.8 ± 0.9	5.3 ± 0.8 NS	3.7 ± 1.1 *†	3.3 ± 0.9 NS	4.2 ± 1.2 NS	5.0 ± 1.5 NS
IV. NSS + CTX (n=5)	6.8 ± 0.8	6.7 ± 1.2 NS	2.6 ± 1.7 NS	2.3 ± 1.4 NS	3.2 ± 1.5 NS	2.9 ± 1.5 NS
V. Ver + CTX (n=4)	5.0 ± 0.9	5.9 ± 1.6 NS	3.9 ± 1.0 NS	5.4 ± 0.7 NS	5.5 ± 0.5 NS	6.0 ± 0.8 NS
<b>U<sub>x</sub>V (μEq/min/kg.bw.)</b>						
II. NSS + CV (n=4)	1.0 ± 0.1	1.0 ± 0.1 NS	0.6 ± 0.2 NS	1.0 ± 0.3 NS	0.9 ± 0.2 NS	1.0 ± 0.1 NS
III. Ver + CV (n=6)	1.3 ± 0.4	1.1 ± 0.2 NS	0.8 ± 0.2 NS	1.1 ± 0.3 NS	1.1 ± 0.5 NS	1.0 ± 0.4 NS
IV. NSS + CTX (n=5)	1.4 ± 0.4	1.6 ± 0.4 NS	0.5 ± 0.3 NS	0.5 ± 0.3 NS	1.2 ± 0.5 NS	1.0 ± 0.5 NS
V. Ver + CTX (n=4)	1.1 ± 0.3	1.1 ± 0.3 NS	1.0 ± 0.4 NS	1.1 ± 0.3 NS	1.1 ± 0.3 NS	1.2 ± 0.3 NS
<b>FE<sub>x</sub> (%)</b>						
II. NSS + CV (n=4)	20.9 ± 3.7	19.7 ± 2.1 NS	22.5 ± 2.7 NS	31.3 ± 7.0 NS	23.8 ± 1.3 NS	22.0 ± 1.0 NS
III. Ver + CV (n=6)	20.6 ± 3.4	21.2 ± 3.3 NS	19.3 ± 2.8 NS	40.2 ± 16.6 NS	23.4 ± 3.9 NS	19.3 ± 2.0 NS
IV. NSS + CTX (n=5)	19.9 ± 2.8	23.6 ± 2.0 NS	26.7 ± 4.7 NS	24.2 ± 5.9 NS	33.5 ± 10.3 NS	45.9 ± 15.0 NS
V. Ver + CTX (n=4)	21.4 ± 4.2	19.0 ± 2.7 NS	24.7 ± 6.3 NS	19.9 ± 4.4 NS	19.2 ± 4.1 NS	18.5 ± 2.2 NS

Values are mean ± SEM. Abbreviations: CV, crude cobra venom; CTX, cardiotoxic fraction; Ver, verapamil; P<sub>x</sub>, plasma potassium concentration; GFRxP<sub>x</sub>, filtered load of potassium; U<sub>x</sub>V, urinary potassium excretion; FE<sub>x</sub>, fractional excretion of potassium.

Significant difference values using paired t-test are indicated by \* P<0.05 different from control values, † P<0.05 different from pretreatment values and NS = not significant.



Table 11. Changes in chloride excretion in response to crude cobra venom and/ or its cardiotoxic fraction administration in four groups.

	Control	Venom administration				
		Pretreatment	15 min	30 min	45 min	60 min
<b>P<sub>Cl</sub> (mEq/L)</b>						
II. NSS + CV (n=4)	122.0 ± 1.1	123.8 ± 1.9 NS	124.0 ± 3.0 NS	124.8 ± 1.6 NS	123.0 ± 0.8 NS	125.5 ± 0.6 *
III. Ver + CV (n=6)	117.3 ± 3.5	121.2 ± 1.6 NS	119.8 ± 2.5 NS	123.0 ± 2.2 NS	124.3 ± 2.4 NS	123.8 ± 2.1 NS
IV. NSS + CTX (n=5)	121.6 ± 1.8	123.0 ± 0.7 NS	120.8 ± 1.7 NS	123.8 ± 0.7 NS	122.4 ± 1.7 NS	123.4 ± 1.0 NS
V. Ver + CTX (n=4)	121.3 ± 1.4	119.0 ± 1.5 NS	121.0 ± 3.2 NS	120.5 ± 4.0 NS	120.3 ± 2.1 NS	119.3 ± 2.5 NS
<b>GFRxP<sub>Cl</sub> (μEq/min/kg.bw.)</b>						
II. NSS + CV (n=4)	180.0 ± 16.5	203.0 ± 18.4 NS	90.1 ± 35.8 *	153.8 ± 46.0 NS	138.9 ± 22.8 NS	169.8 ± 14.3 NS
III. Ver + CV (n=6)	180.4 ± 26.3	166.6 ± 20.5 NS	113.2 ± 34.2 NS	112.3 ± 28.7 NS	140.5 ± 41.9 NS	175.1 ± 52.1 NS
IV. NSS + CTX (n=5)	234.2 ± 15.5	223.6 ± 29.7 NS	72.7 ± 51.3 *	65.5 ± 45.2 *	103.1 ± 44.7 NS	95.7 ± 44.9 NS
V. Ver + CTX (n=4)	191.9 ± 21.5	210.2 ± 45.9 NS	145.5 ± 28.7 NS	204.3 ± 28.3 NS	217.9 ± 36.7 NS	221.6 ± 26.1 NS
<b>U<sub>Cl</sub>V (μEq/min/kg.bw.)</b>						
II. NSS + CV (n=4)	4.9 ± 1.7	6.3 ± 3.0 NS	1.8 ± 0.6 NS	2.8 ± 1.5 NS	3.3 ± 1.8 NS	3.0 ± 1.6 NS
III. Ver + CV (n=6)	2.3 ± 1.1	2.5 ± 0.9 NS	1.4 ± 0.4 NS	2.4 ± 0.8 NS	2.5 ± 1.1 NS	2.4 ± 1.1 NS
IV. NSS + CTX (n=5)	4.4 ± 1.5	5.8 ± 2.3 NS	1.2 ± 0.4 NS	0.7 ± 0.4 NS	1.4 ± 0.6 NS	2.3 ± 1.1 NS
V. Ver + CTX (n=4)	2.1 ± 1.3	3.8 ± 3.0 NS	4.0 ± 3.2 NS	5.3 ± 4.3 NS	3.2 ± 2.2 NS	3.2 ± 1.8 NS
<b>FE<sub>Cl</sub>(%)</b>						
II. NSS + CV (n=4)	2.9 ± 1.1	3.0 ± 1.2 NS	2.9 ± 1.6 NS	1.8 ± 0.7 NS	1.9 ± 1.0 NS	1.6 ± 0.8 NS
III. Ver + CV (n=6)	1.3 ± 0.6	1.7 ± 0.7 NS	1.4 ± 0.5 NS	2.5 ± 1.1 NS	1.8 ± 0.7 *	1.2 ± 0.5 NS
IV. NSS + CTX (n=5)	1.8 ± 0.5	2.3 ± 0.7 NS	3.3 ± 1.1 NS	2.1 ± 0.7 NS	2.0 ± 0.7 NS	4.3 ± 1.8 NS
V. Ver + CTX (n=4)	1.0 ± 0.6	1.5 ± 1.2 NS	2.5 ± 2.1 NS	2.0 ± 1.5 NS	1.5 ± 1.0 NS	1.3 ± 0.7 NS

Values are mean ± SEM. Abbreviations: CV, crude cobra venom; CTX, cardiotoxic fraction; Ver, verapamil; P<sub>Cl</sub>, plasma chloride concentration; GFRxP<sub>Cl</sub>, filtered load of chloride; U<sub>Cl</sub>V, urinary chloride excretion; FE<sub>Cl</sub>, fractional excretion of chloride. Significant difference values using paired t-test are indicated by \* P<0.05 different from control values, † P<0.05 different from pretreatment values and NS = not significant.

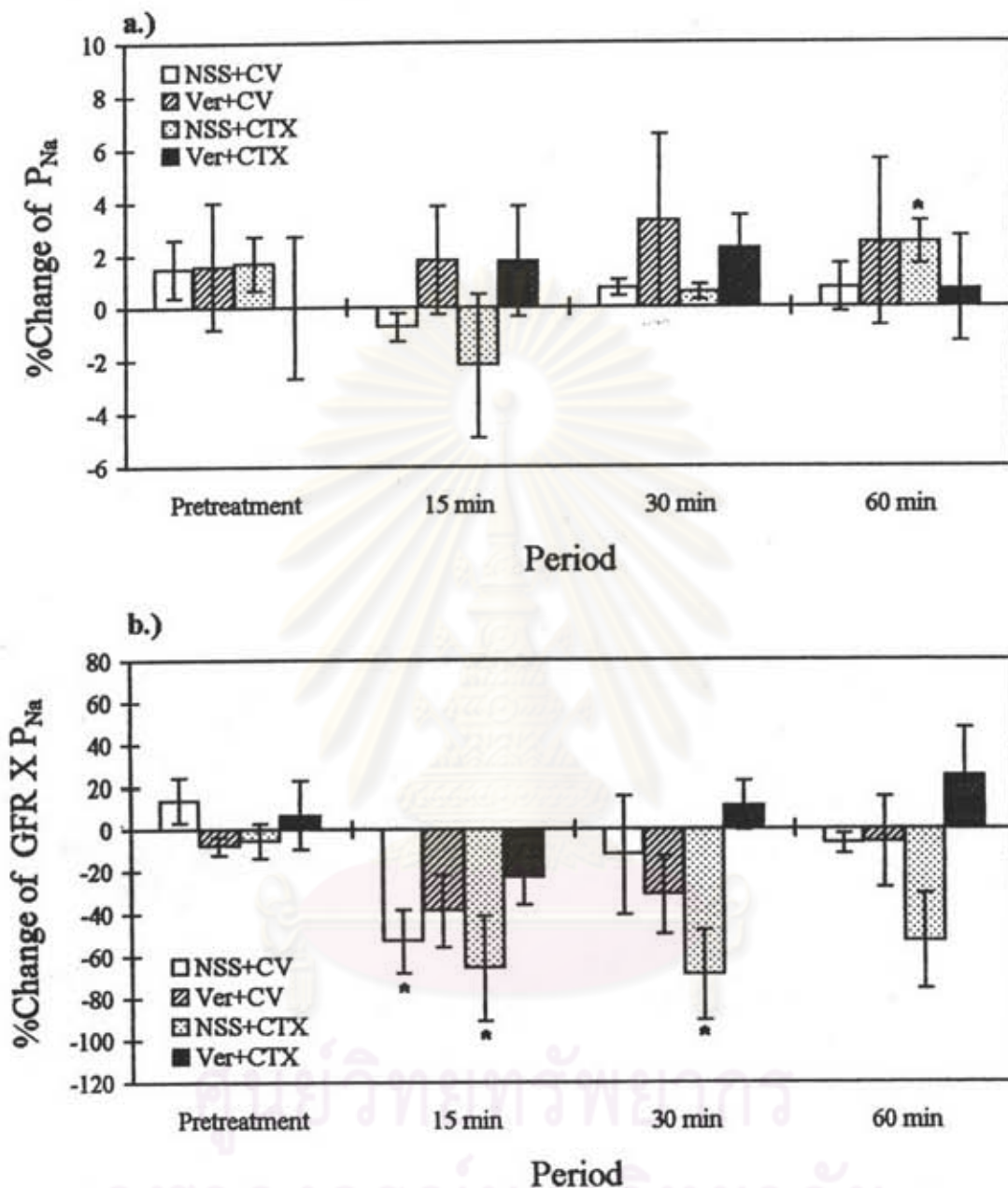


Figure 11. Percentage changes in plasma sodium concentration ( $P_{Na}$ )(a.) and filtered load of sodium ( $GFR \times P_{Na}$ )( b.) in response to crude cobra venom ( CV ) and / or its cardiotoxic fraction ( CTX) and pretreated intravenous infusion with normal saline solution (NSS) and/or verapamil (Ver). Significant difference values using paired t-test are indicated by \*  $P < 0.05$  compared to the control value and †  $P < 0.05$  compared to the pretreatment value of each group.

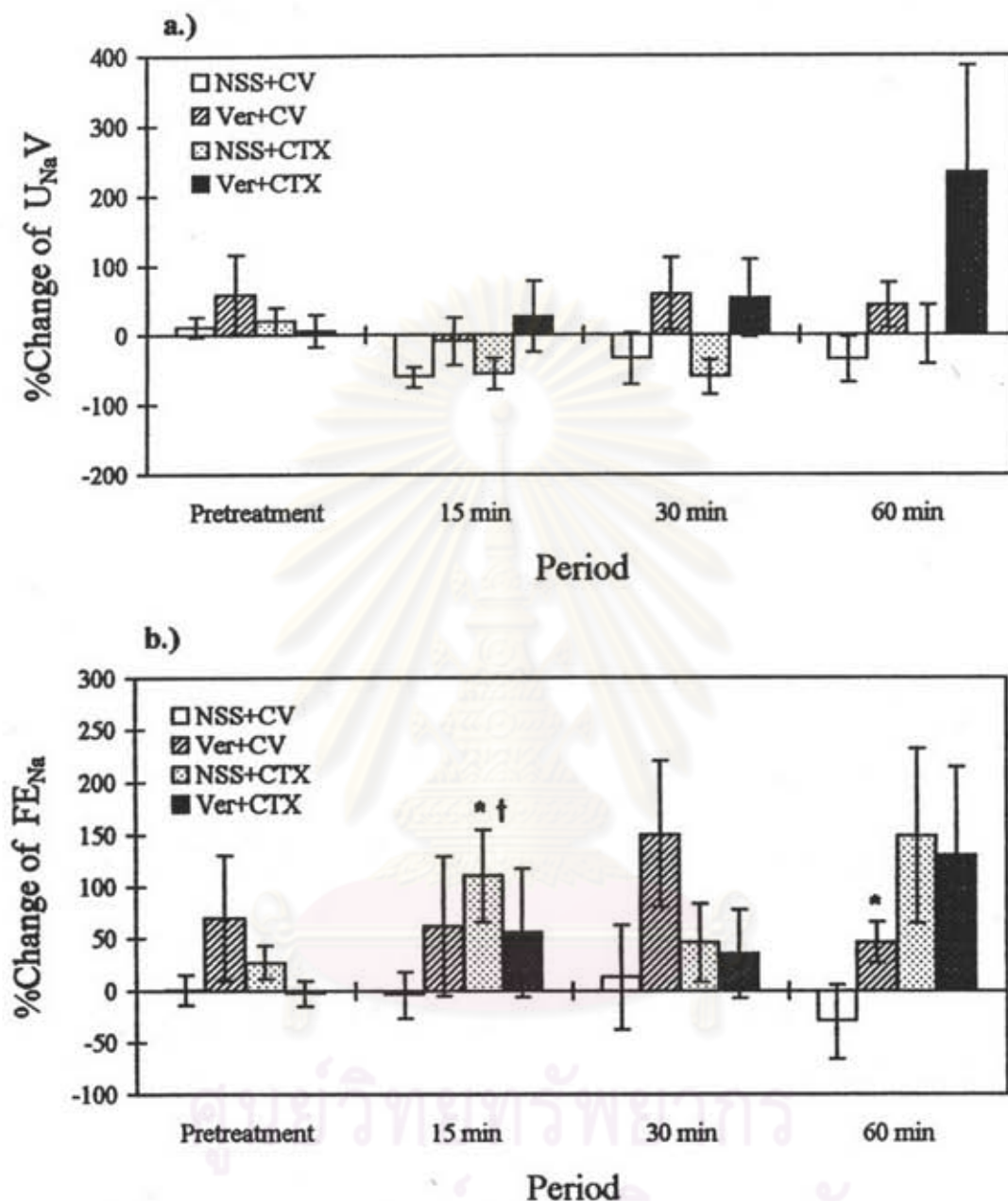


Figure 12. Percentage changes in urinary sodium excretion ( $U_{Na}V$ ) (a.) and fractional excretion of sodium ( $FE_{Na}$ ) (b.) in response to crude cobra venom (CV) and / or its cardiotoxic fraction (CTX) and pretreated intravenous infusion with normal saline solution (NSS) and/or verapamil (Ver). Significant difference values using paired t-test are indicated by \*  $P < 0.05$  compared to the control value and †  $P < 0.05$  compared to the pretreatment value of each group.



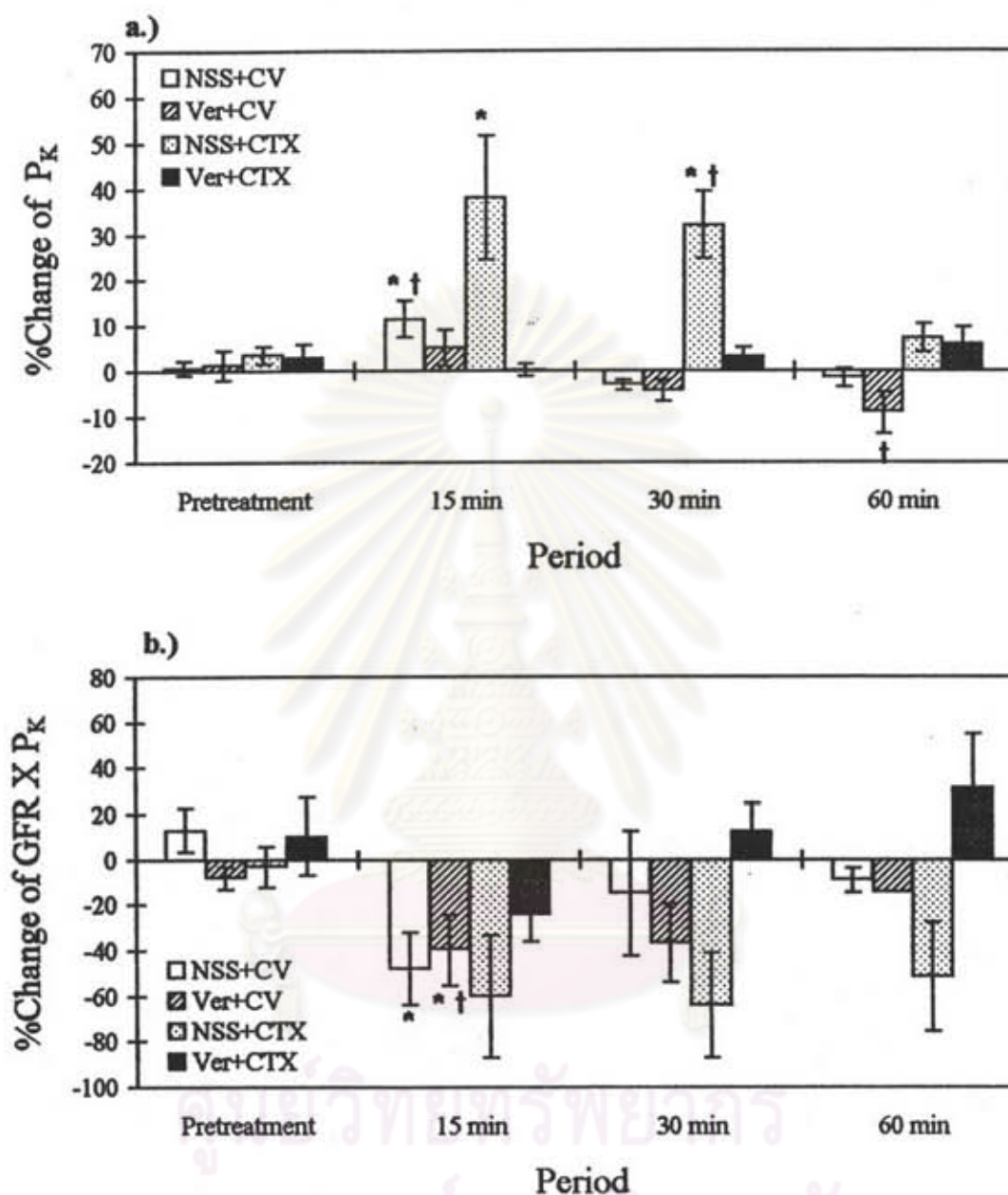


Figure 13. Percentage changes in plasma potassium concentration ( $P_K$ ) (a.) and filtered load of potassium ( $GFR \times P_K$ ) (b.) in response to crude cobra venom (CV) and / or its cardiotoxic fraction (CTX) and pretreated intravenous infusion with normal saline solution (NSS) and/or verapamil (Ver).

Significant difference values using paired t-test are indicated by \*  $P < 0.05$  compared to the control value and †  $P < 0.05$  compared to the pretreatment value of each group.

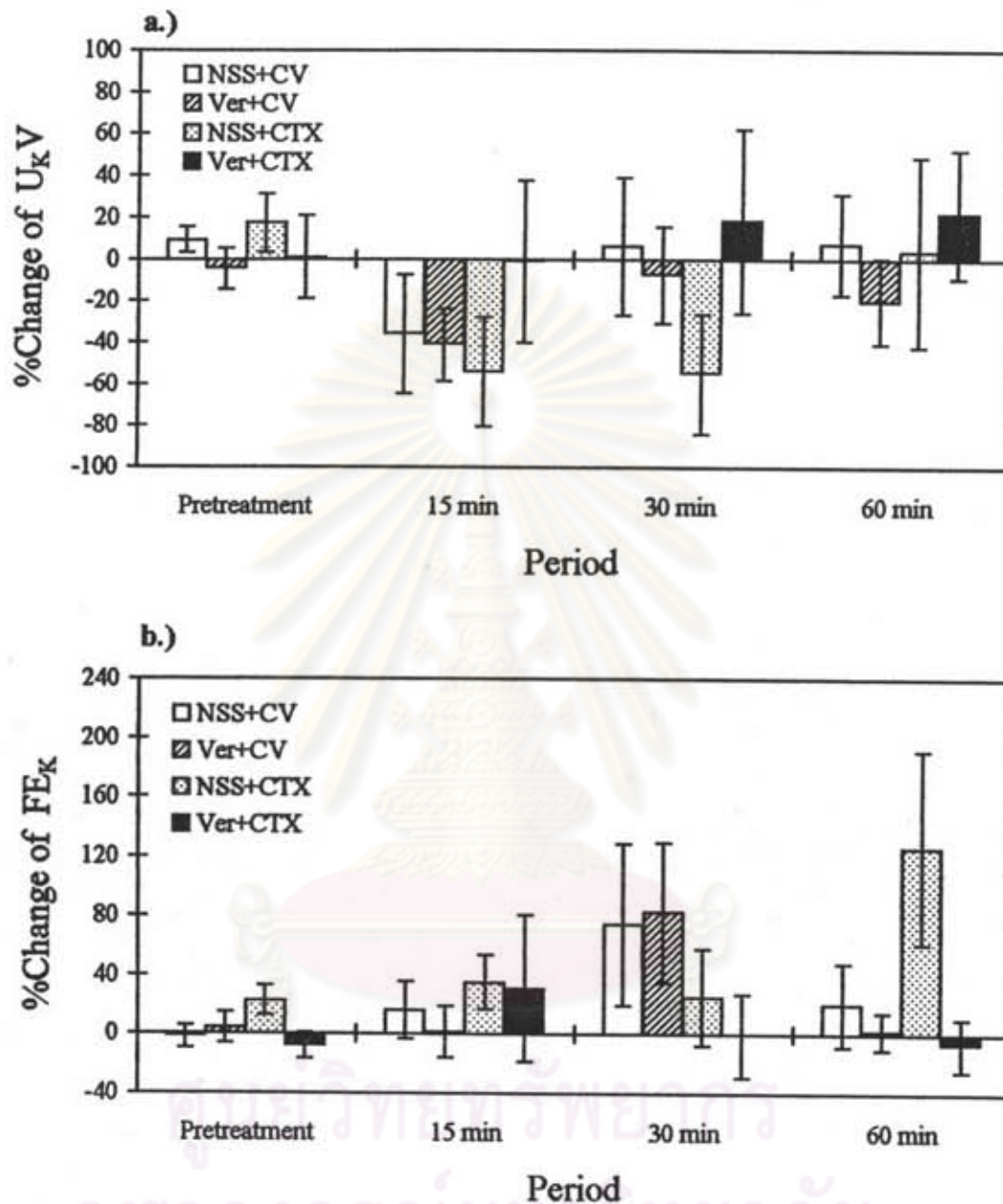


Figure 14. Percentage changes in urinary potassium excretion ( $U_KV$ )(a.) and fractional excretion of potassium ( $FE_K$ ) ( b.) in response to crude cobra venom ( CV ) and / or its cardiotoxic fraction (CTX) and pretreated intravenous infusion with normal saline solution (NSS) and/or verapamil (Ver). Significant difference values using paired t-test are indicated by \*  $P < 0.05$  compared to the control value and †  $P < 0.05$  compared to the pretreatment value of each group.

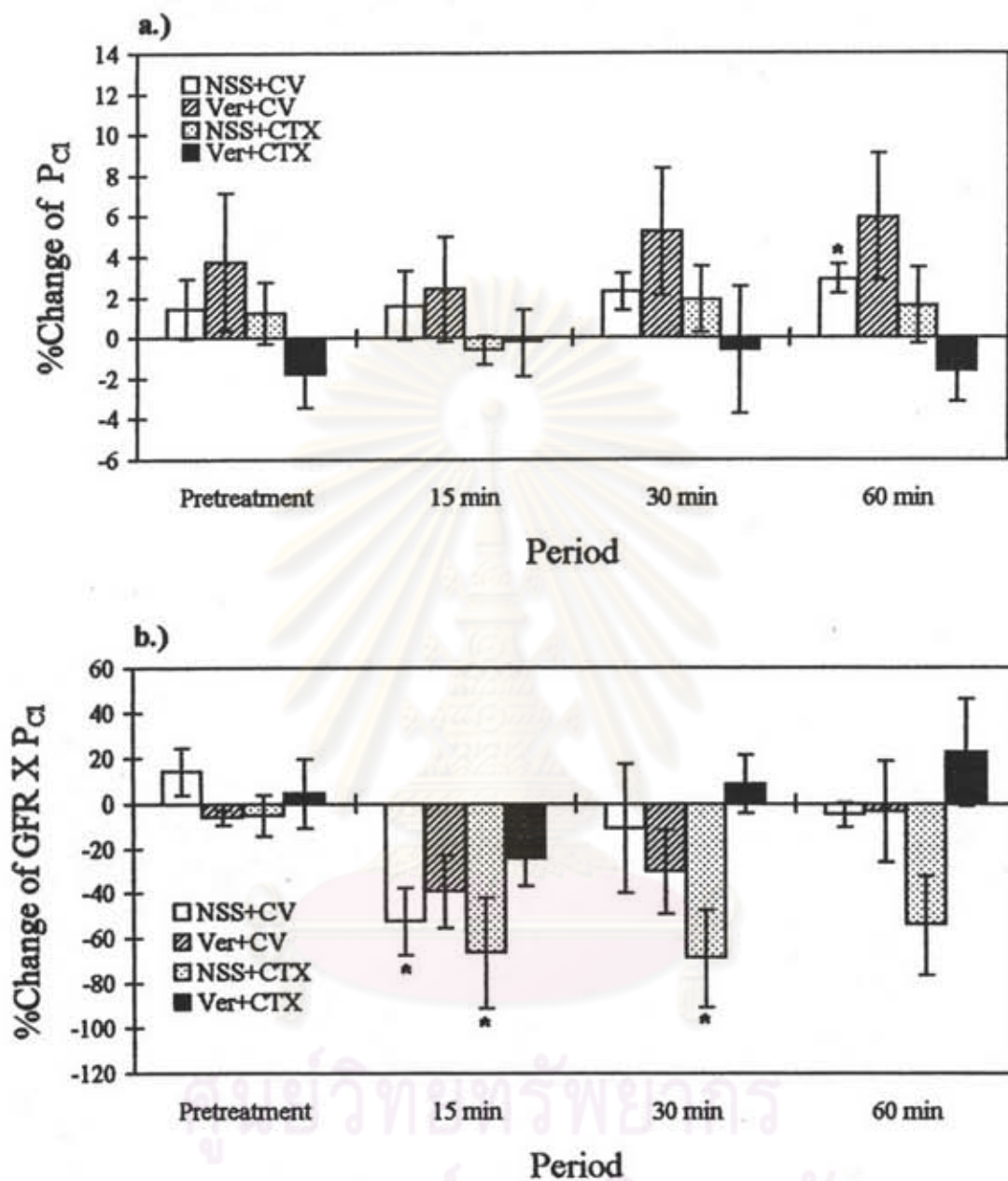


Figure 15. Percentage changes in plasma chloride concentration ( $P_{Cl}$ )(a.) and filtered load of chloride ( $GFR \times P_{Cl}$ )( b.) in response to crude cobra venom ( CV ) and / or its cardiotoxic fraction ( CTX) and pretreated intravenous infusion with normal saline solution (NSS) and/or verapamil (Ver). Significant difference values using paired t-test are indicated by \*  $P < 0.05$  compared to the control value and †  $P < 0.05$  compared to the pretreatment value of each group.



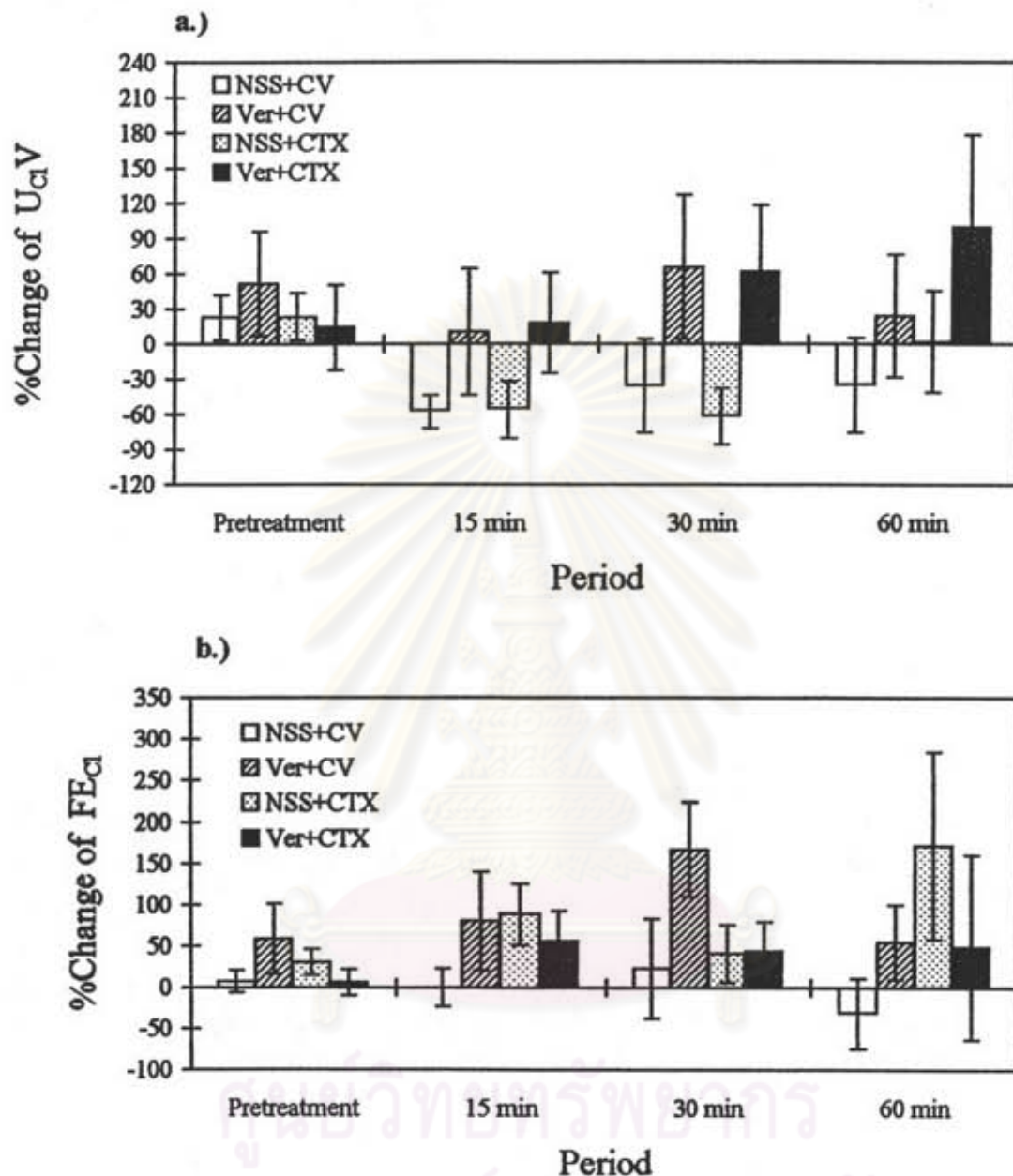


Figure 16. Percentage changes in urinary chloride excretion ( $U_{ClV}$ ) (a.) and fractional excretion of chloride ( $FE_{Cl}$ ) ( b.) in response to crude cobra venom ( CV ) and / or its cardiotoxic fraction (CTX) and pretreated intravenous infusion with normal saline solution (NSS) and / or verapamil (Ver).

Significant difference values using paired t-test are indicated by \*  $P < 0.05$  compared to the control value and †  $P < 0.05$  compared to the pretreatment value of each group.

### Effects of Cobra Venom on Urinary Acid-Base Excretion.

The results of changes in urinary acid-base excretion in dogs given cobra venom are shown in Table 12 - Table 14 and Figure 17 - Figure 19.

Group I : Control animal.

As shown in Table 12, the results are expressed by mean $\pm$ SEM. In the control group after intravenous saline infusion, all parameters did not indicate the significant changes throughout the experimental period.

Group II : Effects of crude cobra venom injection.

Animal pretreated with intravenous saline infusion before crude cobra venom injection caused no changes in any of the measurements made (Table 13 and Table 14). After envenomation, there were no significant changes from the control value in blood pH (Figure 17). Blood concentration of bicarbonate insignificantly decreased from the control value throughout the experiment (Figure 17). Urine pH slightly increased whereas urinary titratable acid excretion ( $U_{TA}V$ ) significantly decreased to  $81.1\pm 7.8\%$  of the control value at 15 minutes after envenomation. Urinary ammonium excretion ( $U_{NH_4}V$ ) showed no significant decrease from the control value (Figure 18). Urinary acid excretion (UAE) significantly decreased to  $75.8\pm 15.2\%$  of the control value at 15 minutes after envenomation and slightly decreased in the other periods (Figure 19).

Group III : Animal pretreated with verapamil before crude cobra venom injection.

Intravenous infusion of verapamil caused a significant raise in blood pH from  $7.3\pm 0.02$  to  $7.31\pm 0.02$  and significant fell in blood bicarbonate to  $13.6\pm 2\%$  of the control value (Table 13). After envenomation, there was no significant change of blood pH in compared to the control value. Blood concentration of bicarbonate significantly decreased to  $12\pm 2.4\%$ ,  $21.1\pm 5.6\%$  and  $18.3\pm 5.3\%$  of the control value at 15 minutes, 30 minutes and 60 minutes after envenomation, respectively (Figure 17). Urine pH significantly increased throughout the experimental period whereas urinary titratable

acid excretion and urinary ammonium excretion slightly decreased (Figure 18 and Figure 19)

Group IV : Effects of cardiotoxic fraction of cobra venom injection.

Animal pretreated with intravenous infusion of normal saline solution caused no changes in any of the measurements made (Table 13 and Table 14). After cardiotoxic fraction administration, there were no significant changes from the control value in blood pH. Blood concentration of bicarbonate significantly decreased to  $12.4 \pm 4.2\%$ ,  $21.6 \pm 7\%$  and  $19.7 \pm 7\%$  of the control value at 15 minutes, 30 minutes and 60 minutes after envenomation, respectively (Figure 17). Urine pH slightly increased throughout the experimental period whereas urinary titratable acid excretion and urinary ammonium excretion insignificantly decreased. Urinary acid excretion insignificantly decreased from the control value in the period of 15 minutes and 30 minutes after envenomation while insignificantly increased in the period of 60 minutes after envenomation (Figure 17 and Figure 18).

Group V : Animal pretreated with verapamil before cardiotoxic fraction of cobra venom injection.

Intravenous infusion of saline caused no changes in any of the measurements made (Table 13 and Table 14). After envenomation, there were no significant changes from the control value in blood pH, and blood concentration of bicarbonate (Figure 17). Urine pH slightly decreased at 15 minutes and 30 minutes after envenomation, and it increased at 60 minutes after envenomation (Figure 19). Urinary titratable acid excretion and urinary ammonium excretion insignificantly increased throughout the experimental period (Figure 18 and Figure 19).



**Table 12. Changes in blood pH, urine pH, blood bicarbonate concentration, urinary titratable acid excretion, urinary ammonium excretion and urinary acid excretion in control dogs (n=6).**

	NSS intravenous infusion		
	Control	NSS intravenous infusion	
		30 min	60 min
Blood pH	7.3 ± 0.0	7.3 ± 0.0 NS	7.3 ± 0.0 NS
Urine pH	6.2 ± 0.2	6.3 ± 0.2 NS	6.5 ± 0.1 NS
Blood <sub>HCO<sub>3</sub></sub> (mmole/L)	14.7 ± 1.3	14.3 ± 1.2 NS	12.9 ± 2.5 NS
UAE (μmole/min/kg. bw.)	0.08 ± 0.03	0.09 ± 0.02 NS	0.07 ± 0.02 NS
U <sub>TA</sub> V (μmole/min/kg. bw.)	0.03 ± 0.01	0.04 ± 0.01 NS	0.04 ± 0.01 NS
U <sub>NH<sub>4</sub></sub> V (μmole/min/kg. bw.)	0.05 ± 0.02	0.05 ± 0.01 NS	0.04 ± 0.01 NS

Values are mean ± SEM. Abbreviations: Blood<sub>HCO<sub>3</sub></sub>, blood bicarbonate concentration, U<sub>TA</sub> V, urinary titratable acid excretion; U<sub>NH<sub>4</sub></sub> V, urinary ammonium excretion; UAE, urinary acid excretion.

Significant difference values using paired t-test are indicated by \* P<0.05 different from control values and NS = not significant.

**Table 13.** Changes in blood pH, urine pH and blood bicarbonate concentration in response to crude cobra venom and/ or its cardiotoxic fraction administration in four groups.

	Control	Venom administration				
		Pre-treatment	15 min	30 min	45 min	60 min
<b>Blood pH</b>						
II. NSS + CV (n=4)	7.27 ± 0.03	7.29 ± 0.03 NS	7.25 ± 0.02 NS	7.24 ± 0.02 NS	7.22 ± 0.01 NS	7.18 ± 0.01 NS
III. Ver + CV (n=6)	7.30 ± 0.02	7.31 ± 0.02 *	7.25 ± 0.02 *†	7.27 ± 0.02 NS	7.26 ± 0.02 NS	7.24 ± 0.03 NS
IV. NSS + CTX (n=5)	7.31 ± 0.02	7.32 ± 0.03 NS	7.28 ± 0.04 NS	7.21 ± 0.05 NS	7.20 ± 0.06 NS	7.21 ± 0.07 NS
V. Ver + CTX (n=4)	7.23 ± 0.02	7.24 ± 0.02 NS	7.25 ± 0.02 NS	7.24 ± 0.02 NS	7.23 ± 0.04 NS	7.23 ± 0.03 NS
<b>Urine pH</b>						
II. NSS + CV (n=4)	6.58 ± 0.28	6.57 ± 0.29 NS	6.68 ± 0.27 NS	7.23 ± 0.31 NS	7.24 ± 0.26 NS	7.10 ± 0.19 NS
III. Ver + CV (n=6)	6.63 ± 0.19	6.70 ± 0.21 NS	6.83 ± 0.21 *†	7.11 ± 0.20 *†	7.21 ± 0.19 *†	7.16 ± 0.20 *†
IV. NSS + CTX (n=5)	6.78 ± 0.23	6.61 ± 0.13 NS	6.98 ± 0.14 NS	7.01 ± 0.10 NS	6.83 ± 0.28 NS	6.80 ± 0.24 NS
V. Ver + CTX (n=4)	6.30 ± 0.30	6.28 ± 0.39 NS	6.24 ± 0.39 NS	6.24 ± 0.43 NS	6.44 ± 0.52 NS	6.75 ± 0.34 NS
<b>Blood<sub>HCO<sub>3</sub></sub> (mmole/L)</b>						
II. NSS + CV (n=4)	19.8 ± 1.7	17.5 ± 1.0 NS	15.0 ± 0.9 NS	16.1 ± 0.6 NS	16.1 ± 0.6 NS	15.8 ± 0.5 NS
III. Ver + CV (n=6)	18.0 ± 0.8	15.5 ± 0.8 *	15.8 ± 0.8 *	14.3 ± 1.4 *	14.7 ± 0.9 *	14.7 ± 1.1 *
IV. NSS + CTX (n=5)	18.1 ± 0.9	17.5 ± 0.7 NS	15.8 ± 0.6 *	14.0 ± 0.9 *†	13.8 ± 0.9 *†	14.4 ± 0.9 *†
V. Ver + CTX (n=4)	16.0 ± 1.0	16.1 ± 0.8 NS	15.5 ± 1.0 NS	15.9 ± 0.7 NS	15.4 ± 1.0 NS	15.6 ± 0.6 NS

Values are mean ± SEM. Abbreviations: CV, crude cobra venom; CTX, cardiotoxic fraction; Ver, verapamil; Blood<sub>HCO<sub>3</sub></sub>, blood bicarbonate concentration.

Significant difference values using paired t-test are indicated by \* P<0.05 different from control values, † P<0.05 different from pretreatment values and NS = not significant.



**Table 14. Changes in urinary titratable acid excretion, urinary ammonium excretion and urinary acid excretion in response to crude cobra venom and/ or its cardiotoxic fraction administration in four groups.**

	Control	Venom administration				
		Pretreatment	15 min	30 min	45 min	60 min
<b>UAE (<math>\mu\text{mole}/\text{min}/\text{kg}.\text{bw}.</math>)</b>						
II. NSS + CV (n=4)	0.059 $\pm$ 0.03	0.110 $\pm$ 0.08 NS	0.011 $\pm$ 0.01 NS	0.034 $\pm$ 0.02 NS	0.036 $\pm$ 0.02 NS	0.037 $\pm$ 0.02 NS
III. Ver + CV (n=6)	0.145 $\pm$ 0.06	0.207 $\pm$ 0.10 NS	0.036 $\pm$ 0.01 NS	0.138 $\pm$ 0.09 NS	0.198 $\pm$ 0.15 NS	0.098 $\pm$ 0.05 NS
IV. NSS + CTX (n=5)	0.076 $\pm$ 0.04	0.140 $\pm$ 0.08 NS	0.008 $\pm$ 0.01 NS	0.008 $\pm$ 0.01 NS	0.020 $\pm$ 0.01 NS	0.020 $\pm$ 0.01 NS
V. Ver + CTX (n=4)	0.039 $\pm$ 0.02	0.084 $\pm$ 0.06 NS	0.202 $\pm$ 0.19 NS	0.229 $\pm$ 0.21 NS	0.091 $\pm$ 0.05 NS	0.080 $\pm$ 0.04 NS
<b>U<sub>TA</sub>V (<math>\mu\text{mole}/\text{min}/\text{kg}.\text{bw}.</math>)</b>						
II. NSS + CV (n=4)	0.013 $\pm$ 0.00	0.018 $\pm$ 0.01 NS	0.002 $\pm$ 0.00 *	0.013 $\pm$ 0.01 NS	0.007 $\pm$ 0.00 NS	0.008 $\pm$ 0.00 NS
III. Ver + CV (n=6)	0.022 $\pm$ 0.01	0.032 $\pm$ 0.01 NS	0.011 $\pm$ 0.00 NS	0.025 $\pm$ 0.01 NS	0.028 $\pm$ 0.02 NS	0.025 $\pm$ 0.01 NS
IV. NSS + CTX (n=5)	0.038 $\pm$ 0.03	0.060 $\pm$ 0.04 NS	0.001 $\pm$ 0.00 NS	0.001 $\pm$ 0.00 NS	0.010 $\pm$ 0.00 NS	0.016 $\pm$ 0.01 NS
V. Ver + CTX (n=4)	0.011 $\pm$ 0.00	0.015 $\pm$ 0.01 NS	0.036 $\pm$ 0.03 NS	0.041 $\pm$ 0.03 NS	0.027 $\pm$ 0.01 NS	0.021 $\pm$ 0.01 NS
<b>U<sub>NH4</sub>V (<math>\mu\text{mole}/\text{min}/\text{kg}.\text{bw}.</math>)</b>						
II. NSS + CV (n=4)	0.046 $\pm$ 0.03	0.092 $\pm$ 0.08 NS	0.008 $\pm$ 0.00 NS	0.021 $\pm$ 0.01 NS	0.028 $\pm$ 0.02 NS	0.029 $\pm$ 0.02 NS
III. Ver + CV (n=6)	0.122 $\pm$ 0.06	0.175 $\pm$ 0.09 NS	0.025 $\pm$ 0.01 NS	0.113 $\pm$ 0.07 NS	0.169 $\pm$ 0.12 NS	0.073 $\pm$ 0.03 NS
IV. NSS + CTX (n=5)	0.044 $\pm$ 0.02	0.068 $\pm$ 0.03 NS	0.006 $\pm$ 0.00 NS	0.005 $\pm$ 0.00 NS	0.026 $\pm$ 0.01 NS	0.085 $\pm$ 0.07 NS
V. Ver + CTX (n=4)	0.029 $\pm$ 0.02	0.069 $\pm$ 0.05 NS	0.165 $\pm$ 0.16 NS	0.188 $\pm$ 0.17 NS	0.065 $\pm$ 0.04 NS	0.060 $\pm$ 0.03 NS

Values are mean  $\pm$  SEM. Abbreviations: CV, crude cobra venom; CTX, cardiotoxic fraction; Ver, verapamil; U<sub>TA</sub>V, urinary titratable acid excretion; U<sub>NH4</sub>V, urinary ammonium excretion; UAE, urinary acid excretion. Significant difference values using paired t-test are indicated by \* P<0.05 different from control values, † P<0.05 different from pretreatment values and NS = not significant.



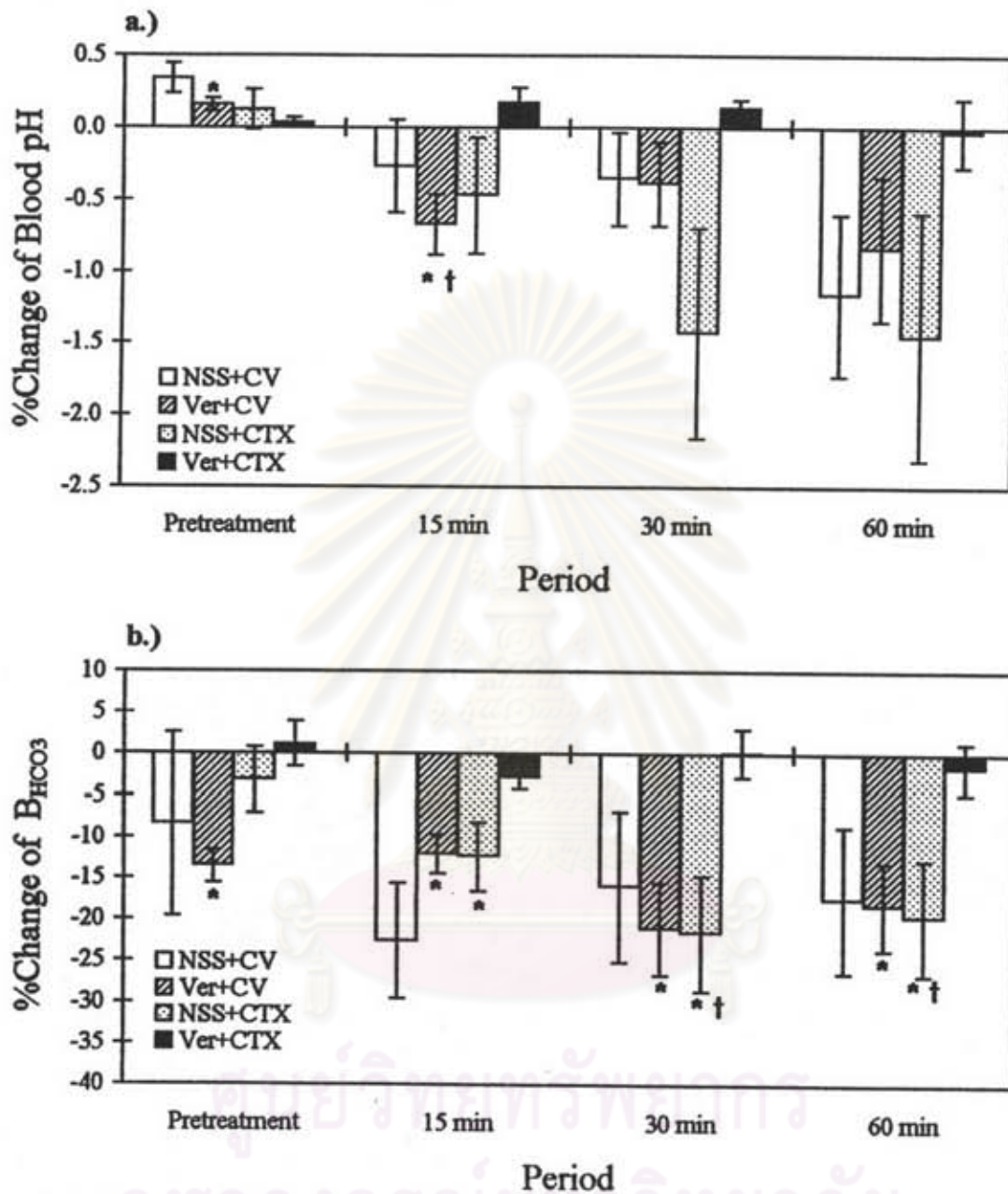


Figure 17. Percentage changes in blood pH (a.) and blood bicarbonate concentration ( $B_{HCO_3}$ ) ( b.) in response to crude cobra venom (CV) and / or its cardiotoxic fraction ( CTX ) and pretreated intravenous infusion with normal saline solution (NSS) and / or verapamil (Ver). Significant difference values using paired t-test are indicated by \*  $P < 0.05$  compared to the control value and †  $P < 0.05$  compared to the pretreatment value of each group.

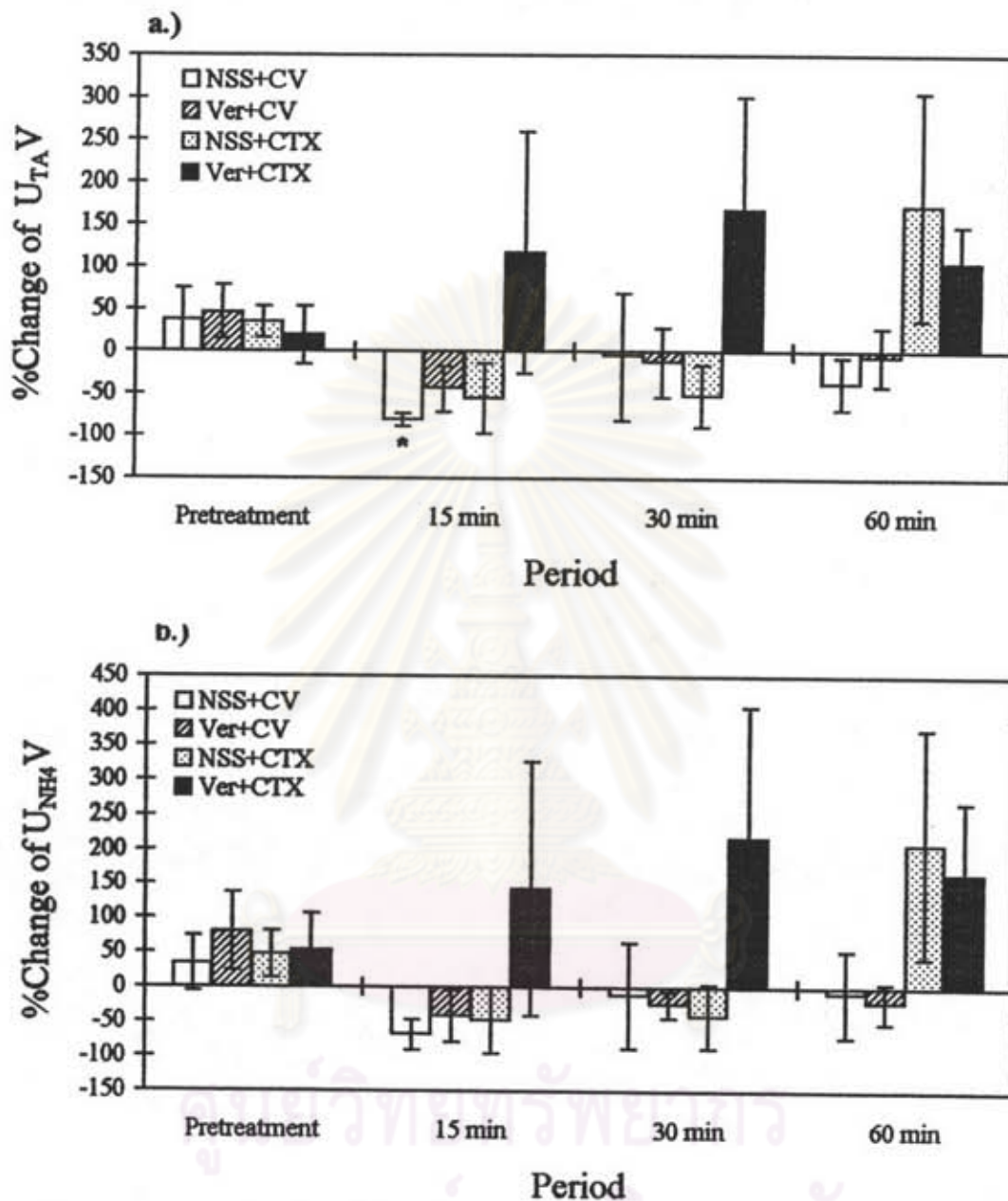


Figure 18. Percentage changes in urinary titratable acid excretion ( $U_{TA}V$ ) (a.) and urinary ammonium excretion ( $U_{NH_4}V$ ) (b.) in response to crude cobra venom (CV) and / or its cardiotoxic fraction (CTX) and pretreated intravenous infusion with normal saline solution (NSS) and / or verapamil (Ver). Significant difference values using paired t-test are indicated by \*  $P < 0.05$  compared to the control value and †  $P < 0.05$  compared to the pretreatment value of each group.

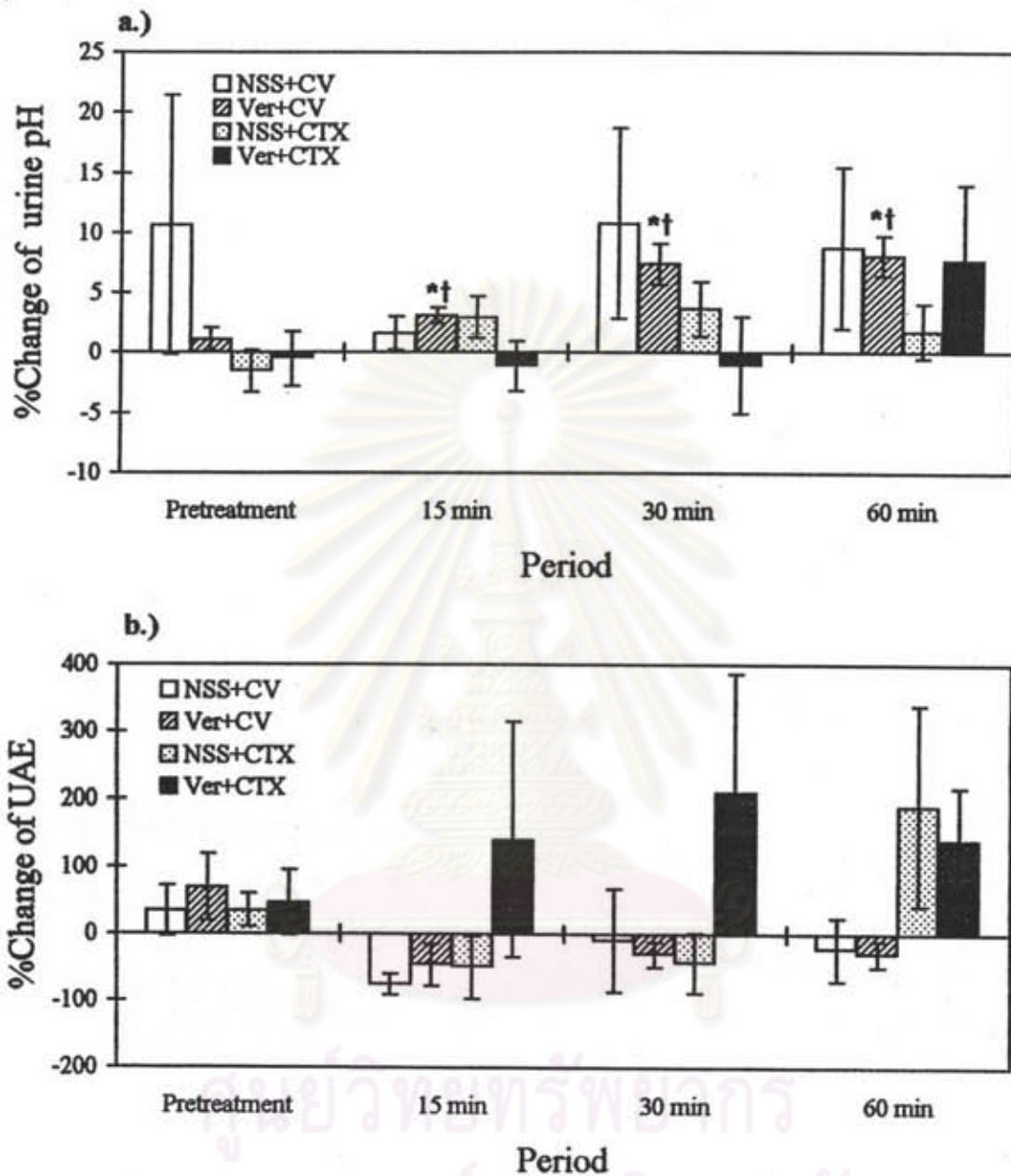


Figure 19. Percentage changes in urine pH (a.) and urinary acid excretion (UAE) (b.) in response to crude cobra venom (CV) and / or its cardiotoxic fraction (CTX) and pretreated intravenous infusion with normal saline solution (NSS) and / or verapamil (Ver).

Significant difference values using paired t-test are indicated by \*  $P < 0.05$  compared to the control value and †  $P < 0.05$  compared to the pretreatment value of each group.



### Effects of Cobra Venom on Urinary Water Excretion.

The results of changes in urinary water excretion in dogs given cobra venom are shown in Table 15 - Table 17 and Figure 20 - Figure 22.

Group I : Control animal.

As shown in Table 15, the results are expressed on mean $\pm$ SEM. After intravenous saline infusion, all parameters did not indicate the significant changes throughout the experimental period.

Group II : Effects of crude cobra venom injection.

Animal pretreated with intravenous infusion normal saline solution before crude cobra venom injection caused no changes in any of the measurements made (Table 16 and Table 17). There were no significant changes in plasma osmolarity. At 15 minutes after envenomation, urinary osmolarity excretion ( $U_{\text{osm}}V$ ) significantly decreased to  $57\pm 13.4\%$  (Figure 20) and osmolarity clearance ( $C_{\text{osm}}$ ) significantly decreased to  $56.7\pm 14\%$  of the control values (Figure 21). Fractional water excretion ( $V/\text{GFR}$ ) slightly increased (Figure 22). Free water clearance ( $C_{\text{H}_2\text{O}}$ ) significantly decreased  $58.7\pm 11.7\%$  of the control value after envenomation (Figure 21).

Group III : Animal pretreated with verapamil before crude cobra venom injection.

Intravenous infusion of verapamil alone caused no changes in any of the measurements made (Table 16 and Table 17). There were no significant changes in plasma osmolarity. Urinary osmolarity excretion and osmolar clearance insignificantly decreased throughout the experimental period whereas fractional water excretion slightly increased. Free water clearance showed no significant changed throughout the experimental period (Figure 20, Figure 21 and Figure 22).

Group IV : Effects of cardiotoxic fraction of cobra venom injection.

Animal pretreated with intravenous infusion of normal saline solution caused no changes in any of the measurements made (Table 16 and Table 17). There was no significant change in plasma osmolarity. Urinary osmolarity excretion and osmolarity clearance insignificantly decreased throughout the experimental period whereas fractional water excretion significantly increased approximately 100% of the control value at 15 minutes after envenomation. Free water clearance decreased from the control value and significance changes at 30 minutes after envenomation (Figure 20, Figure 21 and Figure 22).

Group V : Animal pretreated with verapamil before cardiotoxic fraction of cobra venom injection

Intravenous infusion of verapamil caused no changes in any of the measurements made (Table 16 and Table 17). There were no significant changes in plasma osmolarity. Urinary osmolarity excretion and osmolar clearance showed no difference from the control value whereas fractional water excretion insignificantly increased throughout the experimental period. Free water clearance slightly decreased and gradually increased from the control value (Figure 20, Figure 21 and Figure 22).

Effects of Cobra Venom on Na<sup>+</sup>-K<sup>+</sup> ATPase Activity.

The results of changes in Na<sup>+</sup>-K<sup>+</sup> ATPase activity in dogs given cobra venom are shown in Table 18.

Na<sup>+</sup>-K<sup>+</sup> ATPase activity in dogs given normal saline alone (group I) was 112±17 nmol.P/mg.prot./min. In dogs given crude cobra venom (group II) and cardiotoxic fraction (group III) Na<sup>+</sup>-K<sup>+</sup> ATPase activity decreased to 66±15.4 nmol. P/mg.prot./min. and 72.8±4.3 nmol.P/mg.prot./min., respectively. In comparison to group I activity of Na<sup>+</sup>-K<sup>+</sup> ATPase in group III and group V was also lesser than that of group I but no significant difference.

**Table 15. Changes in plasma osmolarity, osmolar clearance, urinary osmolarity excretion, free water clearance and fractional water excretion in control dogs (n=6).**

	NSS intravenous infusion		
	Control	NSS intravenous infusion	
		30 min	60 min
$P_{\text{osm}}$ (mOsm/L)	281.5 ± 5.1	287.3 ± 6.8 NS	283.8 ± 2.0 NS
$C_{\text{osm}}$ ( $\mu$ l/min/kg.bw.)	32.2 ± 7.2	31.2 ± 6.2 NS	28.8 ± 5.3 NS
$U_{\text{osm}} V$ (mOsm/min/kg.bw.)	9.0 ± 1.9	8.8 ± 1.6 NS	8.2 ± 1.5 NS
Free water clearance ( $\mu$ l/min/kg.bw.)	15.5 ± 15.3	25.3 ± 17.1 NS	22.3 ± 15.1 NS
Fractional water excretion (%)	3.4 ± 1.2	3.7 ± 1.2 NS	3.8 ± 1.1 NS

Values are mean ± SEM. Abbreviations:  $P_{\text{osm}}$ , plasma osmolarity;  $C_{\text{osm}}$ , osmolar clearance;  $U_{\text{osm}} V$ , urinary osmolarity excretion.

Significant difference values using paired t-test are indicated by \*  $P < 0.05$  different from control values and NS = not significant.



**Table 16.** Changes in plasma osmolarity, osmolar clearance and urinary osmolarity excretion in response to crude cobra venom and/ or its cardiotoxic fraction administration in four groups.

	Control	Venom administration				
		Pretreatment	15 min	30 min	45 min	60 min
<b>P<sub>osm</sub> (mOsm/L)</b>						
II. NSS + CV (n=4)	293.8 ± 3.9	299.3 ± 2.5 NS	294.5 ± 1.8 NS	299.3 ± 1.4 NS	297.3 ± 2.9 NS	297.8 ± 2.1 NS
III. Ver + CV (n=6)	286.3 ± 7.8	299.8 ± 2.2 NS	295.3 ± 2.1 NS	296.5 ± 3.7 NS	296.8 ± 1.5 NS	298.3 ± 2.5 NS
IV. NSS + CTX (n=5)	300.8 ± 5.0	301.4 ± 4.3 NS	293.0 ± 3.1 †	304.2 ± 4.9 NS	300.4 ± 3.2 NS	301.0 ± 6.7 NS
V. Ver + CTX (n=4)	297.8 ± 7.6	292.0 ± 5.7 NS	298.5 ± 1.8 NS	287.0 ± 5.5 NS	292.3 ± 6.9 NS	300.5 ± 3.8 NS
<b>C<sub>osm</sub> (μl/min/kg.bw.)</b>						
II. NSS + CV (n=4)	57.0 ± 9.0	66.0 ± 18.3 NS	23.3 ± 6.2 *	40.8 ± 13.8 NS	42.8 ± 14.2 NS	40.5 ± 11.4 NS
III. Ver + CV (n=6)	38.0 ± 6.9	38.2 ± 7.0 NS	24.7 ± 7.7 NS	35.3 ± 8.9 NS	36.3 ± 11.9 NS	37.0 ± 12.6 NS
IV. NSS + CTX (n=5)	56.6 ± 10.8	64.6 ± 15.2 NS	18.8 ± 8.4 NS	12.4 ± 8.1 NS	24.6 ± 9.8 NS	29.4 ± 11.6 NS
V. Ver + CTX (n=4)	35.5 ± 10.9	35.8 ± 11.5 NS	39.0 ± 18.4 NS	56.5 ± 30.4 NS	37.0 ± 10.3 NS	39.3 ± 13.5 NS
<b>U<sub>osm</sub> V (mOsm/min/kg.bw.)</b>						
II. NSS + CV (n=4)	16.7 ± 2.5	19.6 ± 5.3 NS	6.9 ± 1.9 *	12.2 ± 4.2 NS	12.7 ± 4.3 NS	12.1 ± 3.4 NS
III. Ver + CV (n=6)	11.0 ± 2.1	11.4 ± 2.1 NS	7.1 ± 2.2 NS	10.4 ± 2.6 NS	10.7 ± 3.5 NS	11.0 ± 3.8 NS
IV. NSS + CTX (n=5)	17.1 ± 3.4	19.4 ± 4.6 NS	5.6 ± 2.6 NS	3.9 ± 2.6 NS	7.4 ± 2.9 NS	9.0 ± 3.5 NS
V. Ver + CTX (n=4)	10.3 ± 3.0	10.2 ± 3.2 NS	11.6 ± 5.4 NS	16.0 ± 8.6 NS	10.7 ± 2.8 NS	11.7 ± 4.0 NS

Values are mean ± SEM. Abbreviations: CV, crude cobra venom; CTX, cardiotoxic fraction; Ver, verapamil; P<sub>osm</sub>, plasma osmolarity; C<sub>osm</sub>, osmolar clearance; U<sub>osm</sub> V, urinary osmolarity excretion.

Significant difference values using paired t-test are indicated by \* P<0.05 different from control values, † P<0.05 different from pretreatment values and NS = not significant.

**Table 17. Changes in free water clearance and fractional water excretion in response to crude cobra venom and/ or its cardiotoxic fraction administration in four groups.**

	Control	Venom administration				
		Pretreatment	15 min	30 min	45 min	60 min
<b>Free water clearance (<math>\mu\text{l}/\text{min}/\text{kg}.\text{bw}.</math>)</b>						
II. NSS + CV (n=4)	-25.5 $\pm$ 2.1	-26.3 $\pm$ 1.3 NS	-10.0 $\pm$ 2.0 *†	-16.5 $\pm$ 6.0 NS	-16.5 $\pm$ 3.2 NS	-15.5 $\pm$ 3.3 NS
III. Ver + CV (n=6)	5.0 $\pm$ 10.7	14.8 $\pm$ 13.6 *	-4.7 $\pm$ 7.4 NS	4.3 $\pm$ 10.9 NS	6.1 $\pm$ 11.3 NS	0.7 $\pm$ 9.8 NS
IV. NSS + CTX (n=5)	-14.8 $\pm$ 15.0	-37.0 $\pm$ 11.5 NS	-10.8 $\pm$ 5.3 NS	-6.6 $\pm$ 4.0 NS	-7.6 $\pm$ 4.5 NS	-1.6 $\pm$ 9.5 NS
V. Ver + CTX (n=4)	-21.5 $\pm$ 6.1	-13.5 $\pm$ 8.2 NS	-8.3 $\pm$ 10.1 NS	-23.8 $\pm$ 8.3 NS	-12.8 $\pm$ 8.0 NS	-16.3 $\pm$ 10.5 NS
<b>Fractional water excretion (%)</b>						
II. NSS + CV (n=4)	2.3 $\pm$ 0.8	2.3 $\pm$ 0.9 NS	2.6 $\pm$ 1.4 NS	1.9 $\pm$ 0.7 NS	2.0 $\pm$ 0.7 NS	1.8 $\pm$ 0.6 NS
III. Ver + CV (n=6)	3.4 $\pm$ 1.6	4.3 $\pm$ 1.9 NS	2.5 $\pm$ 0.8 NS	6.3 $\pm$ 3.8 NS	3.5 $\pm$ 1.3 NS	2.6 $\pm$ 0.8 NS
IV. NSS + CTX (n=5)	1.3 $\pm$ 0.4	1.5 $\pm$ 0.3 NS	2.4 $\pm$ 0.7 *	2.1 $\pm$ 1.0 NS	2.3 $\pm$ 1.1 NS	4.8 $\pm$ 2.7 NS
V. Ver + CTX (n=4)	0.8 $\pm$ 0.3	1.1 $\pm$ 0.6 NS	2.3 $\pm$ 1.8 NS	1.5 $\pm$ 1.0 NS	1.3 $\pm$ 0.6 NS	1.1 $\pm$ 0.4 NS

Values are mean  $\pm$  SEM. Abbreviations: CV, crude cobra venom; CTX, cardiotoxic fraction; Ver, verapamil. Significant difference values using paired t-test are indicated by \* P<0.05 different from control values, † P<0.05 different from pretreatment values and NS = not significant.

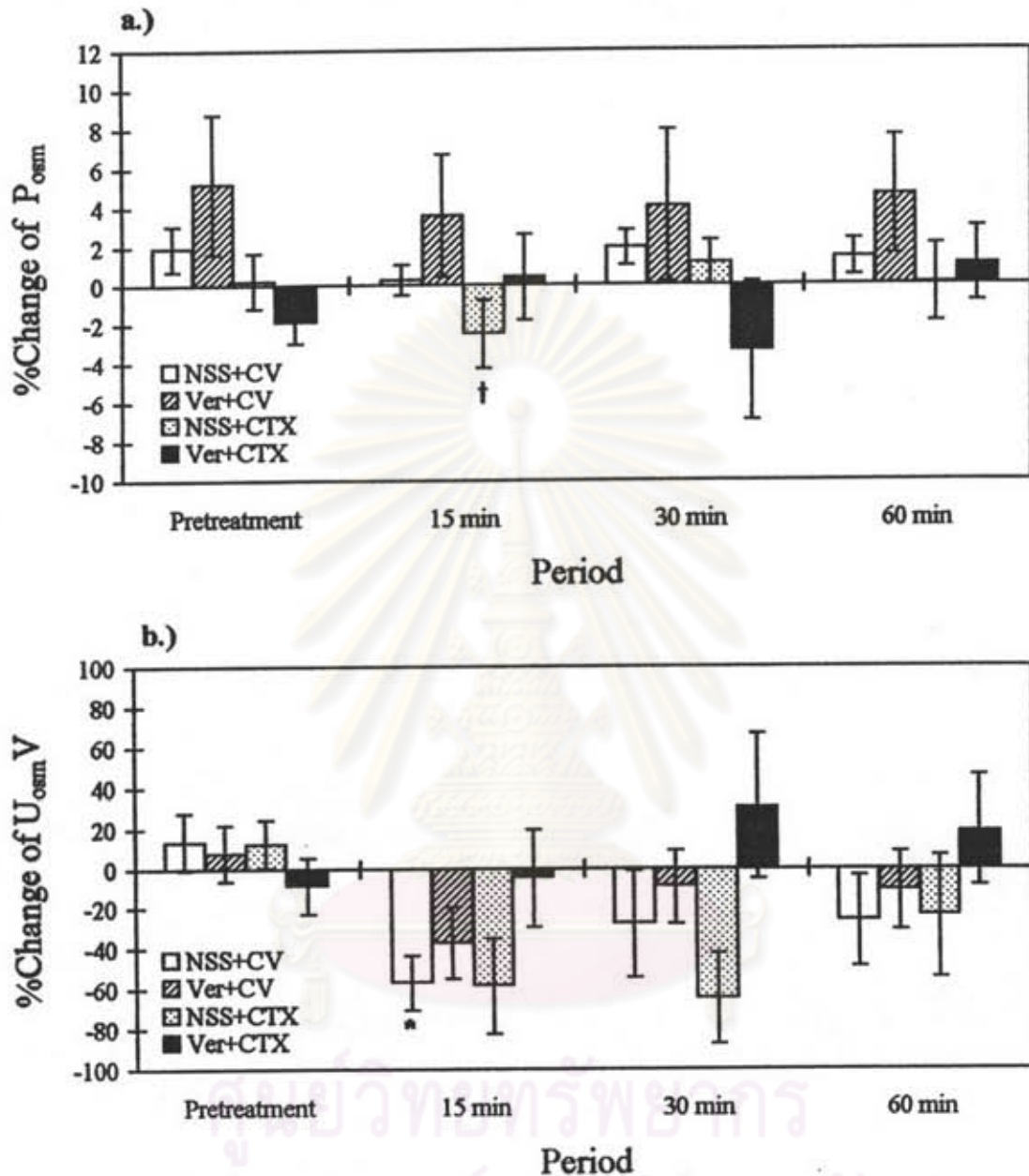


Figure 20. Percentage changes in plasma osmolarity ( $P_{om}$ ) (a.) and urinary osmolarity excretion ( $U_{om}V$ ) (b.) in response to crude cobra venom (CV) and / or its cardiotoxic fraction (CTX) and pretreated intravenous infusion with normal saline solution (NSS) and / or verapamil (Ver).

Significant difference values using paired t-test are indicated by \*  $P < 0.05$  compared to the control value and †  $P < 0.05$  compared to the pretreatment value of each group.



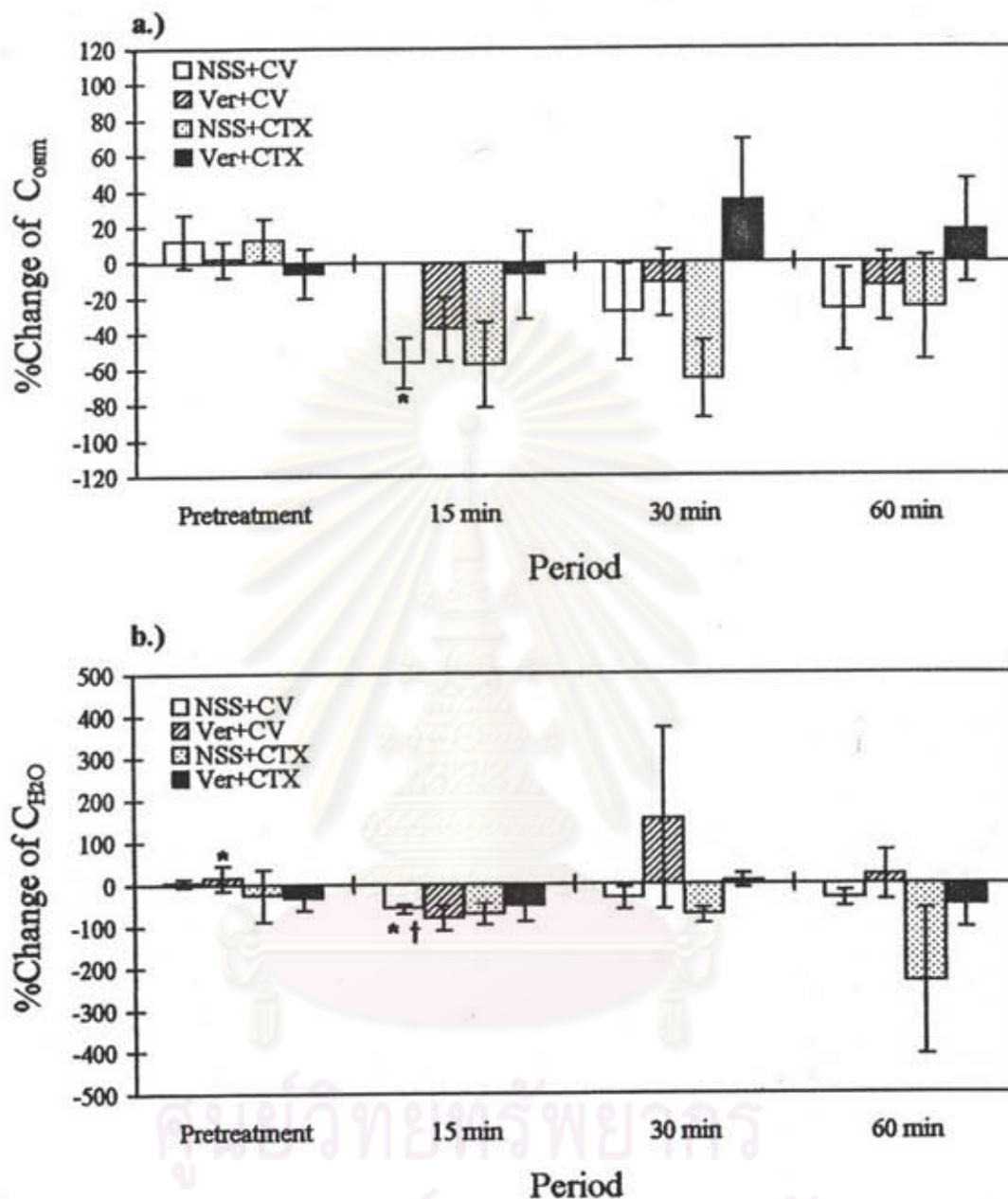


Figure 21. Percentage changes in osmolar clearance ( $C_{om}$ ) (a.) and free water clearance ( $C_{H_2O}$ ) (b.) in response to crude cobra venom (CV) and/or its cardiotoxic fraction (CTX) and pretreated intravenous infusion with normal saline solution (NSS) and/or verapamil (Ver). Significant difference values using paired t-test are indicated by \*  $P < 0.05$  compared to the control value and †  $P < 0.05$  compared to the pretreatment value of each group.

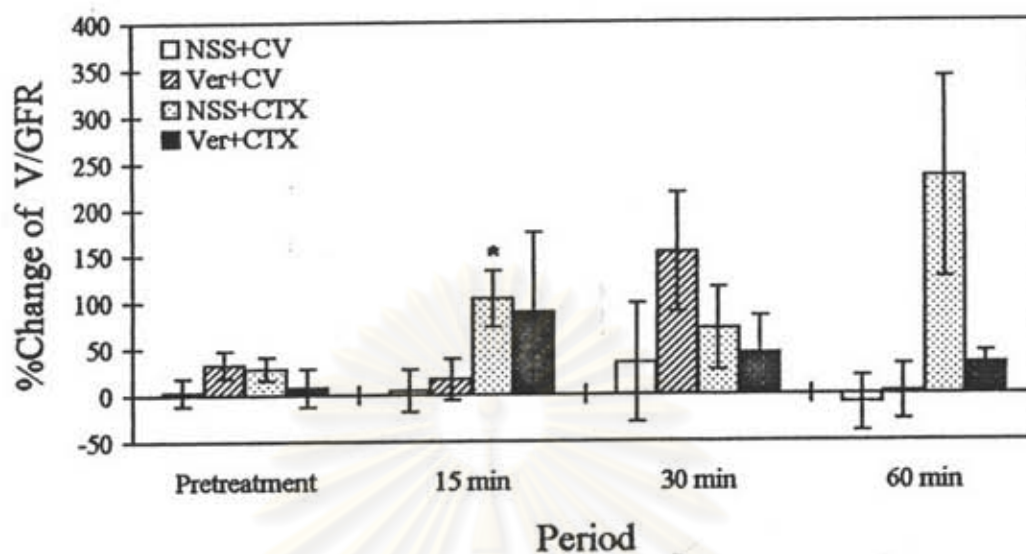


Figure 22. Percentage changes in fractional water excretion (V/GFR) in response to crude cobra venom (CV) and / or its cardiotoxic fraction (CTX) and pretreated intravenous infusion with normal saline solution (NSS) and / or verapamil (Ver). Significant difference values using paired t-test are indicated by \*  $P < 0.05$  compared to the control value and †  $P < 0.05$  compared to the pretreatment value of each group.

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**Table 18.** Changes in Na<sup>+</sup> - K<sup>+</sup> ATPase activity in response to crude cobra venom and/ or its cardiotoxic fraction administration in five groups.

		Na <sup>+</sup> - K <sup>+</sup> ATPase activity (nmol.P <sub>i</sub> /mg.prot./min)
I.	Control (n=7)	112.0 ± 17.0
II.	NSS+CV (n=7)	66.0 ± 15.4 *
III.	Ver+CV (n=6)	70.2 ± 20.1 NS
IV.	NSS+CTX (n=4)	61.3 ± 2.8 *
V.	Ver+CTX (n=4)	72.8 ± 4.3 NS

Values are mean ± SEM. Abbreviations: CV, crude cobra venom; CTX, cardiotoxic fraction; NSS, normal saline solution; Ver, verapamil.

Significant difference values using unpaired t-test are indicated by \* P<0.05 different from control values, and NS = not significant.

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