CHAPTER VI

RESULTS

Epidemiology study

1. *C. rhodostoma* bites

The prospective study collected a total of 145 cases of *C. rhodostoma* snakebites from 10 provinces of higher incidence rates in Thailand (Figure 5). Eighty hosptial charts of snakebite patients from the year 2001 in Prachuab Khiri Khan province were reviewed retrospectively. Most victims came from the southern region which included Trang (33.79 %), Nakorn Si Thammarat (22.07 %), Prachuap Khiri Khan (17.93 %), Surat Thani (15.06 %) and Songkhla (7.50 %). Surprisingly, Lampang, a northern province was provided 2.76 %. We found no *C. rhodostoma* bite victims in Nakorn Ratchasima (North-east), Lop Buri (Central), Ratchaburi (Western) and Nakorn Sawan (Northern) provinces (Table 7). The peak snakebite season was in May and April, early during the monsoon (19.31 % and 18.75 % respectively) (Table 8).

More male patients who were married, had experienced snake bites (50 % and 71 % respectively). The age groups, 41-60 and 21-40, contained more snakebite patients in the prospective and retrospective studies. Most people had primary school education, worked in manual labour positions and had a salary range of 1,000 –3,000 baht (Table 9).

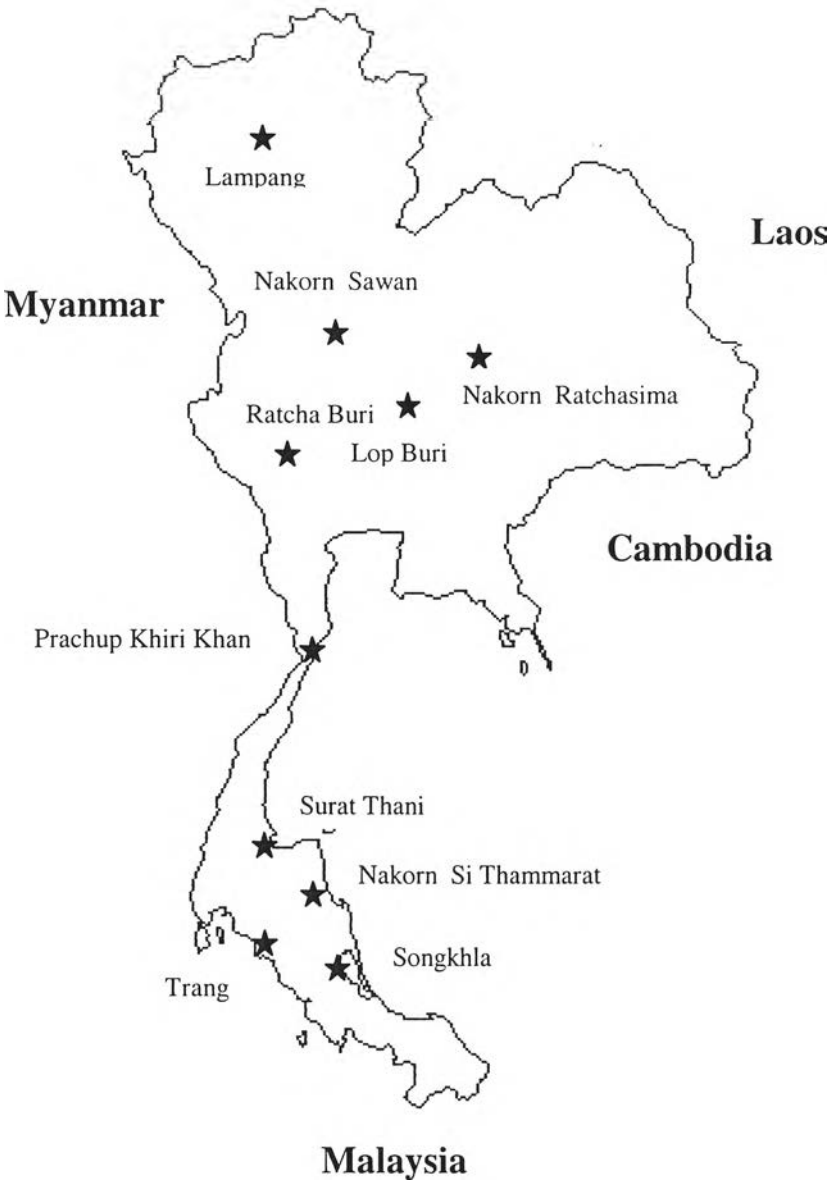


Figure 5. Map of Thailand showing the ten provincial locations.

Table 7. Distribution of snakebite victims among the 10 provincial hospitals, Prospective study.

Province	**Number (n)**	**Frequency (%)**
Lampang	4	2.76
Nakorn Sawan	0	0
Lop Buri	0	0
Nakorn Ratchasima	0	0
Ratcha Buri	0	0
Prachuap Khiri Khan	26	17.93
Surat Thani	23	15.86
Nakorn Si Thammarat	32	22.07
Trang	49	33.79
Songkhla	11	7.59

Table 8. Victims bitten by C. *rhdostoma* during April 2002 – June 2003, seasonal prevalence

Month	Prospective study		Retrospective study	
	Number (n)	Frequency (%)	Number(n)	Frequency (%)
January	2	1.38	10	12.50
February	5	3.45	5	6.25
March	3	2.07	6	7.50
April	14	9.66	15	18.75
May	28	19.31	10	12.50
June	15	10.34	2	2.50
July	12	8.28	3	3.75
August	13	8.97	4	5.00
September	17	11.72	7	8.75
October	14	9.65	5	6.25
November	7	4.83	7	8.75
December	15	8.97	6	7.50

Table 9. Epidemiological data in the prospective and retrospective studies

Variables	Prospective study		Retrospective study	
	Number (n)	Frequency (%)	Number(n)	Frequency (%)
Gender				
Male	73	50.30	50	62.50
Female	72	49.70	30	37.50
Marital Status				
Single	33	22.80	30	37.50
Married	103	71	50	62.50
Divorced	9	6.20	0	0
Age (Years)				
1-10	1	0.69	6	7.50
11-20	13	8.97	8	10.00
21-40	33	22.76	31	38.75
41-60	50	34.48	20	25.00
More than 60	48	33.10	15	18.75
Education				
No education	14	9.70	7	17.50
Primary school	98	67.60	54	67.50
High school	15	10.30	14	17.50
Undergraduate	18	12.40	5	6.25
Occupation				
Unemployed	8	5.52	14	17.50
Labour	102	70.34	46	57.50
Agriculture	12	8.27	5	6.25
Non governmment organizations	5	3.45	1	1.25
Government organizations	8	5.52	5	6.25
Student	10	6.90	9	11.25
Salary (Baht)				
100 - 1,000	15	10.34	25	31.25
1,000 – 3,000	58	40.00	26	32.50
3,001 – 6,000	50	34.48	20	25.00
6,001 – 9,000	9	6.20	3	3.75
9,001 – 12,000	6	4.14	4	5.00
More than 12,000	7	4.83	2	2.50

The distribution of sites bitten were: feet (46.20 – 52.50 %), finger (20.69 – 12.50 %), toe (13.10 – 22.50 %), hand (6.90 - 7.50 %), leg (9.66 – 3.75 %) in the prospective and retrospective study respectively. Most bites occurred in rural areas, outdoors and in dark or dusky places. The offending snakes were killed and available in 50.30 % in the prospective study, and only 7.50 % in the retrospective study. Snakebites occurred throughout the day, but more frequently during 8.01 – 12.00 a.m.; representing the time that victims work in the fields or rubber plantation. The size of snakes, was estimated by the distance between fang marks (1.01 – 2.00 cm. Table 10).

The time between snake bite and arrival at a hospital was 0.01 – 60 min (62.76 % and 76.25 %) in prospective and retrospective studies respectively. Most patients (60.70 in the prospective and 65.75 % in the retrospective studies) had not applied tourniquets. The volume of antivenin administered was 1-5 vials. 23.45 % - 38.75 % did not received antivenin in prospective and retrospective groups. Victims required only wound care to prevent or control infection were 94.50 % and 98.75 %. There was no need for amputation in both groups. Hospital admission ranged from 1-5 days (93.10 % in the prosepctive and 96.25 % in the retrospective studies) (Table 11).

Table 10. The various factors related to snakebites

Variables	Prospective study		Retrospective study	
	Number (n)	Frequency (%)	Number(n)	Frequency (%)
Season				
Rainy	70	48.28	18	22.50
Summer	51	35.17	34	42.50
Winter	24	16.66	28	35.00
Site of bite				
Finger	30	20.69	10	12.50
Hand	10	6.90	6	7.50
Arm	5	3.45	0	0
Toe	19	13.10	18	22.50
Foot	67	46.20	42	52.50
Leg	14	9.66	3	3.75
Buttock	0	0.00	1	1.25
Location				
Urban	25	17.20	26	32.50
Rural	120	82.80	54	67.50
Place of biting				
Indoor	14	9.70	1	1.25
Outdoor	131	90.30	79	98.75
Genous species				
Available	73	50.30	6	7.50
Not available	72	49.70	74	92.50
Time of biting				
00.01 – 05.00 a.m.	15	10.35	7	8.75
05.01 – 08.00 a.m.	22	15.17	8	10.00
08.01 – 12.00 a.m.	37	25.52	16	20.00
00.01 - 04.00 p.m.	21	14.48	15	18.75
04.01 – 08.00 p.m.	30	20.69	24	30.00
08.01 – 12.00 p.m.	20	13.79	10	12.50
The distance between fang marks (c.m.)				
0.01 – 1.00	49	44.95	15	78.95
1.01 – 2.00	41	37.62	3	15.79
2.01 – 3.00	14	12.84	1	5.26
3.01 – 4.00	2	1.84	0	
4.01 – 5.00	3	2.75	0	
	(36 missing record)		(61 missing record)	
Predisposing factors				
Barefoot	20	13.79	21	26.25
Dusky	125	86.21	59	73.75

Table 11. The various factore related to treatment

Variables	Prospective study		Retrospective study	
	Number (n)	Frequency (%)	Number(n)	Frequency (%)
Duration between bite and seeking medical advice (min)				
0.01 – 60	91	62.76	61	76.25
61 – 120	18	12.41	7	8.25
121 - 240	16	11.03	4	5.00
241 – 480	10	6.90	3	3.75
481 – 960	7	4.82	2	2.50
961 – 1440	1	0.70	2	2.50
More than 1440	2	1.38	1	1.25
First Aid Treatment				
Tourniquet	57	39.30	25	34.25
No Tourniquet	88	60.70	48	65.75
			(7 missing record)	
Treatment				
Dressing	137	94.50	79	98.75
Debridgemet	8	5.50	1	1.25
The number of antivenom used (vials)				
0	34	23.45	31	38.75
1 – 5	54	48.64	14	28.57
6 – 10	43	38.74	14	28.57
11 – 15	10	9.00	15	30.61
16 – 20	3	2.70	6	12.24
More than 20	1	0.90	0	0.00
Duration of hospitalization (Days)				
1 – 5	135	93.10	77	96.25
6 – 10	7	4.83	2	2.50
11 – 15	3	2.07	1	1.25

Among all factors effecting tissue necrosis, including demographic data, geographic data, factors related to treatment, have no variables influenced to tissue necrosis in the prospective study (Score 0 : no tissue necrosis ; Score 1-3 : having tissue necrosis) (Table 12). If score 0-1 was no tissue necrosis and score 2-3 having tissue necrosis, it would be shown significant by gender and time of bite in the prospective studies (Table 13). All factors had no effects on tissue necrosis. If the severity level was score 3 (having tissue necrosis) and score 0-2 (no tissue necrosis) (Table 14).

Among victims with coagulopathy, 52.48 % and 35.44 % in the prospective and retrospective studies had severe abnormal coagulation (VCT > 30 min) . The VCT gradually returned to normal by day 5 (Figure 6). A normal range of CPK , 10-180 Units/Litre, was the usual finding in snakebite victims (72.41 %). An abnomal CPK activity ranged from 181 to 856 Units/Litre and presented only on the first day of hospitalization in 27.59 % of patients (Figure 7).

The degree of snake clinical envenomation were calculated by adding each system. The level of severity was noted as : no symptom/sign (score 0-2), Minimal (score 3-5), moderate (score 6-8) and severe (score 9-20). The moderate envenomation was seen in 51.11 % in the 12 hours of hospitalization and gradually decreased to no symptom/sign by day 3. Most victims presented with no symptom/sign to mild clinical envenomation (Figure 8).

Table 12. Various factors effecting tissue necrosis were calculated by multiple logistic regression. The level of local wound severity (score 0-3) divided into 2 parts, no tissue necrosis (score 0) and having tissue necrosis (score 1-3).

Variables	B	Exp(B) : Odd	95 % CI	P value
Demographic data				
Gender	-17.34	0.00	0.00	.99
Age	13.59	801629	0.00	.99
Education	85.95	2.1	0.00	.98
Occupation	-33.41	0.00	0.00	.99
Salary	40.83	5.4	0.00	.99
Geographic data				
Place of bite	-23.83	0.00	0.00	.99
Factors related to snakebite				
Site of bite	20.49	8.0	0.00	.99
Time of bite	4.82	124.34	0.00	.99
Distance between fang marks	7.17	1300.67	0.00	.99
Factors related to treatment				
The number of antivenom used	5.49	242.96	0.00	.99
Duration between bite and seeking medical advice	9.41	2316.45	0.00	.99
First Aid treatment	-26.25	0.00	0.00	.997
Duration of hospitalization	-25.27	0.00	0.00	.99
Constant	**-170.47**			**0.99**

Table 13. Various factors effecting tissue necrosis were calculated by multiple logistic regression. The level of local wound severity (score 0-3) divided into 2 parts, no tissue necrosis (score 0-1) and having tissue necrosis (score 2-3).

Variables	B	Exp(B) : Odd	95 % CI	P value
Demographic data				
Gender	3.24	25.57	2.10-310.83	**0.011**
Age	0.182	1.19	.33-4.31	0.781
Education	1.86	6.45	0.31-1337.26	0.49
Occupation	7.24	1396.74	0.00-1.27	0.934
Salary	0.70	2.02	.864-4.761	0.104
Geographic data				
Place of bite	-0.91	0.40	0.17-9.81	0.57
Factors related to snakebite				
Site of bite	1.03	2.81	0.24-31.67	0.40
Time of bite	0.79	2.21	1.04-4.69	**0.037**
Distance between fang marks	0.10	1.10	0.34-3.50	0.86
Factors related to treatment				
The number of antivenom used	.038	1.47	0.44-4.94	0.52
Duration between bite and seeking medical advice	0.48	1.62	0.81-3.21	0.169
First Aid treatment	0.78	2.17	0.32-14.42	0.42
Duration of hospitalization	1.59	4.94	0.37-65.31	0.225
Constant	**-33.26**			**0.764**

Table 14. Various factors effecting tissue necrosis were calculated by multiple logistic regression. The level of local wound severity (score 0-3) divided into 2 parts, no tissue necrosis (score 0-2) and having tissue necrosis (score 3).

Variables	B	Exp(B) : Odd	95 % CI	P value
Demorgraphic data				
Gender	24.48	4.1	0.00	0.99
Age	4.32	75.18	0.00	1.00
Education	-9.97	0.00	0.00	1.00
Occupation	20.10	5.4	0.00	0.99
Salary	0.73	2.09	0.00	1.00
Geographic data				
Place of bite	-8.40	0.00	0.00	1.00
Factors related to snakebite				
Site of bite	-12.77	0.00	0.00	0.99
Time of bite	2.43	11.37	0.00	1.00
Distance between fang marks	2.11	8.27	0.00	1.00
Factors related to treatment				
The number of antivenom used	3.83	44.82	0.00	1.00
Duration between bite and seeking medical advice	1.62	5.09	0.00	0.99
First Aid treatment	0.13	1.14	0.00	1.00
Duration of hospitalization	25.43	1.2	0.00	0.99
Constant	**157.91**			**0.99**

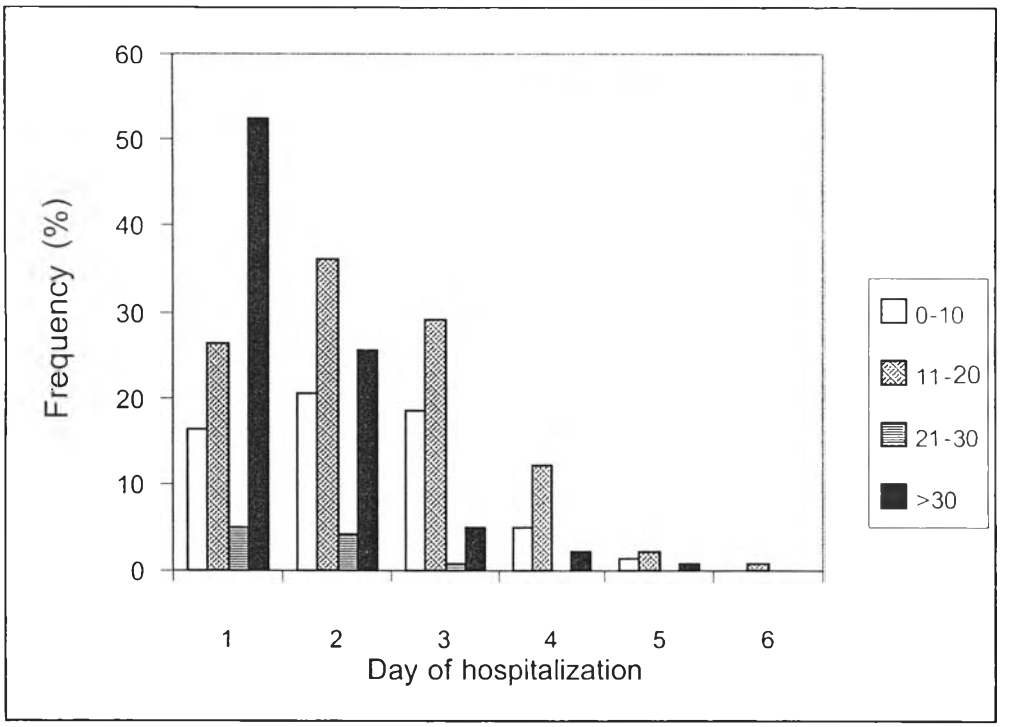


Figure 6. The level of VCT in 6 days of hospitalization (*C. rhodostoma*)

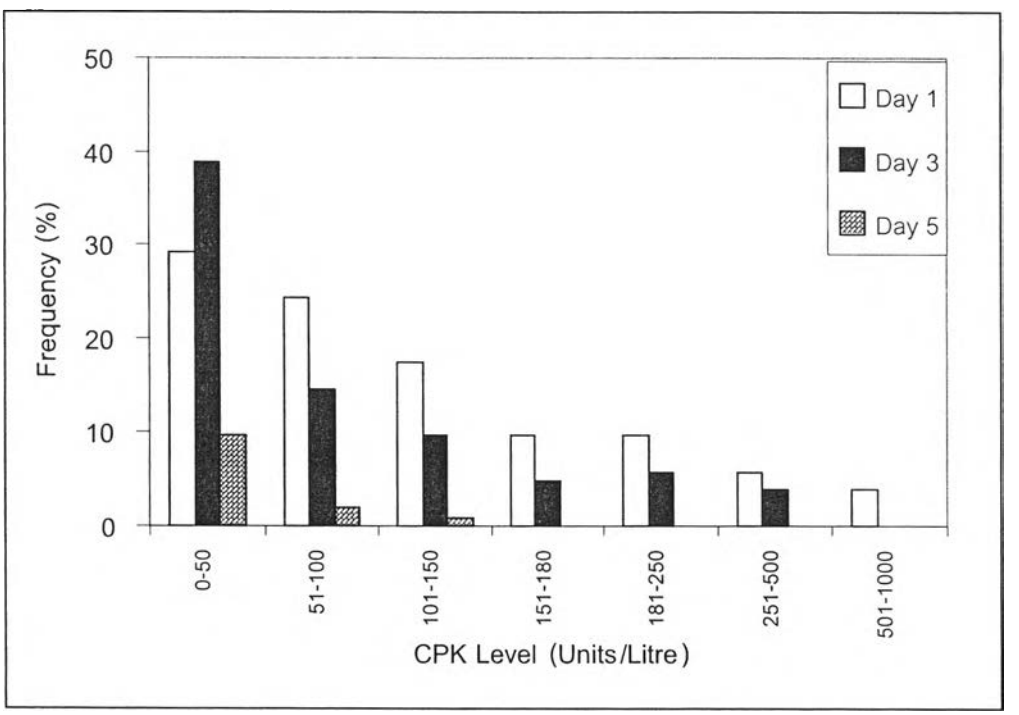


Figure 7. The level of CPK (Units/Litre)

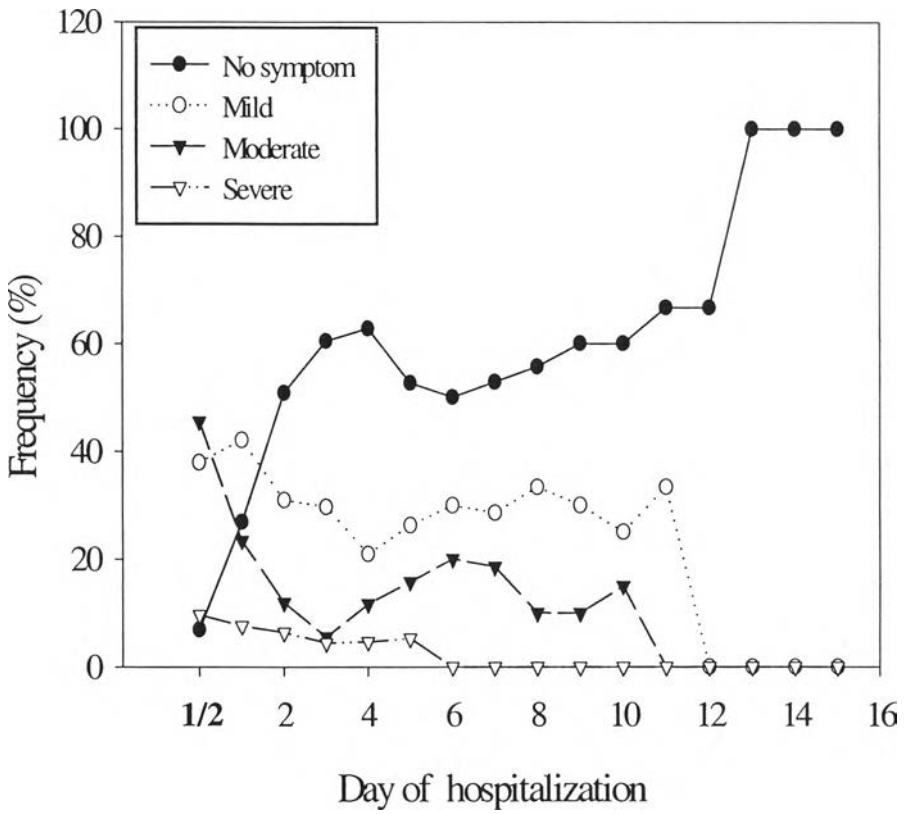


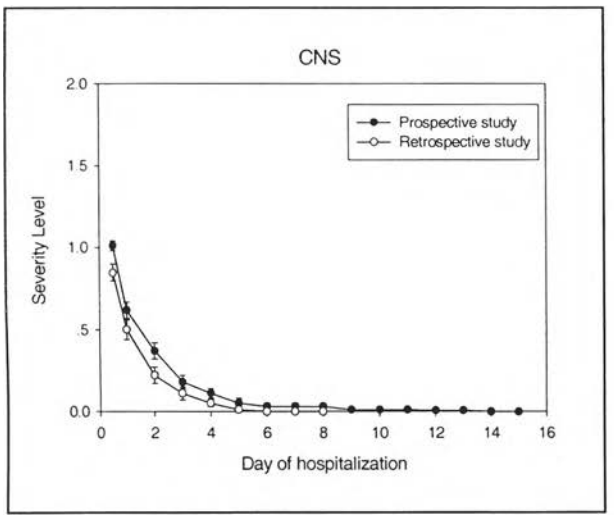
Figure 8. The degree of snake clinical envenomation (*C. rhodostoma*)

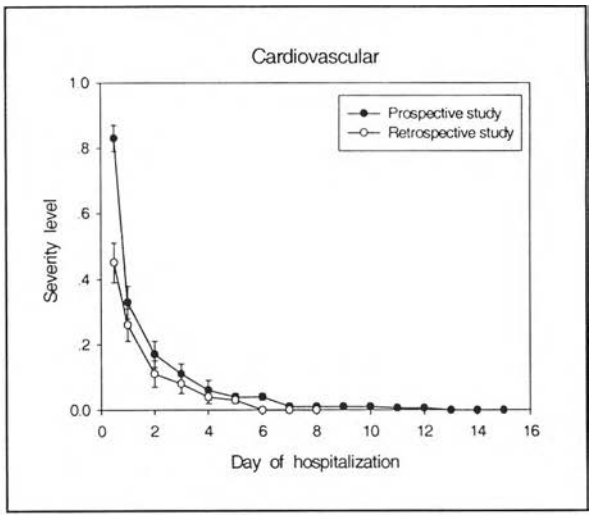
Systemic manifestations involved the central nervous, cardiovascular, pulmonary, gastrointestinal and hematologic systems. An overall analysis of SSS scores revealed 08.00 – 1.00 involving the CNS, 0.40 – 0.90 the pulmonary, 0.03-0.17 the gastrointestinal, 0.45-0.83 the cardiovascular, 1.69-1.84 the hematologic systems and 1.01-1.17 of bite site reactions. The highest score levels appeared 12 hours after hospitalization (Figure 9).

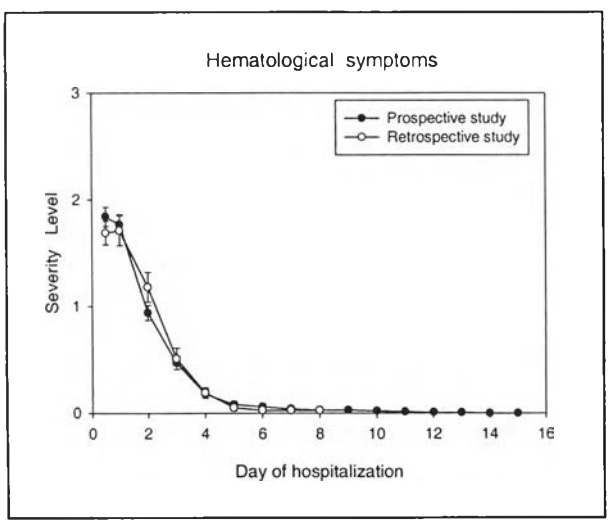
The incidence of tissue necrosis at the bite site from *C. rhodostoma* bites was 95 % in the prospecitv e study and 94 % in the retrospective one. The most common SSS levels were minimal (score 1 in 78.6 % and 86.25 % prosective and retrospective groups) (Figure 10). These patients had local pain and mildly inflammed wounds.

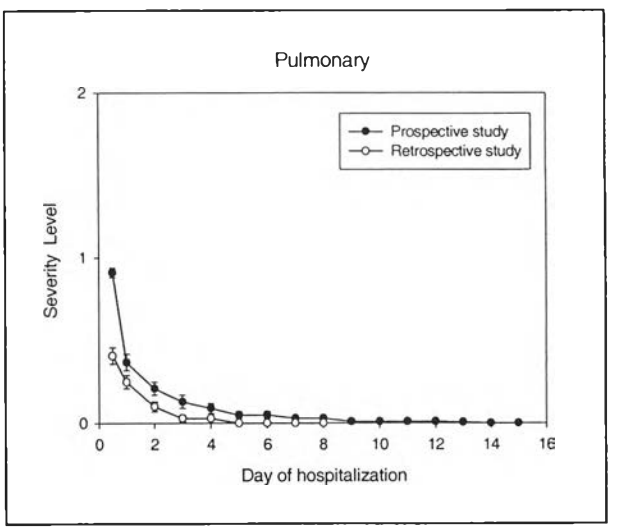
No case developed septicemia but two subjects had the disseminated intravascular coagulation syndrome and died from intracranial hemorrhages. The first case, a 60 year old man, came to hospital comatous and with severe coagulopathy (VCT > 30 min and hematuria). He had been bitten by a MPV 3 days previously and was treated by a traditional healer with herbals and local potions. He was moribund and had moderately severe tissue necrosis (score 2) at the bite site. He was intubated, given 30 mL of antivenin and his VCT returned to normal after 6 hours. However, he had developed an intracranial hemorrhage and never regained conciousness. The second fatality was a 72 year old man. He was admitted to the hospital one hour after having been bitten by a MPV. On the first day of admission, he had pain at the bite site, was very apprehensive but had no abnormal systemic signs/symtoms and no coagulopathy

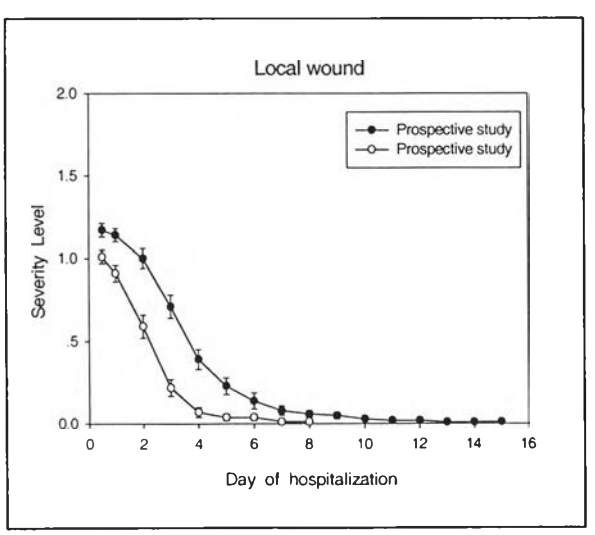
(VCT 10 min). On the morning of his second hospital day, he developed bleeding from gums and had a VCT > 30 min. There was no antivenin available at that time. In the afternoon, he went into shock, lost conciousness and developed, hematuria, hematemesis, and thrombocytopenia. He remained deeply unconscious and required vasopressors. At the afternoon of the second day, when it was decided that his case was hopeless, he was taken home to die.











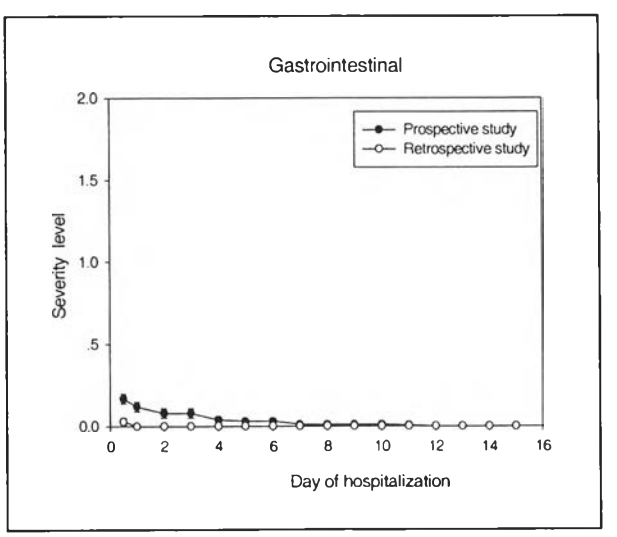


Figure 9. The severity scale of envenomation evaluated by modified SSS (Snake Severity Score).

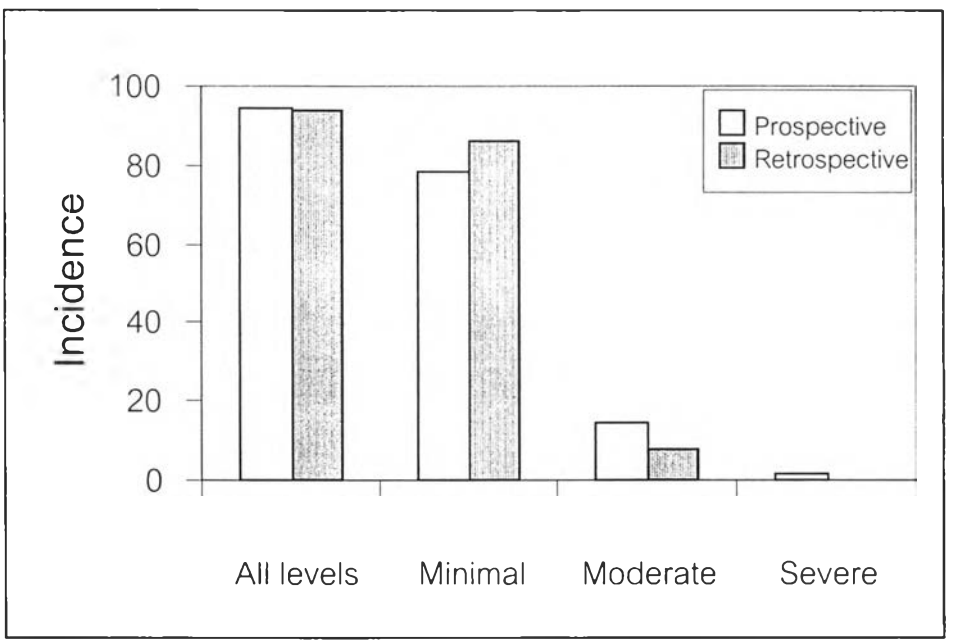


Figure 10. The incidence of tissue necrois in victims after *C. rhodostoma* bites in prospective and retrospective studies.

2. *N. kaouthia* bites

The prospective study collected a total of 45 *Naja kaouthia* snakebite victims from 10 provinces. Forty hospital charts of snake bite patients from the year 1997 to 2000 in Nakorn Sawan, a province north of Bangkok, were also reviewed retrospectively.

Most victims came from Nakhon Si Thammarat (southern) and Nakorn Sawan (northern) province. There were 13 (28.89%) and 11 (24.44 %) respectively (Table 15). The peak prevalence was in May (13 fo 45 ; 28.89 %) which is the early part of the rainy season (Table 16).

Female patients experienced snakebites at 55.56 % and 35 % in the prospective and the retrospective studies respectively. Most patients were of married status and an age range of 21-40 years. They were of low education at primary school level (73.33 % in the prospective and 75 % in the retrospective studies) and worked in manual labour positions with a low salary (1,000 – 3,000 baht) (Table 17).

Snakebites by *N. kaouthia* were distributed throughout the day and were most common between 08.00 – 12.00 a.m. (48.9 0 %) and 0.01 – 04.00 p.m. (17.50 %) in the prospective and retrospective studies respectively. The size of snakes, reflected by the distance between fang marks, was recorded only in the prosepctive study. It ranged between 1.01 –2.00 cm. Bites of lower limbs, especially feet represented by 35 %. Finger and Toe were bitten , 26.67 % and 15.55 % respectively in the prospective study. The number of snakes available for identification was higher ; 60 % in the prospective study. Most bites occurred in rural areas, outdoors and dusky or dark places (Table 18).

The time between bite and arival at a hospital or health center ranged between 0.01 – 60 min (66.67 %) and 61-120 min (30 %) in the prospective and retrospective groups respectively. 27 of 45 victims (60 %) had applied tourniquet in the prospective study and 33 of 40 victims (82.50 %) did so in the retrospective study. The patients required only wound care to prevent or control infection (64.44 – 77.50 %) . Only one case required amputation of the thumb in the retrospective group. Antivenin ranged from 1- 20 vials in the prospective study and a maximum of 29 vials in the retrospective study. Most of them received 1-5 vials of antivenin (45 %). The usual hospitalization was 1-5 days with a maximum of 29 and 20 days in the prospective and retrospective studies (Table 19).

Table 15. The distribution of cobra bite victims among the 10 provincial hospitals in prospective study.

Province	**Number (n)**	**Frequency (%)**
Lampang	4	8.89
Nakorn Sawan	11	24.44
Lop Buri	2	4.45
Nakorn Ratchasima	2	4.45
Ratcha Buri	1	2.22
Prachuap Khiri Khan	1	2.22
Surat Thani	6	13.33
Nakorn Si Thammarat	13	28.89
Trang	3	6.67
Songkhla	2	4.45

Table 16. The seasonal prevalence of cobra bites during April to June 2003 in prospective study and 1997-2000 in retrospective study.

Month	**Prospective study**		**Retrospective study**	
	Number (n)	Frequency (%)	Number(n)	Frequency (%)
January	3	6.67	1	2.50
February	3	6.67	0	0
March	0	0	1	2.50
April	2	4.44	6	15
May	13	28.89	6	15
June	5	11.11	3	7.50
July	1	2.23	4	10
August	6	13.33	2	5
September	5	11.11	7	17.50
October	3	6.67	6	15
November	2	4.4	3	7.50
December	2	4.44	1	2.50

Table 17. The epidemiological data in the prospective and retrospective studies.

Variables	Prospective study		Retrospective study	
	Number (n)	Frequency (%)	Number(n)	Frequency (%)
Gender				
Male	25	44.44	26	65.00
Female	50	55.56	14	35.00
Marital Status				
Single	8	17.78	11	27.50
Married	2	4.44	29	72.50
Divorced	35	77.78	0	0.00
Age (Years)				
1-10	2	4.44	4	10.00
11-20	1	2.22	3	7.50
21-40	19	42.22	11	27.50
41-60	16	35.56	16	40.00
More than 60	7	15.56	5	15.00
Education				
No education	3	6.67	5	12.50
Primary school	33	73.33	30	75.00
High school	7	15.56	5	12.50
Undergraduate	2	4.44	0	0.00
Occupation				
Unemployed	2	4.44	10	25.00
Labour	37	82.23	13	32.50
Agriculture	1	2.22	13	32.50
Non government organizations	0	0.00	0	0.00
Government organizations	2	4.44	0	0.00
Student	3	6.67	4	10.00
Salary (Baht)				
100 - 1,000	11	24.44	5	12.50
1,000 – 3,000	25	55.56	15	37.50
3,001 – 6,000	7	15.56	15	37.50
6,001 – 9,000	1	2.22	5	12.50
9,001 – 12,000	0	0.00	0	0.00
More than 12,000	1	2.22	0	0.00

Table 18. Factors related to *N. kaouthia* bite

Variables	Prospective study		Retrospective study	
	Number (n)	Frequency (%)	Number(n)	Frequency (%)
Season				
Rainy	15	33.33	22	55.00
Summer	20	44.45	13	32.50
Winter	10	22.22	5	12.50
Site of bite				
Finger	12	26.67	9	22.50
Hand	3	6.67	3	7.50
Arm	3	6.67	0	0.00
Toe	7	15.55	7	17.50
Foot	16	35.55	13	32.50
Leg	4	8.89	4	10.00
Eye	0	0.00	4	10.00
Location				
Urban	5	11.11	5	12.50
Rural	40	88.89	35	87.50
Place of biting				
Indoor	13	28.89	11	17.50
Outdoor	32	71.11	29	72.50
Genous species				
Available	27	60.00	12	30.00
Not available	18	40.00	28	70.00
Time of biting				
00.01 – 05.00 a.m.	3	6.67	0	0.00
05.01 – 08.00 a.m.	5	11.11	0	0.00
08.01 – 12.00 a.m.	22	48.89	7	17.50
00.01 - 04.00 p.m.	6	13.33	15	37.50
04.01 – 08.00 p.m.	6	13.33	16	40.00
08.01 – 12.00 p.m.	3	6.67	2	5.00
The distance between fang marks (c.m.)				
0.01 – 1.00	17	44.74	------	------
1.01 – 2.00	19	50.00	------	------
2.01 – 3.00	2	5.26	------	------
	7 miss record			
Predisposing factors				
Barefoot	10	22.22	17	42.50
Dusky	35	77.78	23	57.50

Table 19. Factors related to cobra bite treatment

Variables	Prospective study		Retrospective study	
	Number (n)	Frequency (%)	Number(n)	Frequency (%)
Duration between bite and seeking medical advice (min)				
0.01 – 60	30	66.67	22	55.00
61 – 120	8	17.78	12	30.00
121 - 240	5	11.11	5	12.50
241 – 480	2	4.44	0	0.00
481 – 960	0	0.00	1	2.50
First Aid Treatment				
Tourniquet	27	60.00	33	82.50
No Tourniquet	18	40.00	7	17.50
Treatment				
Dressing	29	64.44	31	77.50
Debridgemet	16	35.56	8	20.00
Amputation			1	2.50
The number of antivenom used (vials)				
0	20	44.44	29	72.50
1 – 5	11	44.00	5	45.56
6 – 10	7	28.00	1	9.09
11 – 15	6	24.00	2	18.18
16 – 20	1	4.00	2	18.18
More than 20			1	9.09
Duration of hospitalization (Days)				
1 – 5	31	68.89	29	72.50
6 – 10	8	17.78	7	17.50
11 – 15	4	8.89	3	7.50
16 – 20	1	2.22	1	2.50
More than 20	1	2.22	0	0.00

Factors effecting tissue necrosis including demorgraphic data, geographic data and factors related to treatment, they were recorded at 3 levels. The results revealed no factors effecting on tissue necrosis after *N. kaouthia* bites (Table 20-22).

The CPK activity indicated the tissue necrosis in patients, was abnormal level (> 180 units/litre) to 24.40 % including 181 – 250 units/litre (2.4 %), 251-500 units/litre (12.20 %) and 501-1000 units/litre (9.80 %), in the first day of hospitalization. Most patients had normal CPK level , especially 101-150 units/litre (34.10 %) (Figure 11).

Table 20. Various factors effecting tissue necrosis were calculated by multiple logistic regression. The level of local wound severity (score 0-3) is divided into 2 parts, no tissue necrosis (score 0) and having tissue necrosis (score 1-3).

Variables	B	Exp(B) : Odd	95 % CI	P value
Demographic data				
Gender	16.37	1.3	0.00	1.00
Age	31.83	6.7	6.68-7.65	0.99
Education	-12.75	0.882	0.00	1.00
Occupation	2.74	15.49	0.00	1.00
Salary	-8.3	0.416	0.0021-0.0024	1.00
Geographic data				
Place of bite	-24.35	0.65	0.00	0.99
Factors related to snakebite				
Site of bite	4.40	81.48	0.00	0.99
Time of bite	39.85	2.0	0.02-3.06	1.00
Distance between fang marks	-144.13	0.524	0.52-2.68	0.99
Factors related to treatment				
The number of antivenom used	66.82	1.1	0.05-2.06	0.99
Duration between bite and seeking medical advice	109.83	2.04	0.03-6.87	0.99
First Aid treatment	-17.98	0.051	0.00	0.99
Duration of hospitalization	1.83	2.04	0.50-4.06	0.99
Constant	**-159.24**			**0.68**

Table 21. Various factors effecting tissue necrosis were calculated by multiple logistic regression. The level of local wound severity (score 0-3) is divided into 2 parts, no tissue necrosis (score 0-1) and having tissue necrosis (score 2-3).

Variables	B	Exp(B) : Odd	95 % CI	P value
Demographic data				
Gender	-32.16	0.017	0.00	0.99
Age	0.861	2.36	0.35-2.36	0.97
Education	26.79	143.52	0.00	0.99
Occupation	-6.18	0.02	0.00	1.00
Salary	-69.86	0.0045	0.00	1.00
Geographic data				
Place of bite	27.64	1.23	0.00	0.99
Factors related to snakebite				
Time of bite	-29.57	0.0014	0.00	1.00
Site of bite	-7.77	0.004	0.00	0.99
Distance between fang marks	37.02	12.36	0.00	1.00
Factors related to treatment				
The number of antivenom used	-1.67	0.189	0-1.89	0.98
Duration between bite and seeking medical advice	-30.98	3.49	0.49-3.49	0.99
First Aid treatment	25.45	1.11	0.00	0.99
Duration of hospitalization	34.54	1.01	0.00	0.99
Constant	**81.48**			**0.89**

Table 22. Various factors effecting tissue necrosis were calculated by multiple logistic regression. The level of local wound severity (score 0-3) is divided into 2 parts, no tissue necrosis (score 0-2) and having tissue necrosis (score 3).

Variables	B	Exp(B) : Odd	95 % CI	P value
Demographic data				
Gender	0.036	1.03	0-1.70	0.99
Age	0.093	1.09	0-1.098	0.99
Education	-0.57	0.945	0-9.45	0.98
Occupation	-11.83	0.025	0-1.7	0.99
Salary	-35.39	0.025	0-1.04	0.98
Geographic data				
Place of bite	35.63	3.05	0-2.98	0.99
Factors related to snakebite				
Time of bite	-26.35	1.35	0.00	1.00
Site of bite	-0.05	0.995	0.00	1.00
Distance between fang marks	-23.37	0.079	0-4.93	0.99
Factors related to treatment				
The number of antivenom used	23.65	1.02	0.00	1.00
Duration between bite and seeking medical advice	35.40	2.4	0-2.38	0.99
First Aid treatment	0.082	1.08	0-1.68	0.98
Duration of hospitalization	35.40	2.40	0-2.86	0.97
Constant	**46.68**			**0.65**

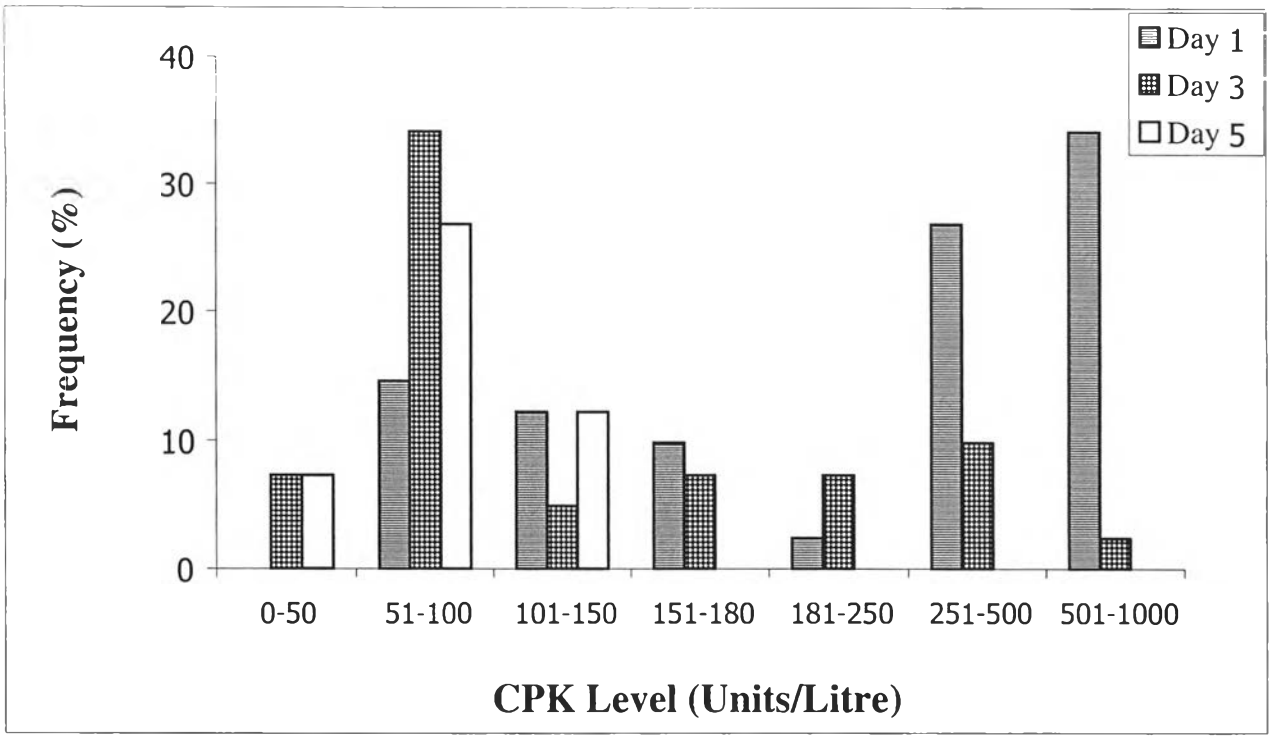


Figure 11. The CPK levels in victims (Units/Litre).

The degree of snake clinical envenomation was calculated by adding each system separated into 3 levels: no symptom/sign (score 0-2), minimal (score 3-5), moderate (score 6-8) and severe (score 9-20). Most patients presented with mild clinical envenomation (51.11 %) at the 12 hour after admission to the hospital and shifted to no symptom/sign after that (Figure 12).

Clinical manifestations involved the central nervous (CNS), cardiovascular, pulmonary, gastrointestinal, hematologic systems and injury at the bite site (Figure 13). An overall analysis of SSS scores revealed 1.05 –1.62 involving the CNS, 1.07-1.42 the pulmonary, 0.13-0.27 the gastrointestinal, 0.55-0.89 cardiovascular, 0 the hematologic systems and 0.70-0.98 of bite site injury. The highest score level was observed at 12 hours after hospitalization and most patients presented with none or only mild signs and symptoms. These gradually decreased to level 0 (no sign/symptom) after 5 days of hospitalization in both prospective and retrospective studies (Figure 13).

Tissue necrosis at the bite site was seen in 91.11 % (41 of 45 cases) and 65 % (26 of 40 cases) in the prospective and retrospective studies respectively. The most common SSS level was minimal tissue necrosis (score1 in 84.5 % and 60 %, prospective and retrospective groups). There was no case of severe tissue necrosis among both groups (Figure 14).

On the first day of hospitalization, 14 of 45 victims (31.11 %) in the prospective and 5 of 40 victims (12.50 %) in the retrospective group, had dysphagia, flaccid paralysis and respiratory failure. They were intubated. Only one patient died from respiratory failure in the prospective study. He was 66 years old, came to the hospital deeply comatous in advanced respiratory failure. He was intubated, given 50 mL of antivenin for the first dose and received the same antivenin dosage 2 hours later. He never regained consciousness over 4 days of hospitalization and was taken home to die at the request of his family.

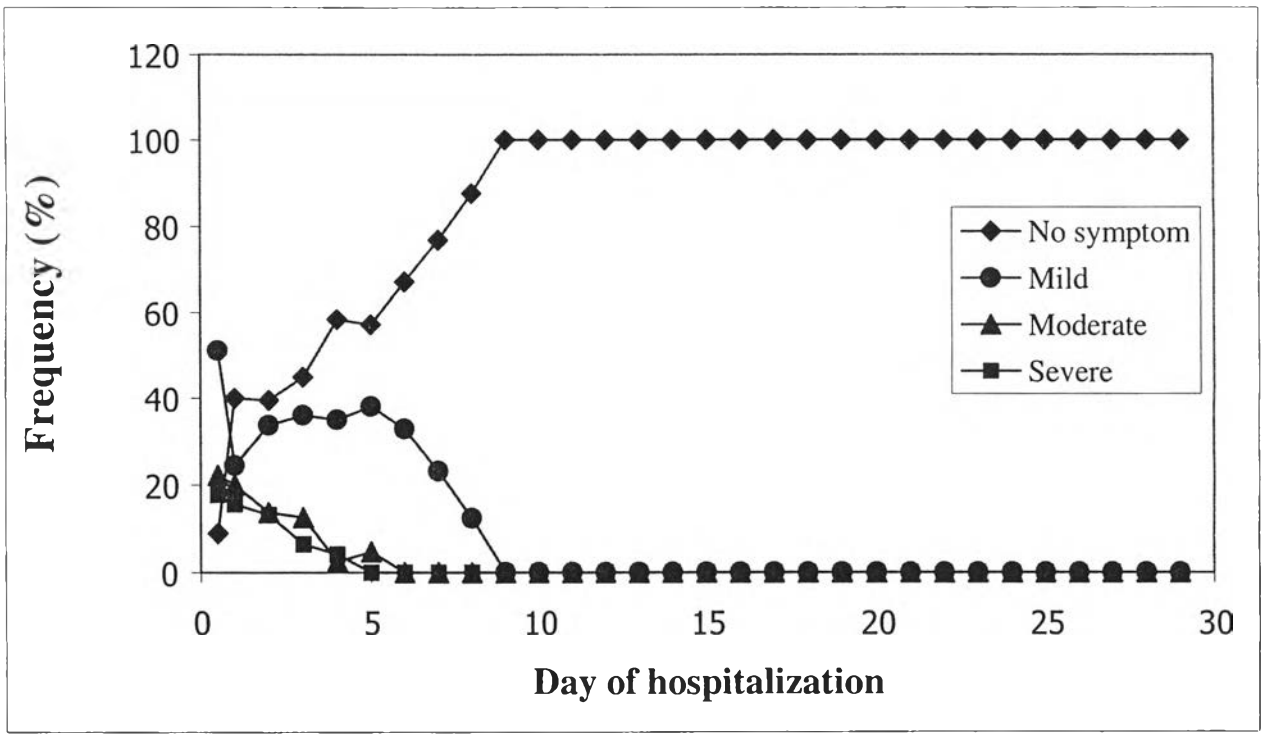


Figure 12. The degree of snake clinical envenomation.

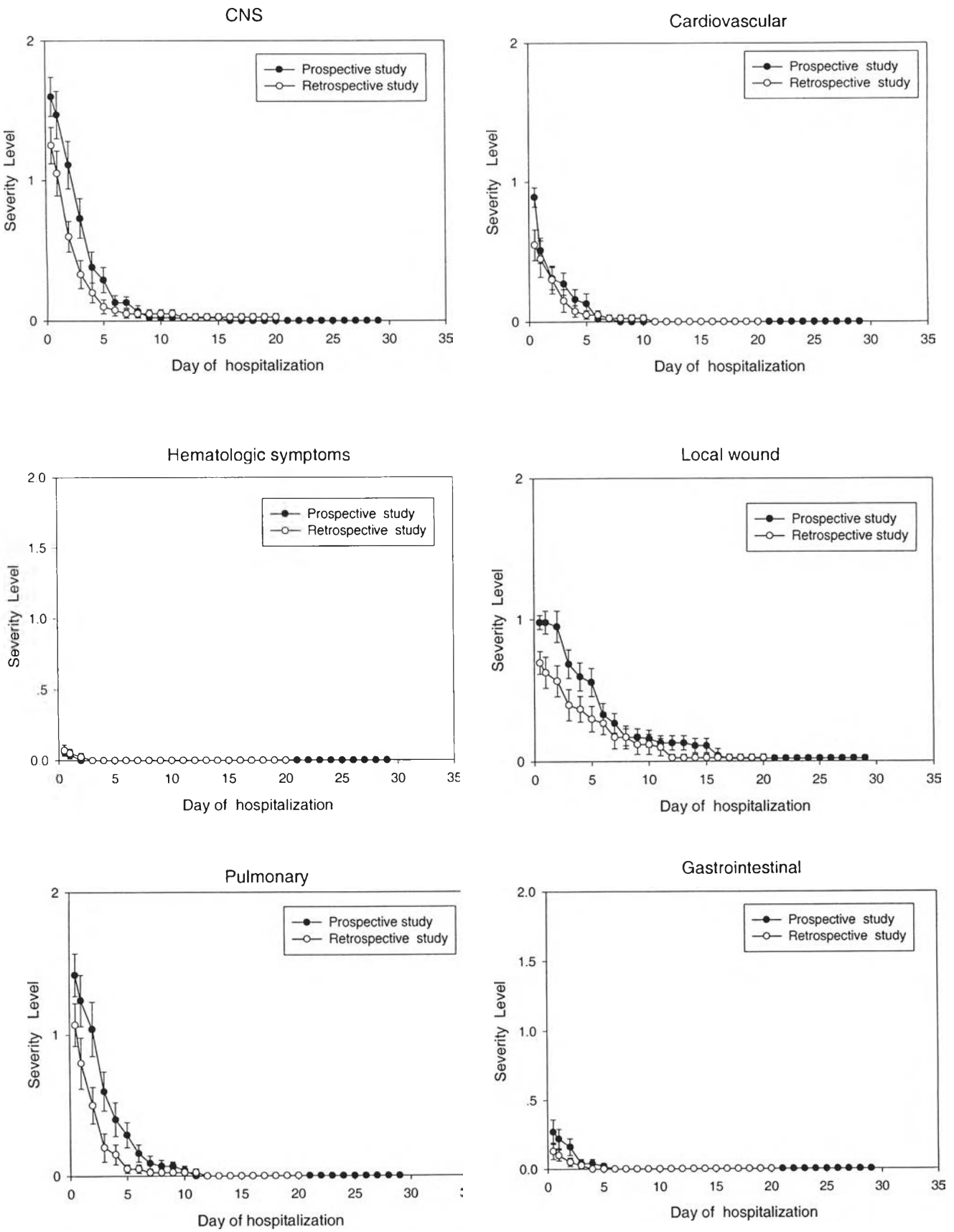


Figure 13. The severity scale of envenomation evaluated by modified SSS (Snakebite Severity Score).

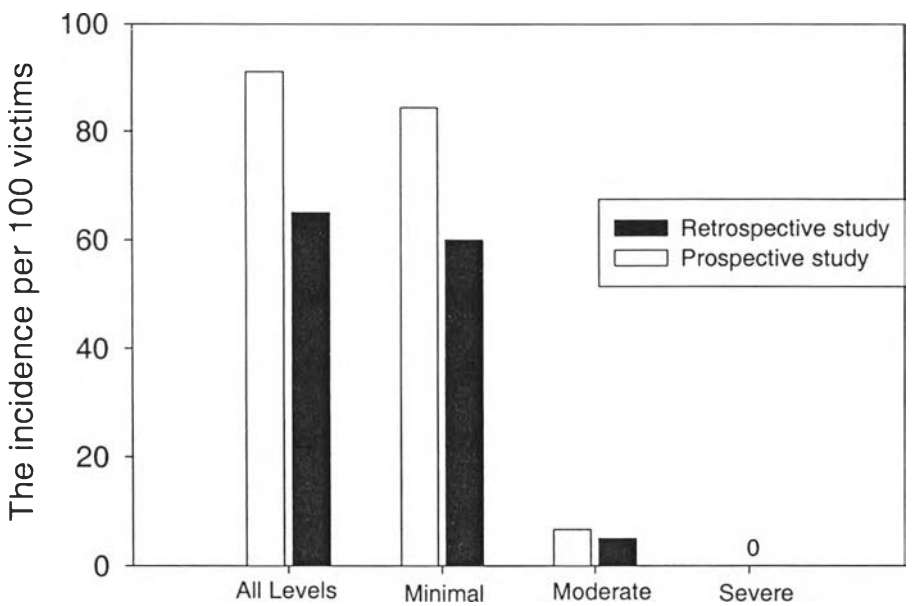


Figure 14. Tissue necrosis after *Naja kaouthia* bites in prospective and retrospective studies.

Laboratory Experiments

1. *In vitro* Experiments

1.1. Determination of inhibitory effects of metalloproteinase and PLA_2 inhibitors on proteolytic and metalloproteinase activities in NK and CR venoms

NK and CR venoms were assayed for proteolytic and metalloproteinase activities using the procedures described in Materials and Methods. Metalloproteinase and phospholipase A_2 inhibitors at various concentrations were preincubated with NK or CR venom for different time (0, 5, and 10 min). The inhibition of the enzymatic activities, determined as percent inhibition, are shown in Tables 23-24.

In the group of chelating reagents, L1, DFO, TEPA and EDTA completely inhibited the proteolytic and metalloproteinase activities in *Naja kaouthia* venom at the following concentrations : 10 mM L1 , 10 mM DFO , 10 mM TEPA and 2 mM EDTA. In *Calloselasma rhodostoma* venom, the metalloproteinase activity was inhibited 86 %, 98 % and 81 % by 10 mM L1 , 20 mM DFO and 20 mM TEPA, respectively. Only N-phenylglycine, at 0.5 mM inhibited proteolytic and metalloproteinase activity by 90 % in NK venom while 20 mM of this reagent completely inhibited the activities in CR venom. PMSF, at the highest concentration studied (5 mM) inhibited less than 10%, and 20 % of the enzymes in NK and CR venoms, respectively (Table 23a-23b).

Quinine, mefloquine and para-bromophenacyl bromide which are phospholipase A_2 inhibitors, partially inhibited of the proteolytic and metalloproteinase activities. Quinine at 10 mM inhibited proteolytic and metalloproteinases activities in CR and NK venoms by 12 % and 36 %, respectively. Mefloquine at 0.50 mM inhibited less than 10 % of proteolytic and metalloproteinase activities of both venoms while 0.50 mM p-BPB inhibited about 20% of the enzyme activities in NK venom (Table 24a-24b).

Table 23a. Effects of metalloproteinase inhibitors at various concentrations and preincubation time on the proteolytic and metalloproteinase activities of *Naja kaouthia* (NK) venom.

Inhibitors	Inhibition of enzymatic activities (%) of NK venom			
	Total Protease			Metalloproteinase
	Preincubation time of inhibitor and venom (min)			
	0	5	10	5
1 mM L1	55.64	47.18	46.67	54.45
5 mM L1	74.67	78.09	94.57	90.13
10 mM L1	100	100	100	100
1 mM DFO	31.85	40.97	47.25	47.28
5 mM DFO	40.51	52.77	55.92	60.91
10 mM DFO	100	100	100	100
20 mM DFO	100	100	100	100
1 mM TEPA	28.32	47.14	23.23	54.40
5 mM TEPA	46.45	52.12	60.15	60.15
10 mM TEPA	93.46	100	95.95	100
20 mM TEPA	89	89	75	100
0.50 mM EDTA	45.23	50.24	56.08	57.98
1 mM EDTA	54.97	56.05	72.95	64.69
2 mM EDTA	71.37	88.64	100	100
5 mM EDTA	86.02	100	100	100
1 mM PMSF	0	0	0	0
2 mM PMSF	1.75	1.75	1.75	2.01
5 mM PMSF	6.14	6.14	6.14	7.08
0.125 mM N-phenylglycine	42.91	60.78	73.18	70.15
0.25 mM N-phenylglycine	54.78	66.75	77.36	77.04
0.50 mM N-phenylglycine	55.60	77.95	93.86	89.98

Table 23b. Effects of metalloproteinase inhibitors at various concentrations and preincubation time on the proteolytic and metalloproteinase activities of *Calloselasma rhodostoma* (CR) venom.

Inhibitors	Inhibition of enzymatic activities (%) of CR venom			
	Total Protease			Metalloproteinase
	Preincubation time of inhibitor and venom (min)			
	0	5	10	5
1 mM L1	48	49.21	49.56	51.39
5 mM L1	61.11	63	63.07	65.80
10 mM L1	72.23	83	92.78	86.69
1 mM DFO	12.14	11.93	3.35	12.46
5 mM DFO	24.87	30.65	47.23	32.01
10 mM DFO	49.83	59.29	86.22	61.93
20 mM DFO	93.42	94.30	100	98.50
1 mM TEPA	10.01	11.65	12.89	12.16
5 mM TEPA	27.22	32.12	32.88	33.55
10 mM TEPA	76.26	81.55	84	85.18
20 mM TEPA	75	78	80	81.47
1 mM EDTA	39.91	46.11	55.97	48.21
2 mM EDTA	63.13	78.37	88	81.85
5 mM EDTA	88.57	96.60	95.73	100
1 mM PMSF	3.28	3.86	4.14	4.03
2 mM PMSF	6.56	8.58	9.37	8.96
5 mM PMSF	14.10	18.64	19.75	19.47
1 mM N-phyenylglycine	5.10	8.29	7.32	8.65
5 mM N-phenylglycine	46.58	52.13	52.26	54.45
10 mM N-phenylglycine	53.05	74.45	70.39	77.77
20 mM N-phenylglycine	100	100	100	100

Table 24a. Effects of phospholipase A2 inhibitors at various concentrations and 5 min preincubation time on the proteolytic and metalloproteinase activities of *Naja kaouthia* (NK) venom.

Phospholipase A_2 Inhibitors	**NK venom/ Enzyme inhibition (%)**	
	Proteolytic activity	**Metalloproteinase activity**
1 mM Quinine	3.24	3.73
5 mM Quinine	4.39	5.06
10 mM Quinine	10.64	12.28
0.125 mM Mefloquine	0	0
0.25 mM Mefloquine	4.86	5.60
0.50 mM Mefloquine	7.40	8.54
0.125 mM p-BPB	5.09	5.87
0.25 mM p-BPB	16.89	19.49
0.50 mM p-BPB	20.13	23.23

Table 24b. Effects of phosplolipase A_2 inhibitors at various concentrations and 5 min preincubation time on the proteolytic and metalloproteinase activities of *Calloselasma rhodostoma* (CR) venom.

Phospholipase A_2 Inhibitors	**CR venom/ Enzyme inhibition (%)**	
	Proteolytic activity	**Metalloproteinase activity**
1 mM Quinine	30.84	32.21
5 mM Quinine	41.52	43.37
10 mM Quinine	34.62	36.16
0.125 mM Mefloquine	5.35	5.58
0.25 mM Mefloquine	2.70	2.82
0.50 mM Mefloquine	3.29	3.43
0.125 mM p-BPB	3.12	3.25
0.25 mM p-BPB	7.93	8.28
0.50 mM p-BPB	3.54	3.69

1.2. Determination of inhibitory effects of metalloproteinase and PLA_2 inhibitors on phospholipase A_2 activity in NK and CR venoms

PLA_2 inhibitors at the highest concentrations studied, mefloquine 0.50 mM, p-BPB (0.50 mM) and quinine (10 mM) inhibited 78.84 %, 100 % and 97.21 % of the PLA_2 activity in CR venom and 68.84 %, 72.93 %, 98.79 % of the activity in NK venom respectively. EDTA at 0.50 mM completely inhibited PLA_2 in CR venom while 2 mM was needed to inhibit the NK enzyme. Prolonged preincubation time only slightly increased the percent inhibition of PLA_2 (Table 25a-25b).

Chelating agents including 20 mM DFO, 20 mM TEPA and 20 mM N-phenylglycine inhibited PLA_2 activity in NK venom by 1.85 %, 100 %, 100 % and inhibited 16.75 %, 100 %, 100 % the PLA_2 in CR venom respectively. The percent inhibition was slightly increased at longer preincubation time and higher doses (Table 26a-26b).

Table 25a. Effects of various phospholipase A_2 inhibitors at various concentrations and preincubation time on the phospholipase A_2 activity of NK venom.

Inhibitors	**Inhibition of phospholipase A_2 activities (%) of NK venom**		
	Preincubation time of inhibitor and venom (min)		
	0	**5**	**10**
Quinine 1 mM	5.65	5.73	10.14
Quinine 5 mM	87.71	90.07	91.65
Quinine 10 mM	86.15	95.95	98.79
Mefloquine 0.125 mM	0	0	0
Mefloquine 0.25 mM	19.92	23.43	35.53
Mefloquine 0.50 mM	64.36	64.86	68.84
p-BPB 0.125 mM	7.16	47.50	47.91
p-BPB 0.25 mM	21.88	53.17	63.75
p-BPB 0.50 mM	34.81	72	72.93
EDTA 0.50 mM	20.17	35.68	40.32
EDTA 1 mM	57.16	64.17	72.35
EDTA 2 mM	84.38	100	100

Table 25b. Effects of various phospholipase A_2 inhibitors at various concentrations and preincubation time on the phospholipase A_2 activity of CR venom.

Inhibitors	**Inhibition of phospholipase A_2 activities (%) of CR venom**		
	Preincubation time of inhibitor and venom (min)		
	0	**5**	**10**
Quinine 1 mM	0	0	0
Quinine 5 mM	77.33	83.82	84.91
Quinine 10 mM	92.99	94.76	97.21
Mefloquine 0.125 mM	0	0	0
Mefloquine 0.25 mM	8.79	9.93	17.53
Mefloquine 0.50 mM	75.05	76.81	78.74
p-BPB 0.125 mM	72.65	100	100
p-BPB 0.25 mM	97.03	100	100
p-BPB 0.50 mM	100	100	100
EDTA 0.125 mM	14.08	22.46	20.27
EDTA 0.25 mM	56.47	59.18	59.40
EDTA 0.50 mM	100	100	100

Table 26a. Effects of various metalloproteinase inhibitors at various concentrations and preincubation time on the phospholipase A_2 activity of NK venom.

Inhibitors	**Inhibition of phospholipase A_2 activities (%) of NK venom**		
	Preincubation time of inhibitor and venom (min)		
	0	**5**	**10**
TEPA 1 mM	0	0	0
TEPA 5 mM	10.89	14.54	22.35
TEPA 10 mM	68.81	69.24	72.88
TEPA 20 mM	95.32	95.66	100
DFO 1 mM	0	0	0
DFO 5 mM	0.32	0.57	0.95
DFO 10 mM	0.34	0.65	1.43
DFO 20 mM	1.15	1.47	1.85
N-phenylglycine 1 mM	0	0	0
N-phenylglycine 5 mM	5.68	8.35	19.66
N-phenylglycine 10 mM	52.19	57.25	72.34
N-phenylglycine 20 mM	86.74	93.85	100

Table 26b. Effects of various metalloproteinase inhibitors at various concentrations and preincubation time on the phospholipase A_2 activity of CR venom.

Inhibitors	**Inhibition of phospholipase A_2 activities (%) of CR venom**		
	Preincubation time of inhibitor and venom (min)		
	0	**5**	**10**
TEPA 1 mM	0	0	0
TEPA 5 mM	18.36	10.95	12.50
TEPA 10 mM	71.77	74.23	76
TEPA 20 mM	100	100	100
DFO 1 mM	0	0	0
DFO 5 mM	0.23	0.71	1.61
DFO 10 mM	1.63	4.33	4.97
DFO 20 mM	10.37	12.86	16.75
N-phenylglycine 1 mM	0	0	0
N-phenylglycine 5 mM	3.13	5.79	17.13
N-phenylglycine 10 mM	51.70	57.41	71.98
N-phenylglycine 20 mM	87.57	94.77	100

2. *In vivo* Experiments

2.1. Effects of PLA_2 and metalloproteinase inhibitors on edema, hemorrhage and myonecrosis induced by CR venom.

Edema, hemorrhage and myonecrosis induced by CR venom injection were determined as described in Materials and Methods. The effects of the inhibitors on the local tissue necrosis were studied using 2 types of experimental design.

a. Pre-incubation type experiment

The experiment was carried out by incubating a constant amount of NK venom with various amounts of enzyme inhibitor for 5 min at 37°C before the mixture was injected into groups of four mice.

b. Independent inoculation experiment

The independent inoculation experiment was done by injecting a constant amount of CR venom followed at different time intervals by the injection of PLA_2 and/or metalloproteinase inhibitors into the same site of venom injection.

In these experiments, the concentration of venom or enzyme inhibitor injected was 2x while the volume was ½ those used in pre-incubation expriments. Therefore total doses and volumes of the venom/inhibitor were exactly the same as those used in the pre-incubation experiments.

2.1.1. Pre-incubation type experiments

- **Edema**

Edema was found to rapidly develop after venom injection, reaching its highest value at 1 hour and gradually decreased. CR venom at a dose of 2 μg / mouse, increased the weight of footpad from 14.24 ± 0.24 to 67.77 ± 7.61 mg (Table 27 and Figure 15).

In the preincubation experiment, the metalloproteinase inhibitors TEPA and N-phenylglycine significantly decreased edema induced by CR venom. However, even at lower doses, N-phenylglycine was more effective and the inhibition was over 70% (Table 27).

Among the PLA_2 inhibitors, EDTA significantly inhibited the CR venom induced edema by about 60%. Mefloquine and p-BPB at the highest doses studied (10.60 μg and 6.96 μg, respectively) significantly reduced the edema (Table 28 and Figure 16).

The 'Inhibitor mixture' was highly effective in decreasing the extent of edema induced by CR venom (Table 27).

- **Myonecrosis**

Myonecrosis caused by venom injection was evaluated by measuring the increase in serum creatine phosphokinase (CPK) activity after venom injection and was expressed as Mean ± S.E. of CPK activity (Sigma units/ml). In a group of mice injected with CR venom (25 μg), the serum CPK activity increased from 105.20 ± 22 to 846.06 ± 18.06 units/ml (Table 29 and Figure 17).

TEPA and N-phenylglycine, two of the metalloproteinase inhibitors, significantly decreased the CPK activity by 80% and 61.92% respectively (Table 29). DFO at 328.50 μg was less effective in this regard.

In the group of phospholipase A_2 inhibitors, Mefloquine (10.60 μg), p-BPB (6.96 μg) and EDTA (93.05 μg) inhibited myonecrosis by 85.99 %, 78.62 % and 90.65 % respectively.

CPK activity was significantly inhibited by 94.37 % when CR venom was preincubated with the 'Inhibitor mixture' (Table 29).

- **Hemorrhage**

CR venom at a dose of 10 μg/mouse induced hemorrhagic spots with diameter of 13.74 $\pm$ 0.52 mm. Two metalloproteinase inhibitors: TEPA and N-phenylglycine completely inhibited hemorrhage caused by venom injection. DFO was less effective (Table 30 and Figure 18).

Among the PLA_2 inhibitors, p-BPB, EDTA, quinine and mefloquine significantly reduced hemorrhagic spot by 20-90% (Table 31 and Figure 19).

Hemorrhage was almost completely inhibited when CR venom was preincubated with the 'Inhibitor mixture' (Table 30).

Table 27. Effects of various metalloproteinase inhibitors on edema induced by CR venom in a pre-incubation type experiment.

Treatment +	**Increment in weight (mg) Mean ± S.E.**	**Inhibition (%)**
2 μg CR venom only	67.77 ± 7.61	
NSS injection only	14.24 ± 0.24	
75.60 μg N-phenylglycine only	10.23 ± 0.35	-
34.75 μg L1 only	10.36 ± 0.21	-
328.50 μg DFO only	9.35 ± 0.32	-
185.80 μg TEPA only	10.39 ± 0.32	-
'Inhibitor mixture' ≠ only	10.25 ± 0.27	-
2 μg CR venom + 37.80 μg N-phenylglycine	17.78 ± 6.63 *	73.76
2 μg CR venom + 75.60 μg N-phenylglycine	14.59 ± 6.59 *	78.46
2 μg CR venom + 151.20 μg N-phenylglycine	18.75 ± 2.88 *	72.32
2 μg CR venom + 164.25 μg DFO	67.75 ± 4.98	3.02
2 μg CR venom + 328.50 μg DFO	60.92 ± 3.50	10.10
2 μg CR venom + 657 μg DFO	55.77 ± 2.38	17.72
2 μg CR venom + 92.90 μg TEPA	30.19 ± 2.70 *	55.45
2 μg CR venom + 185.80 μg TEPA	31.92 ± 3.17 *	52.89
2μg CR venom + 371.60 μg TEPA	38.33 ± 6.22 *	43.44
2μg CR venom + 'Inhibitor mixture'	17.76 ± 3.07 *	86.72

+ L1: Desferiprone; DFO: Desferrioxamine; TEPA: Tetraethylenepentamine.

≠ 'Inhibitor mixture' contained 195 μg sodium aurothiomalate, 37.80 μg N-phenylglycine and 93.05 μg EDTA.

* statistically significant, $p < 0.05$

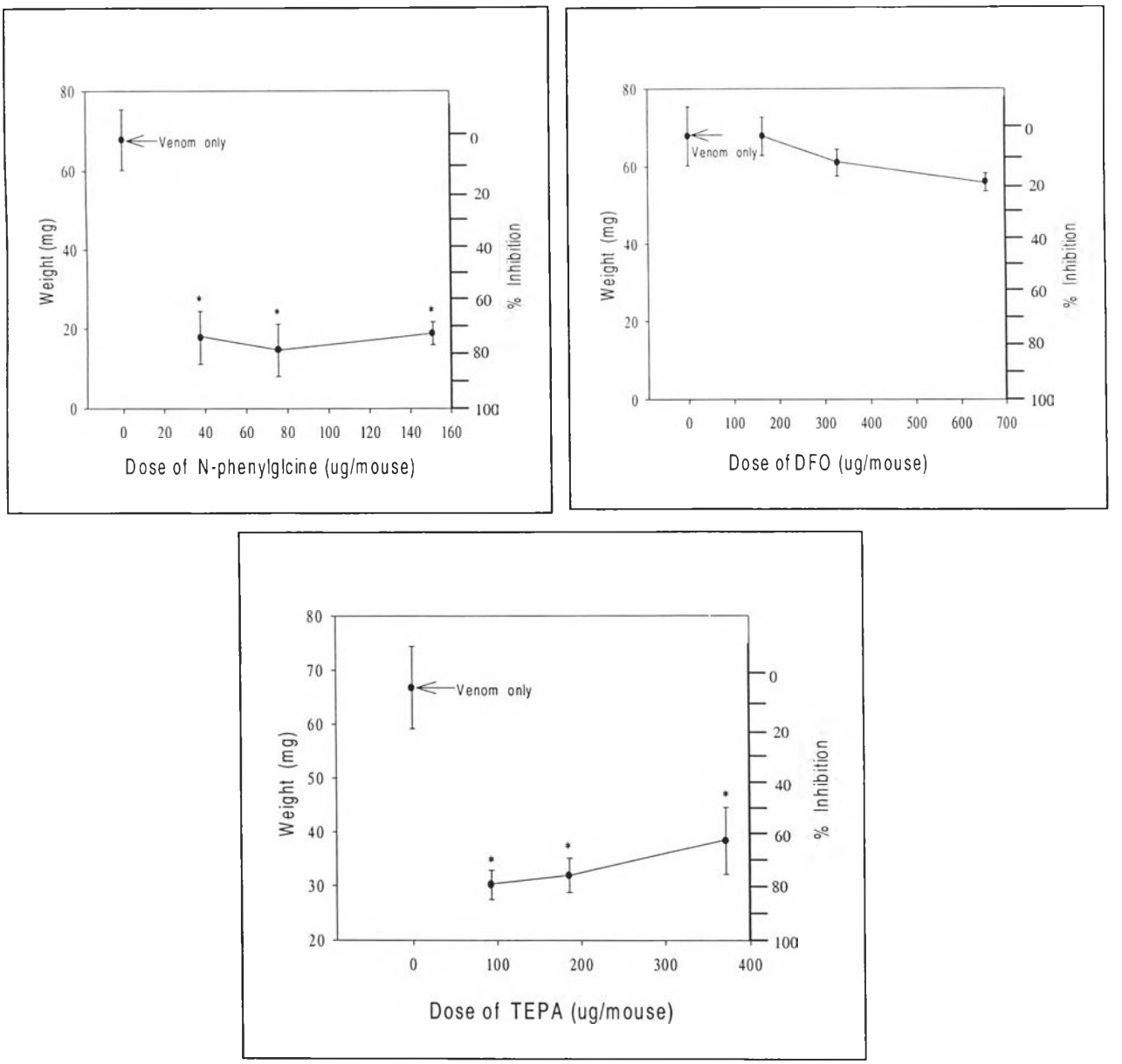


Figure 15. Effects of various metalloproteinase inhibitors on edema induced by CR venom. CR venom (2 μg) was mixed with each inhibitor and incubated for 5 min at room temperature before injection. Each point represents the mean ± S.E. of results obtained from five mice.

* statistically significant, $p < 0.05$

Table 28. Effects of various phospholipase A_2 inhibitors on edema induced by CR venom in a pre-incubation type experiment.

Treatment +	**Increment in weight (mg) Mean ± S.E.**	**Inhibition (%)**
2μg CR venom only	67.77 ± 7.61	
NSS injection only	14.24 ± 0.24	
90.25 μg Quinine only	10.65 ± 0.25	
3.48 μg p-BPB only	10.32 ± 0.31	
5.30 μg Mefloquine only	10.48 ± 0.29	
186.10 μg EDTA only	10.39 ± 0.25	
2 μg CR venom + 18.05 μg Quinine	65.66 ± 10.80	3.11
2 μg CR venom + 90.25μg Quinine	65.53 ± 18.69	3.31
2 μg CR venom + 180.50 μg Quinine	63.33 ± 12.32	6.56
2 μg CR venom + 1.74 μg p-BPB	62.48 ± 9.11	7.81
2 μg CR venom + 3.48 μg p-PBP	60.34± 5.50	10.97
2 μg CR venom + 6.96 μg p-BPB	52.22 ± 4.95 *	22.94
2 μg CR venom + 2.65 μg Mefloquine	64.59 ± 4.93	4.69
2 μg CR venom + 5.30 μg Mefloquine	62.11 ± 12.61	8.35
2 μg CR venom + 10.60 μg Mefloquine	45.50 ± 17.22 *	32.70
2 μg CR venom + 93.05 μg EDTA	25.23 ± 4.52 *	62.77
2 μg CR venom + 186.10 μg EDTA	23.95 ± 2.42 *	64.65
2 μg CR venom + 372.20 μg EDTA	38.75 ± 3.14 *	42.82

+ p-BPB: para-bromophenacyl bromide; EDTA: Ethylenediamine tetraacetic acid.

* statistically significant , $p < 0.05$.

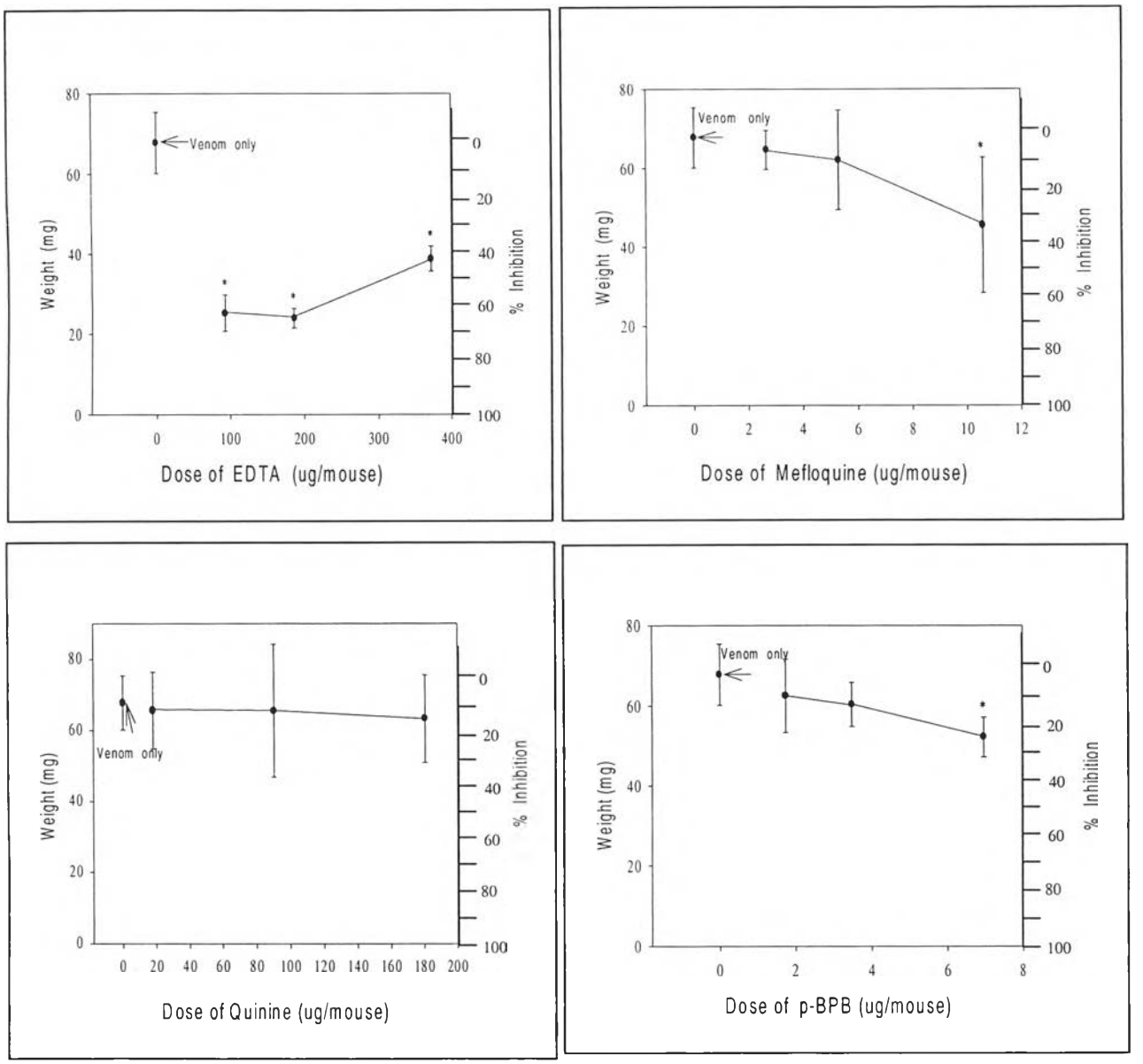


Figure 16. Effects of various phospholipase A_2 inhibitors on edema induced by CR venom. CR venom (2 μg) was mixed with each inhibitor and incubated for 5 min at room temperature before injection. Each point represents the mean ± S.E. of results obtained from five mice.

* statistically significant, $p < 0.05$

Table 29. Effects of metalloproteinase inhibitors, phospholipase A_2 inhibitors and 'inhibitor mixture' on the myonecrosis induced by CR venom in a pre-incubation experiment.

Treatment	**CPK (Sigma units/ml) Mean ± S.E.**	**Inhibition (%)**
25 μg CR venom only	864.06 ± 18.06	
NSS injection only	105.20 ± 22.00	
DMSO injection only	111.20 ± 7.50	
37.80 μg N-phenylglycine	121.80 ± 24.00	
92.90 μg TEPA	163.85 ± 16.50	
328.50 μg DFO	117.80 ± 7.50	
90.25 μg Quinine	105.80 ± 20.00	
10.60 μg Mefloquine	190.32 ± 26.88	
6.96 μg p-BPB	180.50 ± 7.30	
93.05 μg EDTA	102.49 ± 3.86	
'Inhibitor mixture' only	159.97 ± 27.22	
25 μg CR venom + 37.80 μg N-phenylglycine	329.06 ± 36.66 *	61.92
25 μg CR venom + 92.90 μg TEPA	168.89 ± 13.16 *	80.68
25 μg CR venom + 328.50 μg DFO	606.73 ±12.67 *	29.78
25 μg CR venom + 90.25 μg Quinine	704.65 ± 48.59	18.44
25 μg CR venom + 10.60 μg Mefloquine	120.91 ± 18.00 *	85.99
25 μg CR venom + 6.96 μg p-BPB	184.73 ± 23.33 *	78.62
25 μg CR venom + 93.05 μg EDTA	80.78 ± 14.32 *	90.65
1.25 μg CR venom + 'Inhibitor mixture'	48.60 ± 10.18 *	94.37

+ DFO: Desferrioxamine; TEPA: Tetraethylenepentamine; p-BPB : para-bromophenacyl bromide; EDTA : Ethylenediamine tetraacetic acid.

≠ 'Inhibitor mixture' contained 195 μg sodium aurothiomalate, 37.80 μg N-phyenylglycine and 93.05 μg EDTA.

* statistically significant, $p < 0.05$

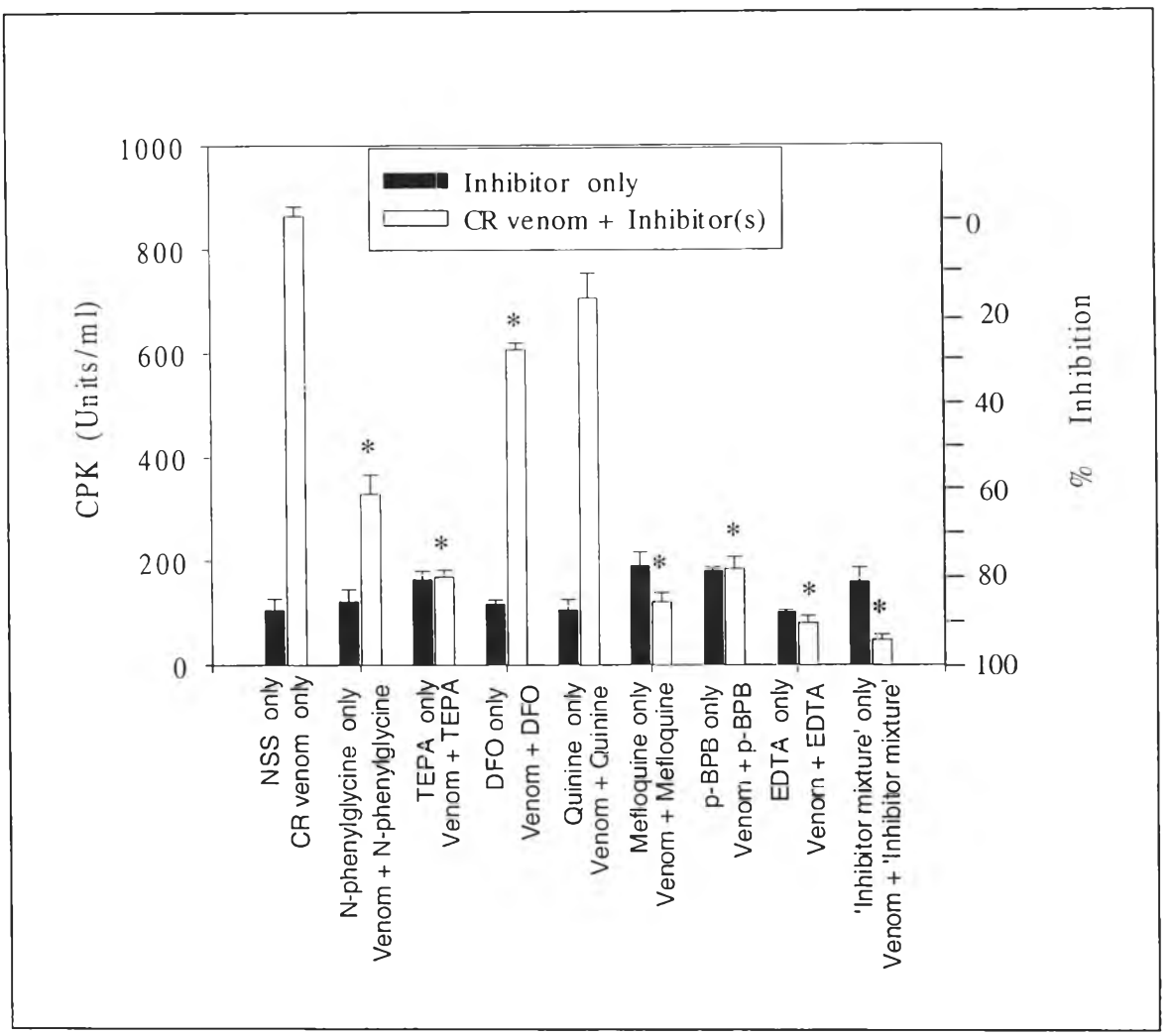


Figure 17. The effect of EDTA (93.05 μg), TEPA (92.09 μg), N-phenylglycine (37.80 μg), p-BPB (6.96 μg), mefloquine (10.60 μg), quinine (90.25 μg) and 'Inhibitor mixture' (37.80 μg N-phenylglycine + 195 μg sodium aurothiomalate + 93.05 μg EDTA) on CPK activity induced by CR venom. The venom (25 μg) and the inhibitor were preincubated at room temperature for 5 min before injection. Each bar represents the mean ± S.E. of results obtained from five mice.

* statistically significant, $p < 0.05$

Table 30. Effects of various metalloproteinase inhibitors and 'inhibitor mixture' on hemorrhage induced by CR venom in a pre-incubation type experiment.

Treatment +	**Hemorrhagic spot (mm) Mean ± S.E.**	**Inhibition (%)**
10 μg CR venom only	13.74 ±0.52	
NSS injection only	0	
151.20 μg N-phenylglycine only	0	
657 μg DFO only	0	
376.10 μg TEPA only	0	
'Inhibitor mixture'≠ only	0	
10 μg CR venom + 75.60 μg N-phenylglycine	0 *	100
10 μg VR venom + 151.20 μg N-phenylglycine	0 *	100
10 μg CR venom + 302.40 μg N-phenylglycine	0 *	100
10 μg CR venom + 328.50 μg DFO	11 ± 0.40 *	19.79
10 μg CR venom + 657 μg DFO	11.75 ± 1.31	14.32
10 μg CR venom + 1,314 μg DFO	12.25 ± 0.85	10.67
10 μg CR venom + 188.05 μg TEPA	0 *	100
10 μg CR venom + 376.10 μg TEPA	0 *	100
10 μg CR venom + 752.20 μg TEPA	0.25 ± 0.05 *	98.17
10 μg CR venom + 'Inhibitor mixture'	0 *	100

+ DFO: Desferrioxamine; TEPA: Tetraethylenepentamine.

≠ 'Inhibitor mixture' contained 390.10 μg sodium aurothiomalate, 75.60 μg N-phenylglycine and 186.10 μg EDTA.

* statistically significant, $p < 0.05$

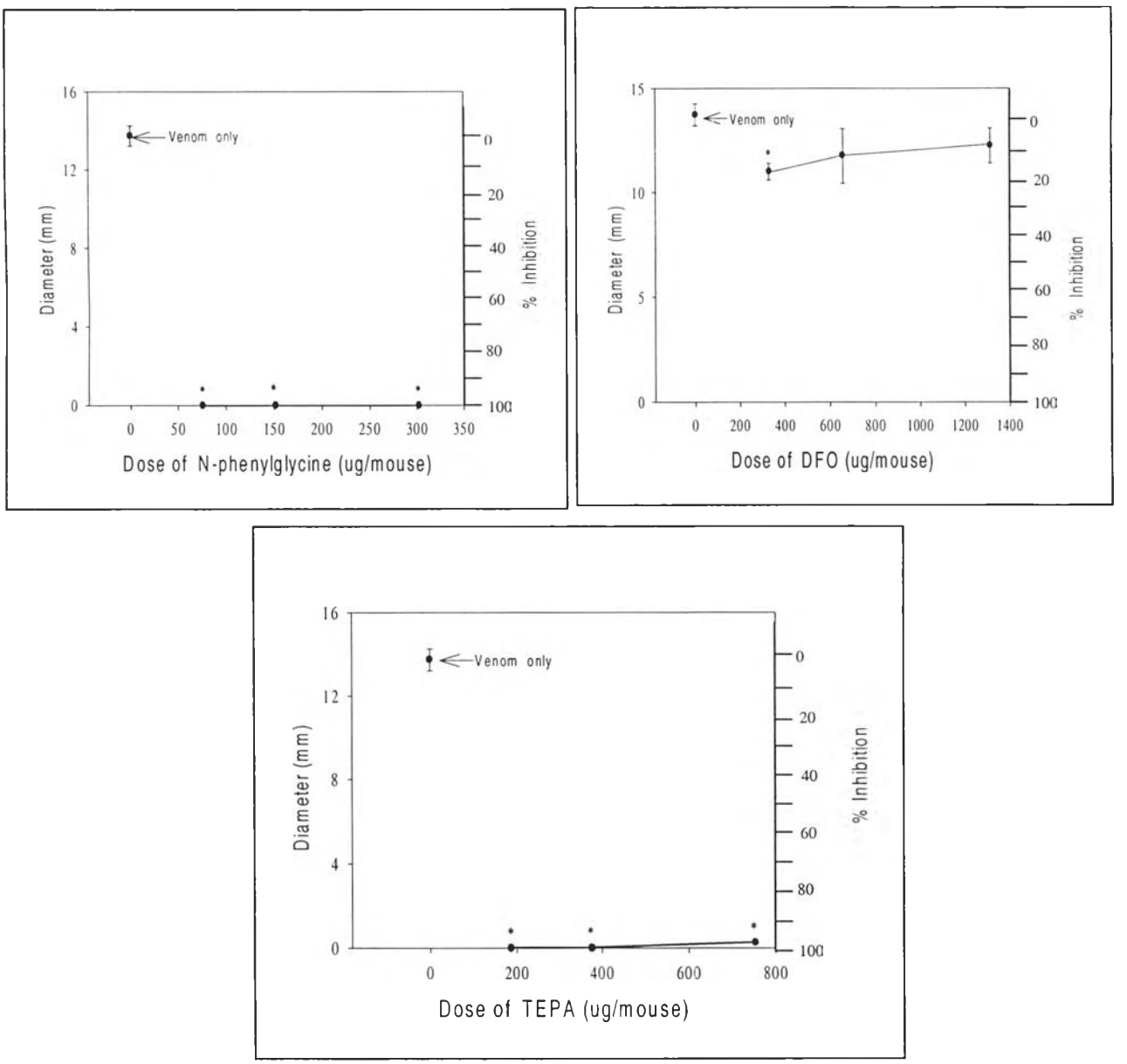


Figure 18. Effects of various metalloproteinase inhibitors on hemorrhage induced by CR venom. CR venom (10 μg) was mixed with each inhibitor and incubated for 5 min at room temperature before injection. Each point represents the mean ± S.E. of results obtained from five mice.

* statistically significant, $p < 0.05$

Table 31. Effects of various phospholipase A_2 inhibitors on the hemorrhage induced by CR venom in a pre-incubation type experiment.

Treatment +	Hemorrhagic spot (mm) Mean ± S.E.	Inhibition (%)
10 μg CR venom only	13.71 ± 0.51	
NSS injection only	0	
180.45 μg Quinine only	0	
6.94 μg p-BPB only	0	
10.60 μg Mefloquine only	0	
372.20 μg EDTA only	0	
10 μg CR venom + 36.09 μg Quinine	11.75 ± 1.31	14.32
10 μg CR venom + 180.45μg Quinine	12.25 ± 0.85	10.67
10 μg CR venom + 360.09 μg Quinine	8.25 ± 0.40 *	39.84
10 μg CR venom + 3.47 μg p-BPB	10.00 ± 0.81 *	27.08
10 μg CR venom + 6.94 μg p-PBP	10.00 ± 1.54 *	27.08
10 μg CR venom + 13.88 μg p-BPB	5.25 ± 1.25 *	61.71
10 μg CR venom + 5.30 μg Mefloquine	6.75 ± 1.37 *	50.78
10 μg CR venom + 10.60 μg Mefloquine	3.75 ± 1.75 *	72.65
10 μg CR venom + 21.20 μg Mefloquine	1.00 ± 0.57 *	92.70
10 μg CR venom + 186.10 μg EDTA	3.75 ± 0.75 *	72.65
10 μg CR venom + 372.20 μg EDTA	2.00 ± 1.09 *	85.41
10 μg CR venom + 744.40 μg EDTA	2.00 ± 0.31 *	85.41

+ p-BPB : para-bromophenacyl bromide; EDTA : Ethylenediamine tetraacetic acid

* statistically significant, $p < 0.05$

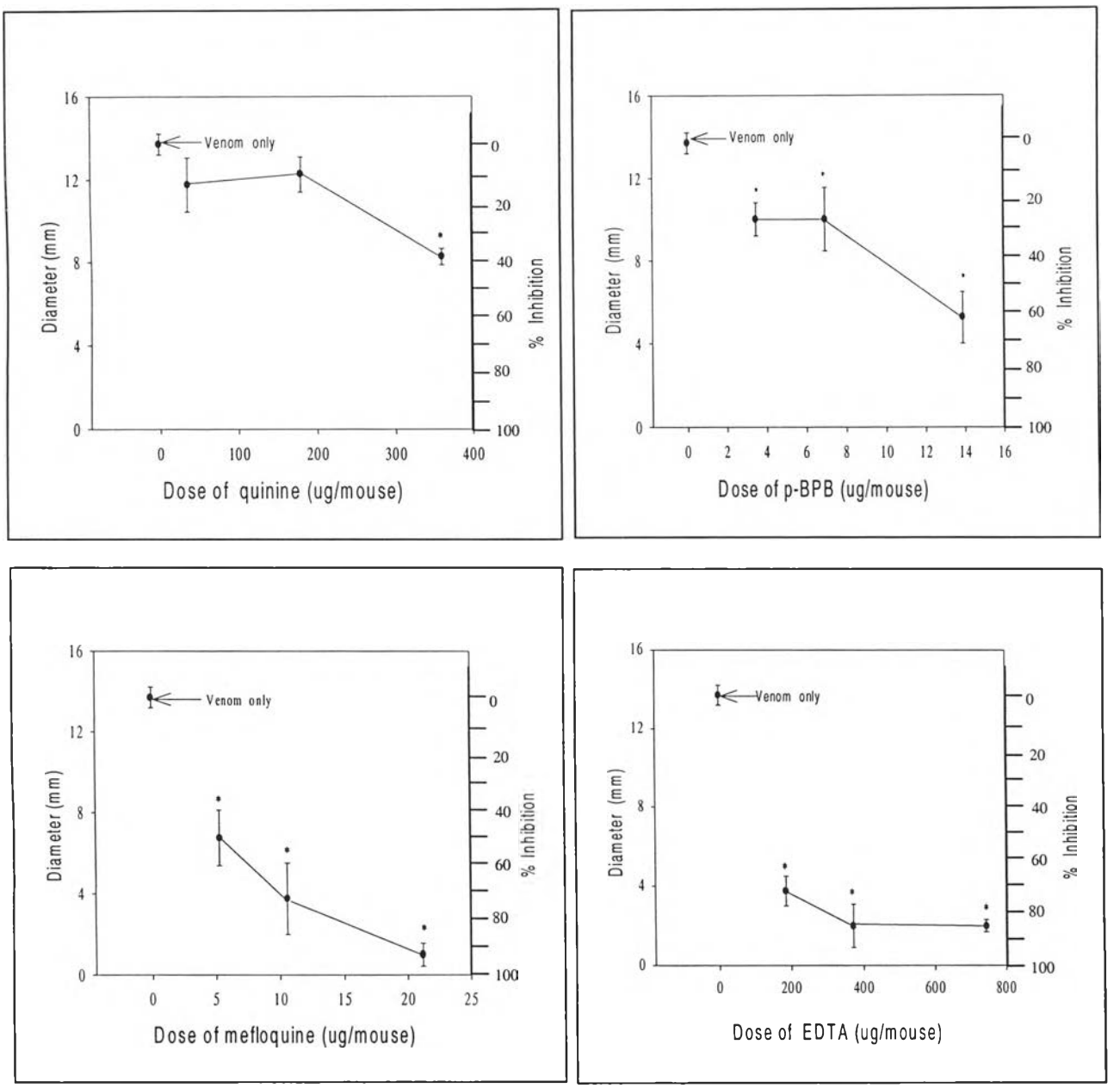


Figure 19. Effects of various phospholipase A_2 inhibitors on hemorrhage induced by CR venom. CR venom (10 μg) was mixed with each inhibitor and incubated for 5 min at room temperature before injection. Each point represents the mean ± S.E. of results obtained from four mice.

* statistically significant, $p < 0.05$

2.1.2. Independent inoculation experiment

- **Edema**

CR venom induced conspicuous edema on the footpad which weighed 99.17 ± 4.07 mg cf. 67.77 mg observed in the preincubation experiment. This was most likely due to the exposure of the footpad to higher concentration of CR venom used in these experiments.

In these experiments, only N-phenylglycine and EDTA which were highly effective in the preincubation experiments, were studied. N-phenylglycine at 37.80 µg significantly reduced edema even when injected 3 min after venom injection. EDTA was effective only when injected within 1 min after CR venom injection (Table 32).

The 'Inhibitor mixture' significantly decreased edema even when injected 10 min after venom injection.

- **Myonecrosis**

CR venom injection induced myonecrosis resulting in an increase in serum CPK activity from 105.20 ± 22 to 1809.61 ± 82.17 units/ml. This was higher than that observed with CR venom alone in the preincubation experiment (Table 11) and was most likely due to exposure of the muscle to higher concentration of the venom used in this type of experiment.

EDTA and N-phenylglycine were shown to significantly reduced myonecrosis induced by CR venom even when injected 3 min after the venom injection. The 'Inhibitor mixture' was highly effective when it was injected within 1 min but myonecrosis was still significantly reduce if the mixture was injected 10 min later (Table 33).

- **Hemorrhage**

EDTA and N-phenylglycine significantly reduced the hemorrhage by 45.65 % and 39.13 % respectively if injected within 1 min after venom injection. This inhibition was only 26.08 % and 23.91 % respectively if the injection of EDTA and N-phenylglycine was delayed to 3 min (Table 34).

The 'Inhibitor mixture' was very effective in reducing hemorrhage, 78.26% inhibition was achieved when injected within one min after the injection of CR venom.

Table 32. Effects of various enzyme inhibitors on edema induced by CR venom in an independent inoculation experiment.

Treatment	Delay in inhibitor administration (min)	Increment in weight (mg) Mean ± S.E.	Inhibition (%)
2 µg CR venom only		99.17 ± 4.07	
93.05 µg EDTA only		10.10 ± 0.67	
37.80 µg N-phenylglycine only		10.00 ± 0.56	
'Inhibitor mixture' ≠ only		10.25 ± 0.27	
2 µg CR venom + 93.05 µg EDTA	1	74.74 ± 4.17 *	24.63
	3	85.48 ± 2.34	13.79
	10	88.46 ± 1.93	10.79
2 µg CR venom + 37.80 µg N-phenylglycine	1	64.21 ± 8.81 *	35.25
	3	67.75 ± 1.37 *	31.67
	10	69.49 ± 5.30	29.93
2 µg CR venom + 'Inhibitor mixture'	1	60.85 ± 3.20 *	38.64
	3	61.05 ± 4.28 *	38.44
	10	64.25 ± 6.39 *	35.23

≠ 'Inhibitor mixture' contained 195 µg sodium aurothiomalate, 37.80 µg N-phyenylglycine and 93.05 µg EDTA.

* statistically significant, $p < 0.05$

Table 33. Effects of various enzyme inhibitors on myonecrosis induced by CR venom in an independent inoculation experiment.

Treatment	**Delay in inhibitor administration (min)**	**CPK (Sigma units/ml) Mean ± S.E.**	**Inhibition (%)**
25 μg CR venom only		1809.61 ± 82.17	
NSS injection only		105.20 ± 22.00	
93.05 μg EDTA only		102.49 ± 3.86	
37.80 μg N-phenylglycine only		121.80 ± 24.00	
'Inhibitor mixture'≠ only		159.97 ± 27.22	
25 μg CR venom + 93.05 μg EDTA	1	602.49 ± 19.97 *	66.70
	3	1348.97 ± 46.60 *	25.46
	10	1564.71 ± 48.74	13.53
25μg CR venom +37.80 μg N-phenylglycine	1	832.59 ± 132.00 *	53.99
	3	1359.68 ± 41.67 *	24.86
	10	1571.65 ± 166.96	13.14
25 μg CR venom + 'Inhibitor mixture'	1	436.88 ± 46.18 *	75.87
	3	1275.88 ± 186.61 *	29.49
	10	1340.31 ± 199.80 *	25.93

≠ 'Inhibitor mixture' contained 195 μg sodium aurothiomalate, 37.80 μg N-phyenylglycine and 93.05 μg EDTA.

* statistically significant ; $p < 0.05$

Table 34. Effects of various enzyme inhibitors on hemorrhage induced by CR venom in an independent inoculation experiment.

Treatment	Delay in inhibitor administration (min)	Hemorrhagic spot (mm) Mean ± S.E.	Inhibition (%)
10 μg CR venom only		15.33 ± 0.33	
186.10 μg EDTA only		0	
75.60 μg N-phenyglycine only		0	
'Inhibitor mixture' ≠ only		0	
10 μg CR venom + 186.10 μg EDTA	1	8.33 ± 0.88 *	45.65
	3	11.33 ± 0.66 *	26.08
	10	12.66 ± 0.50	17.39
10 μg CR venom +75.60 μg N-phenylglycine	1	9.33 ± 1.45 *	39.13
	3	11.66 ± 1.66 *	23.91
	10	13.00 ± 1.52	15.27
10 μg CR venom + 'Inhibitor mixture'	1	3.33 ± 0.88 *	78.26
	3	10.66 ± 0.88 *	30.43
	10	11.66 ± 0.88 *	23.91

≠ 'Inhibitor mixture' contained 390.10 μg sodium aurothiomalate, 75.60 μg N-phyenylglycine and 186.10 μg EDTA.

* statistically significant, $p < 0.05$

2.2. Effects of PLA_2 and metalloproteinase inhibitors on edema and myonecrosis induced by NK venom.

NK venom, unlike CR venom, does not cause hemorrhage in mice and therefore only edema and myonecrosis were studies. Edema and myonecrosis induced by NK venom injection were determined as described in Materials and Methods. The effects of the inhibitors on the local tissue necrosis were studied using 2 types of experimental design.

a. Pre-incubation type experiment

The experiment was carried out by incubating a constant amount of NK venom with the various amounts of enzyme inhibitor for 5 min at 37°C before the mixture was injected into groups of four mice.

b. Independent inoculation experiment

The independent inoculation experiment was done by injecting a constant amount of CR venom followed at different time intervals by the injection of PLA_2 and/or metalloproteinase inhibitors into the same site of venom injection.

In these experiments, the concentration of venom or enzyme inhibitor injected was 2x while the volume was ½ those used in pre-incubation expriments. Therefore the doses and total volumes of the venom/inhibitor were exactly the same as those used in the pre-incubation experiments.

2.2.1. Pre-incubation type experiments

- **Edema**

Edema was found to rapidly develop after NK venom injection, reaching its highest values at 1 hour and gradually decreased. NK venom at a dose of 2.5 μg increased the weight of mouse footpad from 14.24 ± 0.24 mg to 63.42 ± 3.73 mg (Table 35).

In the preincubation experiments, the metalloproteinase inhibitors DFO (164.25-657 μg), L1 (6.95 – 69.50 μg), TEPA (92.90 – 371.60 μg) and N-phenylglycine (37.80 – 151.20 μg) decreased edema significantly. (Table 35 and Figure 20).

Among the PLA_2 inhibitors, only quinine at the highest concentration tested (180.50 μg) and EDTA (93.05 μg – 372.20 μg) significantly reduced edema induced by the NK venom (Table 36 and Figure 21).

An 'Inhibitor mixture' containing sodium aurothiomalate (195 μg), N-phenylglycine (37.80 μg) and EDTA (93.05 μg) when preincubated with NK venom, significantly reduced edema by 57.13 % (Table 35).

- **Myonecrosis**

Myonecrosis was evaluated by measuring the activity of serum creatine phosphokinase (CPK) after venom injection and was expressed as Mean ± S.E. of CPK activity (Materials and Methods). In a group of mice injected with NK (5 μg), the serum CPK increased from 105.20 ± 22 to 628 ± 2 units/ml (Table 37). Among the metalloproteinase inhibitors TEPA (92.90 μg) and N-phenylglycine (37.80 μg) inhibited

myonecrosis by over 60%. The PLA_2 inhibitors, p-BPB (6.96 μg) and EDTA (93.05 μg) inhibited myonecrosis by 31 and 53 %, respectively.

The 'Inhibitor mixture', after preincubated with NK venom, reduced the CPK activity by 69.74 % (Table 37 and Figure 22).

Table 35. Effects of various metalloproteinase inhibitors on edema induced by NK venom in a pre-incubation type experiment.

Treatment +	Increment in weight (mg) Mean ± S.E.	Inhibition (%)
2.5 μg NK venom only	63.42 ± 3.73	-
NSS injection only	14.24 ± 0.24	-
75.60 μg N-phenylglycine only	10.23 ± 0.35	-
34.75 μg L1 only	10.36 ± 0.21	-
328.50 μg DFO only	9.35 ± 0.32	-
185.80 μg TEPA only	10.39 ± 0.32	-
'Inhibitor mixture' ≠ only	10.25 ± 0.27	-
2.5 μg NK venom + 37.80 μg N-phenylglycine	35.32 ± 3.18 *	47.46
2.5 μg NK venom + 75.60 μg N-phenylglycine	36.24 ± 1.37 *	42.85
2.5 μg NK venom + 151.20 μg N-phenylglycine	55.07 ± 3.72	13.16
2.5 μg NK venom + 6.95 μg L1	48.80 ± 2.99 *	23.05
2.5 μg NK venom + 34.75 μg L1	44.99 ± 6.01 *	29.06
2.5 μg NK venom + 69.50 μg L1	37.04 ± 7.75 *	41.06
2.5 μg NK venom + 164.25 μg DFO	48.24 ± 4.59 *	23.92
2.5 μg NK venom + 328.50 μg DFO	37.29 ± 5.46 *	41.19
2.5 μg NK venom + 657 μg DFO	44.45 ± 6.01 *	29.90
2.5 μg NK venom + 92.90 μg TEPA	47.24 ± 3.70 *	25.50
2.5 μg NK venom + 185.80 μg TEPA	38.25 ± 7.18 *	39.68
2.5 μg NK venom + 371.60 μg TEPA	39.93 ± 5.52 *	37.03
2.5 μg NK venom + 'Inhibitor mixture'	22.19 ± 2.58 *	65.01

+ L1 : Desferiprone; DFO : Desferrioxamine; TEPA : Tetraethylenepentamine.

≠ 'Inhibitor mixture' contained 195 μg sodium aurothiomalate, 37.80 μg N-phenylglycine and 93.05 μg EDTA.

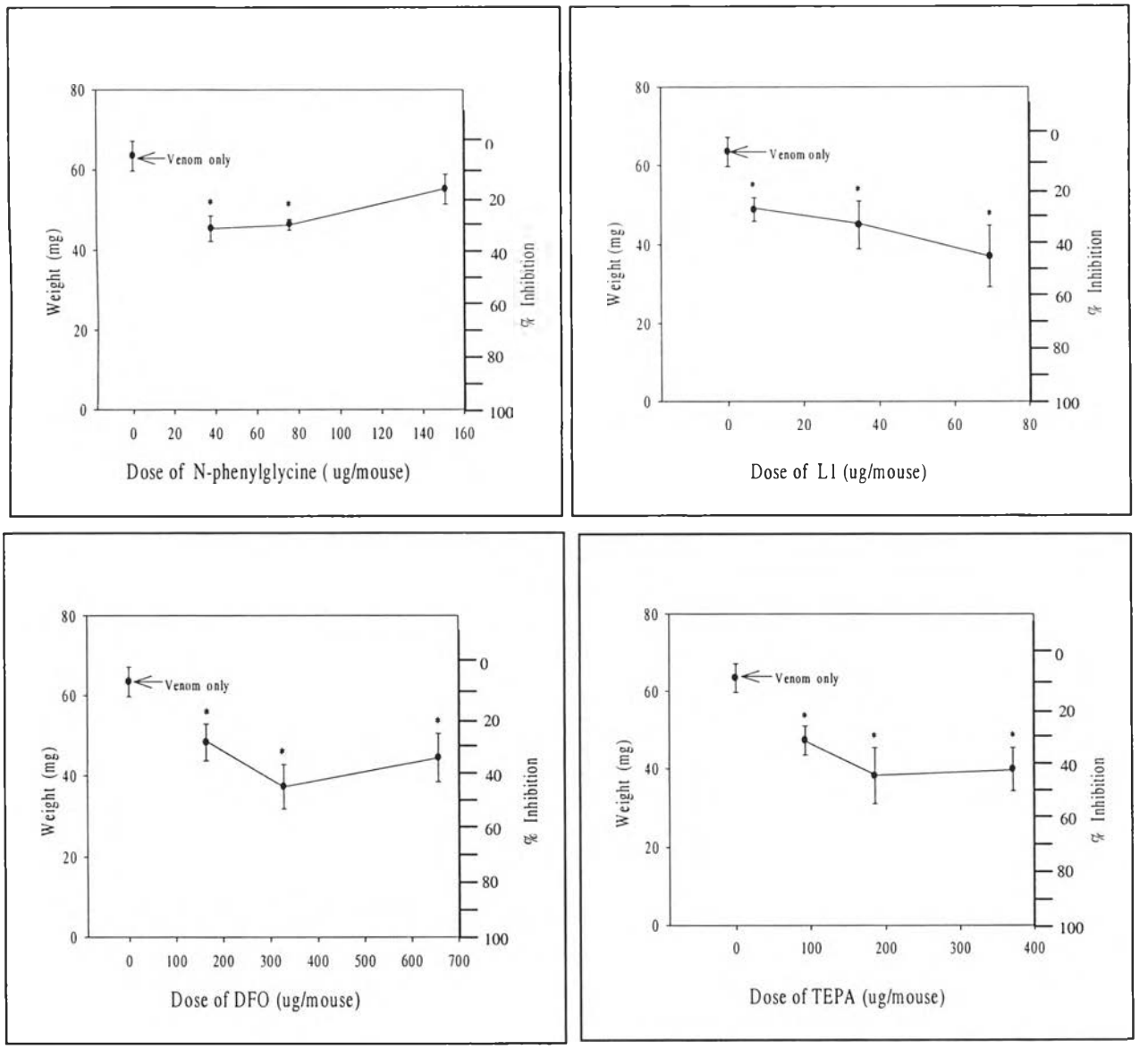


Figure 20. Effects of various metalloproteinase inhibitors on edema induced by NK venom. NK venom (2.5 μg) was mixed with each inhibitor and incubated for 5 min at room temperature before injection. Each point represents the mean ± S.E. of results obtained from four mice.

* statistically significant, $p < 0.05$

Table 36. Effects of various phospholipase A_2 inhibitors on edema induced by NK venom in a pre-incubation type experiment.

Treatment +	**Increment in weight (mg) Mean ± S.E.**	**Inhibition (%)**
2.5 µg NK venom only	63.42 ± 3.73	
NSS injection only	14.24 ± 0.24	
90.25 µg Quinine only	10.65 ± 0.25	
3.48 µg p-BPB only	10.32 ± 0.31	
5.30 µg Mefloquine only	10.48 ± 0.29	
186.10 µg EDTA only	10.39 ± 0.25	
2.5 µg NK venom + 18.05 µg Quinine	62.00 ± 1.29	2.23
2.5 µg NK venom + 90.25 µg Quinine	53.59 ± 6.52	15.50
2.5 µg NK venom + 180.50 µg Quinine	41.53 ± 6.33 *	34.55
2.5 µg NK venom + 1.74 µg p-BPB	63.21 ± 3.78	0.34
2.5 µg NK venom + 3.48 µg p-PBP	56.11 ± 8.09	11.52
2.5 µg NK venom + 6.96 µg p-BPB	59.74 ± 9.54	5.80
2 µg NK venom + 2.65 µg Mefloquine	60.63 ± 4.11	4.40
2 µg NK venom + 5.30 µg Mefloquine	60.39 ± 1.52	4.78
2 µg NK venom + 10.60 µg Mefloquine	61.09 ± 8.08	3.67
2 µg NK venom + 93.05 µg EDTA	38.20 ± 1.71 *	39.77
2 µg NK venom + 186.10 µg EDTA	39.22 ± 1.77 *	38.16
2 µg NK venom + 372.20 µg EDTA	56.67 ± 4.23	11.45

+ p-BPB: para-bromophenacyl bromide; EDTA: Ethylenediamine tetraacetic acid.

* statistically significant , $p < 0.05$

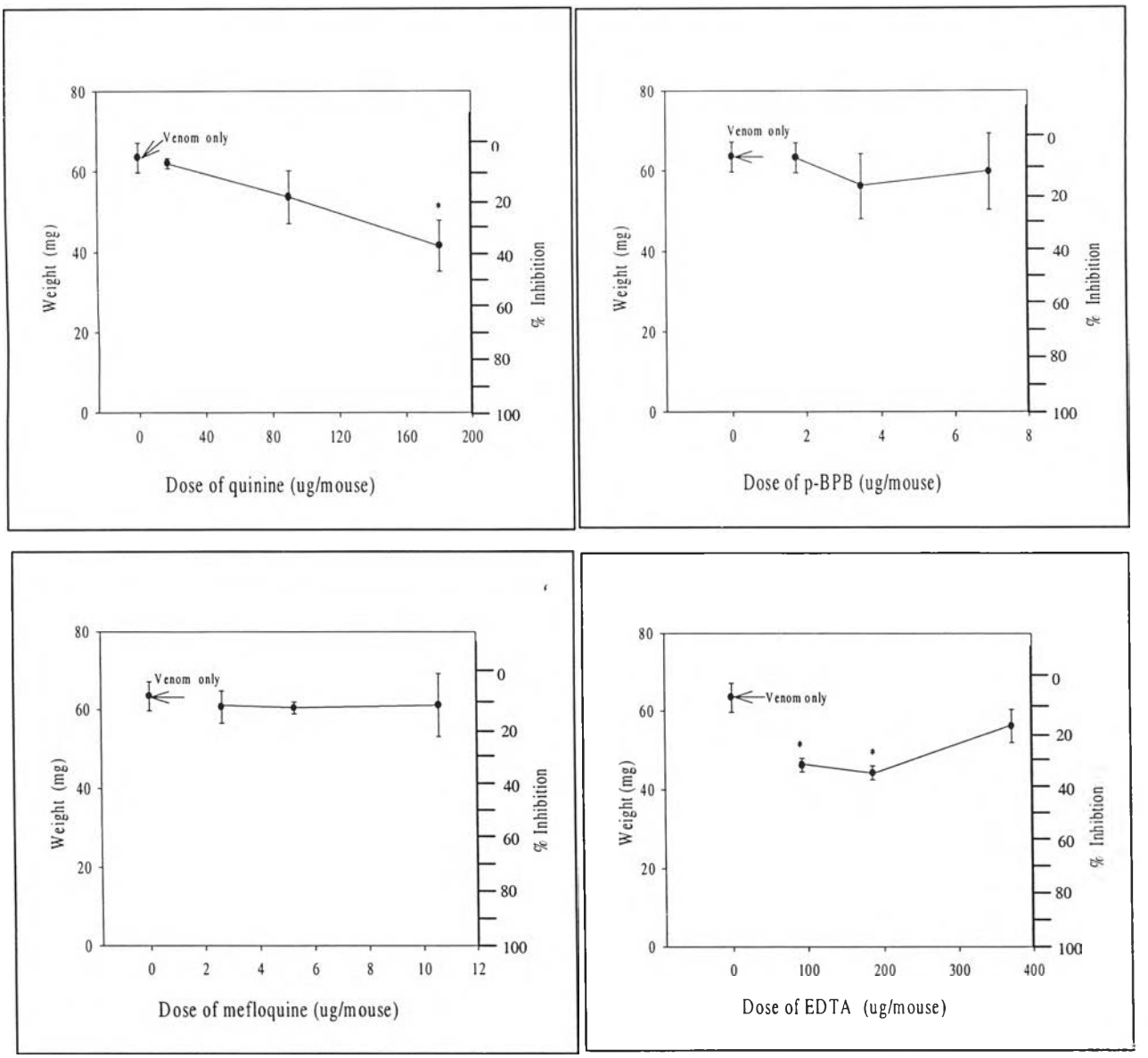


Figure 21. Effects of various phospholipase A_2 inhibitors on edema induced by NK venom. NK venom (2.5 μg) was mixed with each inhibitor and incubated for 5 min at room temperature before injection. Each point represents the mean ± S.E. of results obtained from four mice.

* statistically significant, $p < 0.05$

Table 37. Effects of metalloproteinase inhibitors, phospholiase A_2 inhibitors and 'inhibitor mixture' on the myonecrosis induced by NK venom in a pre-incubation type experiment.

Treatment +	**CPK (Sigma units/ml) Mean ± S.E.**	**Inhibition (%)**
5 μg NK venom only	628.00 ± 2.00	
NSS injection only	105.20 ± 22.00	
DMSO injection only	111.20 ± 7.50	
37.80 μg N-phenylglycine	121.80 ± 24.00	
4.65 μg TEPA	163.85 ± 16.50	
92.90 μg DFO	117.80 ± 7.50	
328.50 μg Quinine	105.80 ± 20.00	
10.60 μg Mefloquine	190.32 ± 26.88	
6.96 μg p-BPB	180.50 ± 7.30	
93.05 μg EDTA	102.49 ± 3.86	
'Inhibitor mixture'	159.97 ± 27.22	
5 μg NK venom + 37.80 μg N-phenylglycine	240.63 ± 21.81 *	61.68
5 μg NK venom + 92.80 μg TEPA	212.65 ± 62.21 *	66.13
5 μg NK venom + 328.50 μg DFO	556.89 ± 79.68	11.32
5 μg NK venom + 90.25 μg Quinine	620.60 ± 13.18	1.28
5 μg NK venom + 10.60 μg Mefloquine	460.93 ± 71.51	26.60
5 μg NK venom + 6.96μg p-BPB	431.43 ± 43.23 *	31.29
5 μg NK venom + 93.05 μg EDTA	294.08 ± 43.01 *	53.17
5 μg NK venom + 'Inhibitor mixture' ≠	190.04 ± 28.36 *	69.74

+ DFO: Desferrioxamine; TEPA: Tetraethlylenepentamine; p-BPB: para-bromophenacyl bromide; EDTA: Ethylenediamine tetraacetic acid.

≠ 'Inhibitor mixture' contained 195 μg sodium aurothiomalate, 37.80 μg N-phyenylglycine and 93.05 μg EDTA.

* statistically significant, $p< 0.05$

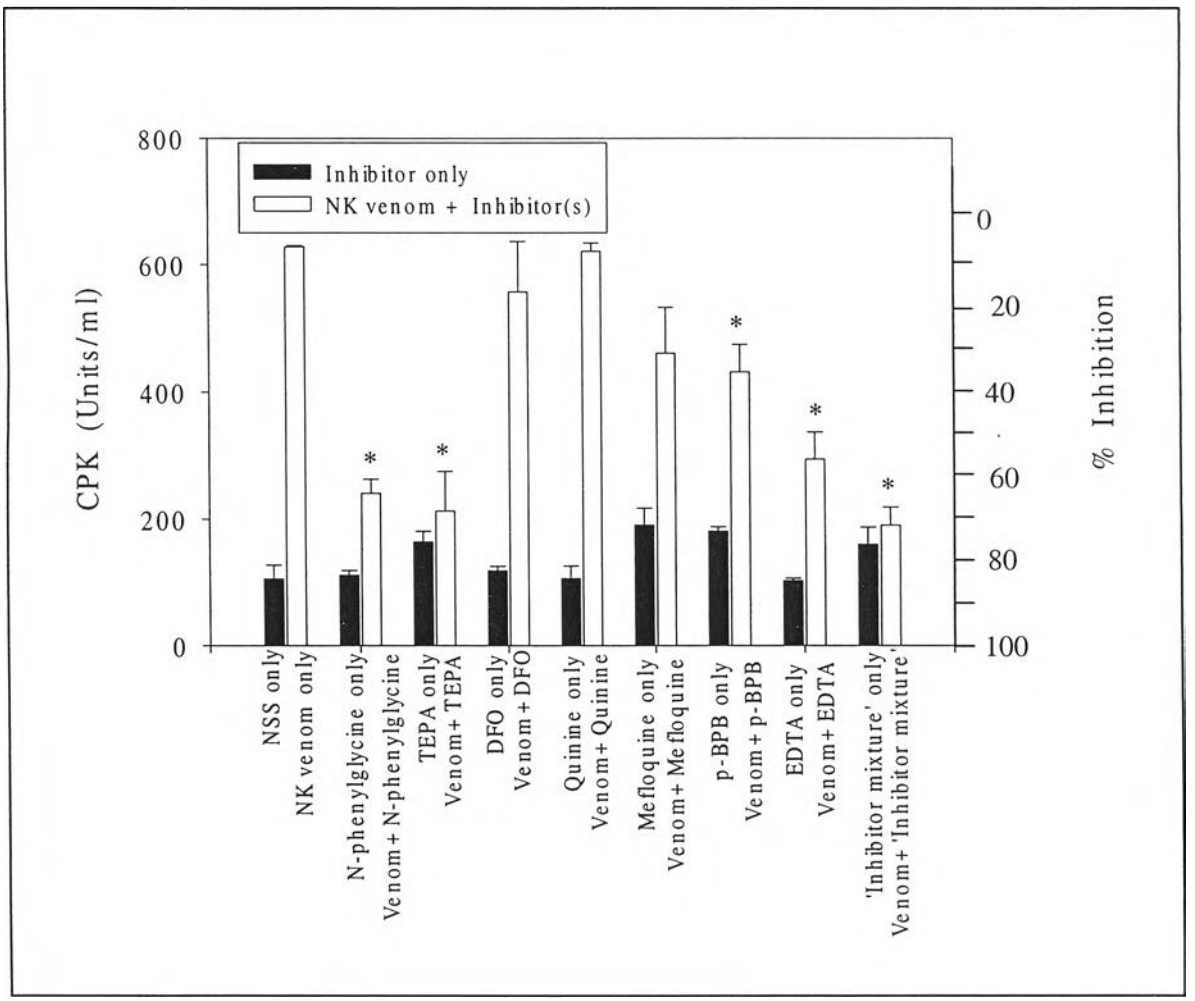


Figure 22. The effect of EDTA (93.05 μg), TEPA (92.09 μg), N-phenylglycine (37.80 μg), p-BPB (6.96 μg), mefloquine (10.60 μg), quinine (90.25 μg) and 'Inhibitor mixture' (37.80 μg N-phenylglycine + 195 μg sodium aurothiomalate + 93.05 μg EDTA) on CPK activity induced by NK venom. The NK venom (5 μg) and the inhibitor was preincubated at room temperature for 5 min before injection. Each bar represents the mean ± S.E. of results obtained from five mice.

* statistically significant, $p < 0.05$

2.2.2. Independent inoculation experiment

- **Edema**

In these experiments, the volume of NK venom injected was ½ while the concentration was 2x those used in preincubation experiment. Under these conditions, NK venom induced conspicuous edema weighing 88.73 ± 4.78 mg (Table 38) cf. 63.42 ± 3.73 mg shown in Table 35.

N-phenylglycine and EDTA were found to be effective in reducing edema if they were injected 1 min after NK venom injection. The 'Inhibitor mixture' significantly reduced edema even when its injection was delayed by 3 min (Table 38).

- **Myonecrosis**

The CPK activity induced by NK venom in the dependent inoculation experiment was much higher (1831.37 ± 54.42 units/ml) than that observed in preincubation experiment (628.20 ± 2.00 units/ml). This is most likely due to exposure of the muscle to higher concentration of the venom.

N-phenylglycine and EDTA significantly inhibited CPK activity when injected 1-3 min after venom injection (Table 39). The 'Inhibtor mixture' significantly reduced myonecrosis even when injected 10 min after venom injection. However, its effectiveness was reduced with longer time delay.

Table 38. Effects of various enzyme inhibitors on edema induced by *Naja kaouthia* venom in an independent inoculation experiment.

Treatment	Delay in inhibitor administration (min)	Increment in weight (mg) Mean ± S.E.	Inhibition (%)
2.5 µg NK venom only		88.73 ± 4.78	
93.05 µg EDTA only		10.10 ± 0.67	
37.80 µg N-phenylglycine only		10.00 ± 0.56	
'Inhibitor mixture'≠ only		10.25 ± 0.27	
2.5 µg NK venom + 93.50 µg EDTA	1	61.80 ± 2.53 *	30.35
	3	65.24 ± 4.59	26.47
	10	69.50 ± 3.73	21.67
2.5 µg NK venom + 37.80 µg N-phenylglycine	1	56.33 ± 2.31*	36.51
	3	61.33 ± 5.28	27.12
	10	69.20 ± 5.33	22.01
2.5 µg NK venom + 'Inhibitor mixture'	1	44.33 ± 2.91 *	50.03
	3	61.55 ± 6.73 *	30.62
	10	67.20 ± 5.8	24.26

≠ 'Inhibitor mixture' contained 195 µg sodium aurothiomalate, 37.80 µg N-phyenylglycine and 93.05 µg EDTA.

* statistically significant, $p < 0.05$

Table 39. Effects of various enzyme inhibitors on myonecrosis induced by *Naja kaouthia* (NK) venom in an independent inoculation experiment.

Treatment	**Delay in inhibitor administration (min)**	**CPK (Sigma units/ml) Mean ± S.E.**	**Inhibition (%)**
5 μg NK venom only		1831.37 ± 54.42	
NSS injection only		105.20 ± 22.00	
93.50 μg EDTA only		102.49 ± 3.86	
37.80 μg N-phenylglycine only		121.80 ± 24.00	
'Inhibitor mixture' ≠ only		159.97 ± 27.22	
5 μg NK venom + 93.05 μg EDTA	1	960.87 ± 39.93 *	47.53
	3	1416.48 ± 169.76	22.65
	10	1530.00 ± 212.72	16.44
5 μg NK venom + 37.80 μg N-phenylglycine	1	1045.62 ± 82.13 *	42.90
	3	1417.40 ± 158.74 *	22.60
	10	1544.73 ± 53.81	15.65
5 μg NK venom + 'Inhibitor mixture'	1	698.22 ± 95.25 *	61.87
	3	1258.89 ± 32.45 *	31.25
	10	1462.96 ± 88.76 *	20.11

≠ 'Inhibitor mixture' contained 195 μg sodium aurothiomalate, 37.80 μg N-phyenylglycine and 93.05 μg EDTA.

* statistically significant; $p < 0.05$

2.3. The effects of *N. kaouthia* and *C. rhodostoma* venoms at various doses on the survival time of mice.

The survival times of mice injected with various doses of NK or CR venom are shown in Table 40.

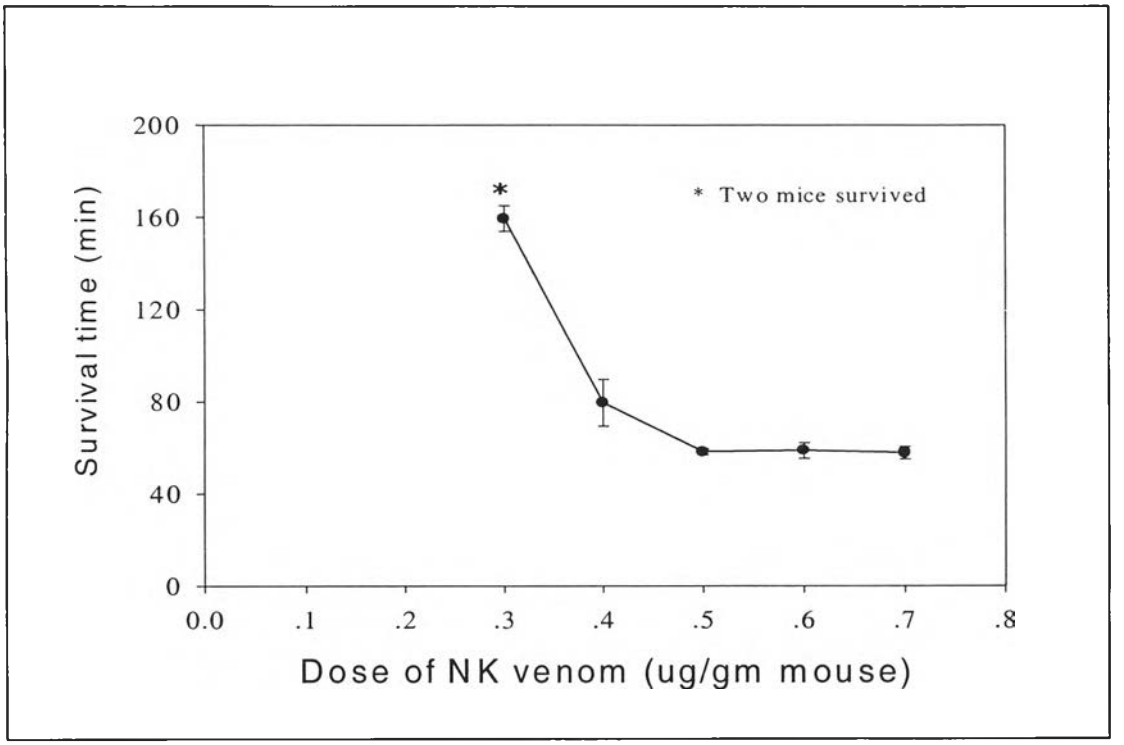
Mice receiving CR 40 μg/gm mouse died within 63.20 ± 12.17 min. Increasing the doses of the venom shortened the survival time. Injection of 30 μg/gm mouse CR venoms resulted in 2 mice survived more than 24 hour.

Similar results were obtained with NK venom. However, the lethal dose of NK was about one-tenth that of the CR venom (Table 40 and Figure 23).

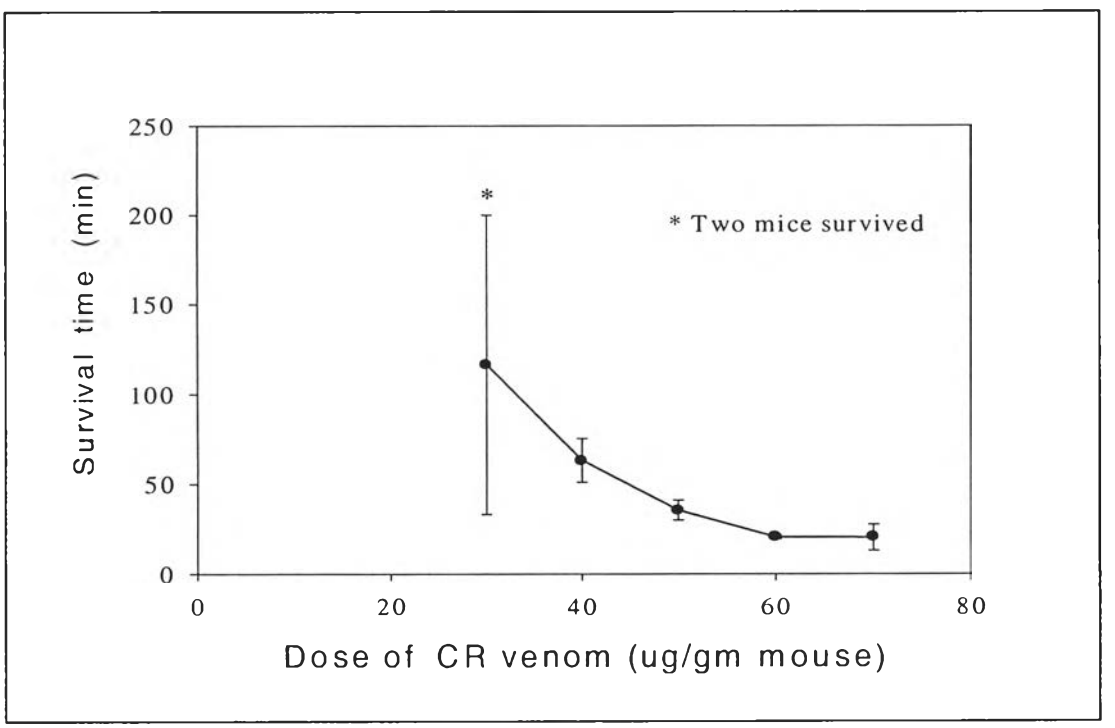
Table 40. The survival time of mice injected with various doses of *Calloselasma rhodostoma* (CR) and *Naja kaouthia* (NK) venom.

NK venom (μg/gm mouse)	**Survival time (min) : Mean ± S.E.**	**Remark**
0.30	159.50 ± 5.50	2 mice survived
0.40	79.50 ± 10.21	
0.50	58.14 ± 1.33	
0.60	58.69 ± 3.42	
0.70	57.72 ± 2.77	

CR venom (μg/gm mouse)	**Survival time (min) : Mean ± S.E.**	**Remark**
30	116.60 ± 83.86	2 mice survived
40	63.20 ± 12.17	
50	35.50 ± 5.43	
60	20.60 ± 1.12	
70	20.33 ± 7.31	



(a)



(b)

Figure 23. Survival time of mice injected with varying doses on *Naja kaouthia* (a) and *Calloselasma rhodostoma* (b) venoms. Each point represents the mean ± S.E. (n=6)

2.3.1. Effect of inhibitors of PLA_2 and metalloproteinase on the survival time of mice injected with *N. kaouthia* or *C. rhodostoma* venom.

i) Effect of metalloproteinase and/or PLA_2 inhibitors on the survival time of mice injected with CR venom

Mice injected with CR venom (40 μg/gm mouse) in the presence and absence of various doses of inhibitors were studied for their survival time. Metalloproteinase inhibitors including TEPA , DFO ,L1 and N-phenylglycine significantly prolonged, in a dose dependent manner, the survival time of the mice injected with the venom(Table 41 and Figure 24).

Among the PLA_2 inhibitors, p-BPB (3.47-13.88), EDTA (372.20 – 744.40 μg) and quinine (360.09 μg) significantly prolonged the survival time of mice (Table 42 and Figure 25). The metalloproteinase and PLA_2 inhibitors prolonged the survival time of mice to the extent that in same cases, a mouse survived more than 24 hour (Table 41 and 42)

ii) Effect of metalloproteinase and PLA_2 inhibitors on the survival time of mice injected with NK venom

Among the metalloproteinase inhibitors, DFO, TEPA, N-phenylglycine and L1 were effective in increasing the survival time of mice injected with 0.4 μg/gm mouse of NK venom. (Table 43 and Figure 26).

The PLA_2 inhibitors, p-BPB at all concentrations, mefloquine (10.60-21.20 μg), quinine (360.09 μg) and EDTA (372.20-744.40 μg) increased the survival time significantly (Table 44 and Figure 27).

iii) **Effect of 'Inhibitor mixture' on the survival time of mice after injected either NK venom or CR venom**

An inhibitor mixture, containing 390.10 μg sodium aurothiomalate, 75.60 μg N-phenylglycine, and 186.10 μg EDTA, were highly effective in increasing the survival time in mice injected with either NK venom (Table 43) or CR venom (Table 41) respectively.

Table 41. Survival time of mice injected with *Calloselasma rhodostoma* (CR) venom in the presence and absence of various metalloproteinase inhibitors.

Treatment +	Survival time (min) (Mean ± S.E.)	Remark
CR venom 40 ug/gm mouse	63.20 ± 12.17	
CR venom 40 μg/gm mouse + DMSO	63.50 ± 13.16	
CR venom + 15.12 μg N-phenylglycine	76.20 ± 17.24	
CR venom + 75.60 μg N-phenylglycine	194.50 ± 63.92 *	
CR venom + 151.20 μg N-phenylglycine	239.00 ± 52.17 *	1 mouse survived
CR venom + 69.50 μg L1	78.63 ± 23.63	
CR venom + 139 μg L1	215.00 ± 74.50 *	1 mouse survived
CR venom + 278 μg L1	235.00 ± 45.00 *	1 mouse survived
CR venom + 8.21 μg/gm mouse DFO	54.60 ± 14.17	
CR venom + 16.42 μg/gm mouse DFO	105.75 ± 9.40 *	
CR venom + 32.84 μg/gm mouse DFO	138.75 ± 3.52 *	
CR venom + 328.50 μg TEPA	58.00 ± 7.50	
CR venom + 657 μg TEPA	128.60 ± 30.23 *	1 mouse survived
CR venom + 1,314 μg TEPA	140.00 ± 39.00 *	1 mouse survived
CR venom + ≠ 'Inhibitor mixture'	280.25 ± 49.08 *	1 mouse survived

+ DMSO : Dimethysulfoxide, L1 : Desferiprone , DFO : Desferrioxamine, TEPA : Tetraethlylenepentamine.

≠ 'Inhibitor mixture' contained 390.10 μg sodium aurothiomalate , 75.60 μg N-phenylglycine and 186.10 μg EDTA.

* statistically significantly, $p < 0.05$

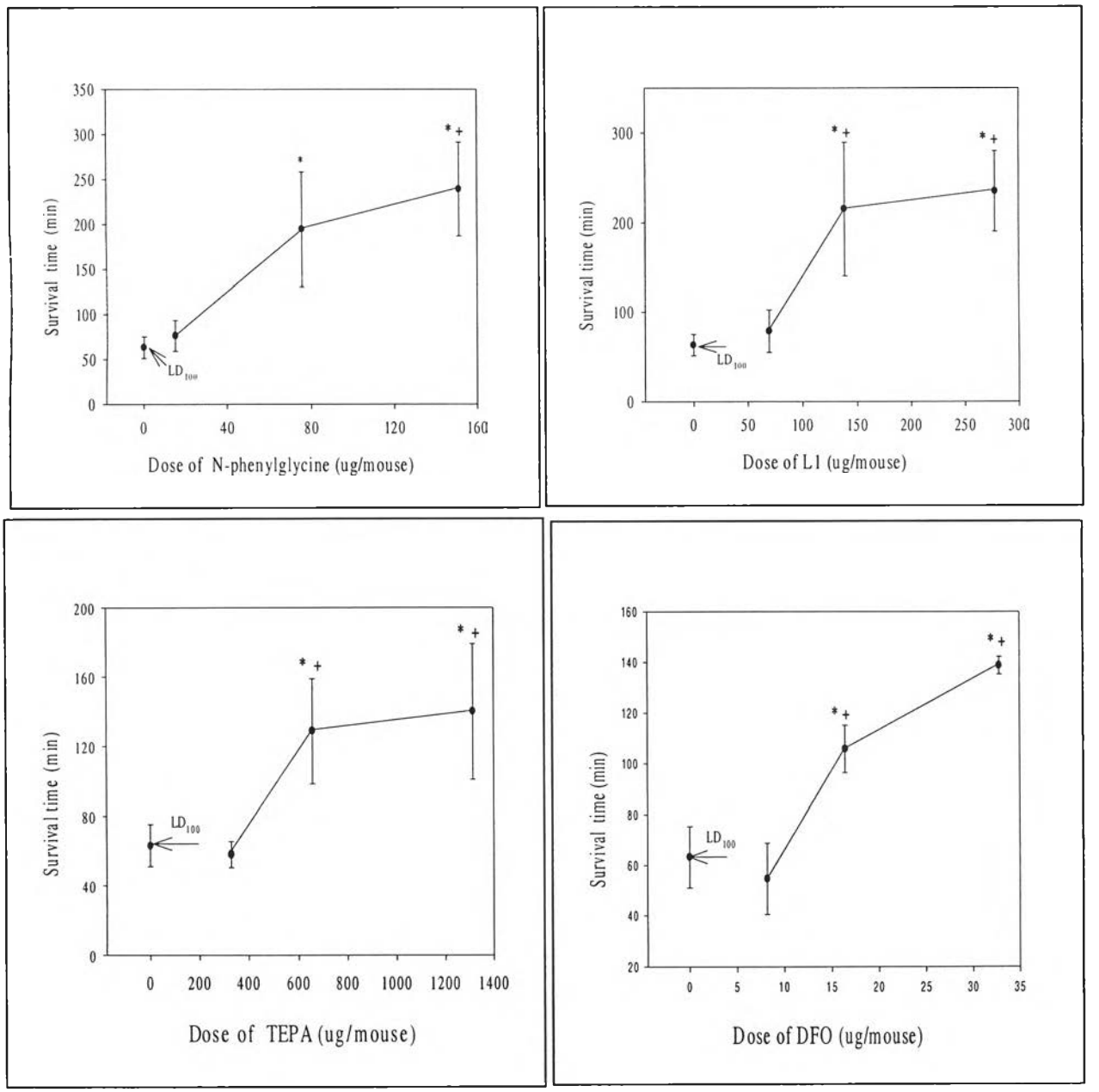


Figure 24. The effects of various metalloproteinase inhibitors on the survival time of mice injected with CR venom. Each point represented the mean ± S.E. (n= 5).

* statistically significant, $p < 0.05$

\+ 1 mouse survived

Table 42. Survival time of mice injected with *Calloselasma rhodostoma* (CR) venom in the presence and absence of various PLA_2 inhibitors.

Treatment +	Survival time (min) (Mean ± S.E.)	Remark
40 ug/gm mouse CR venom	63.20 ± 12.17	
40 μg/gm mouse CR venom + DMSO	63.50 ± 13.16	
CR venom + 36.09 μg Quinine	85.20 ± 24.71	
CR venom + 180.45 μg Quinine	78.00 ± 21.19	
CR venom + 360.09 μg Quinine	182.75 ± 8.87 *	1 mouse survived
CR venom + 3.47 μg p-BPB	171.66 ± 67.54 *	
CR venom + 6.94 μg p-PBP	207.50 ± 120.17 *	1 mouse survived
CR venom + 13.88 μg p-BPB	211.66 ± 81.34 *	1 mouse survived
CR venom + 5.30 μg Mefloquine	60.20 ± 9.57	
CR venom + 10.60 μg Mefloquine	75.20 ± 16.23	
CR venom + 21.20 μg Mefloquine	151.20 ± 19.03 *	
CR venom + 186.10 μg EDTA	49.00 ± 11.26	
CR venom + 372.20 μg EDTA	97.50 ± 6.50 *	2 mice survived
CR venom + 744.40 μg EDTA	117.50 ± 13.50 *	2 mice survived

+ p-BPB : para-bromophenacyl bromide, EDTA : Ethylenediamine tetraacetic acid,

* statistically significant, $p < 0.05$

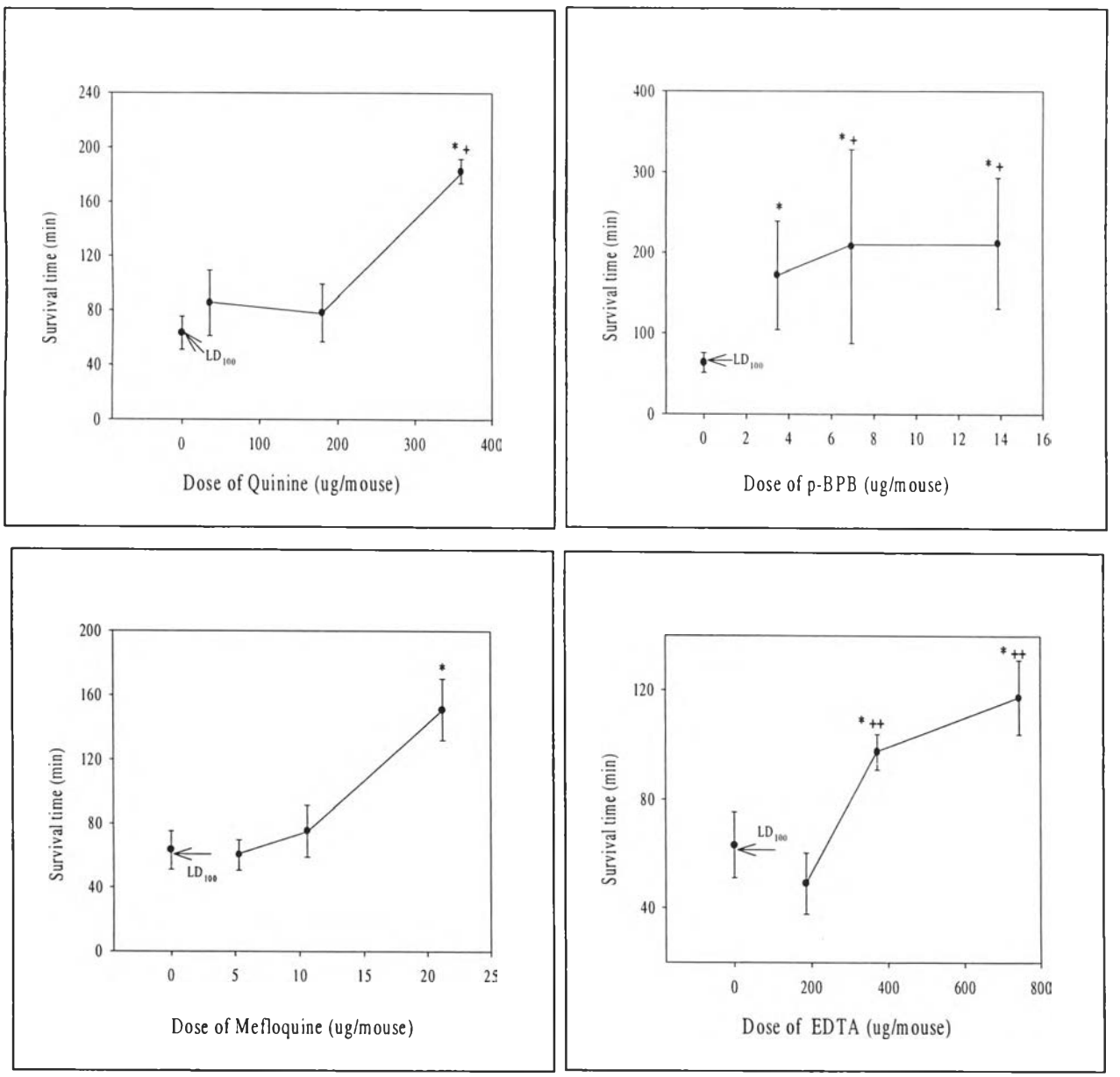


Figure 25. The effects of various phospholipase A_2 inhibitors on the survival time of mice injected with CR venom. Each point represented the mean ± S.E. (n= 5).

* statistically significant, $p < 0.05$

\+ 1 mouse survived

++ 2 mice survived

Table 43. Survival time of mice injected with *Naja kaouthia* (NK) venom in the presence and absence of various metalloproteinase inhibitors.

Treatment +	Survival time (min) (Mean ± S.E.)	Remark
NK venom 0.40 ug/gm mouse	79.50 ± 10.21	
NK venom 0.40 µg/gm mouse + DMSO	80.52 ± 11.30	
NK venom + 15.12 µg N-phenylglycine	111.00 ± 17.54	
NK venom + 75.60 µg/gm mouse N-phenylglycine	154.60 ± 10.30 *	1 mouse survived
NK venom + 151.20 µg/gm mouse N-phenylglycine	186.60 ± 25.02 *	1 mouse survived
NK venom + 69.50 µg L1	93.00 ± 17.00	
NK venom + 139 µg L1	115.00 ± 11.53 *	
NK venom + 278 µg L1	134.50 ± 7.42 *	
CR venom + 328.50 µg DFO	95.44 ± 13.22	
CR venom + 657 µg DFO	142.66 ± 18.06 *	
CR venom + 1,314 µg DFO	145.25 ± 27.92 *	1 mouse survived
CR venom + 188.05 µg TEPA	139.80 ± 21.59 *	
CR venom + 376.10 µg TEPA	178.00 ± 33.55 *	
CR venom + 752.20 µg TEPA	213.00 ± 49.12 *	
NK venom + 'Inhibitor mixture' ≠	253.67 ± 53.94 *	1 mouse survived

+ DMSO : Dimethysulfoxide, L1 : Desferiprone , DFO : Desferrioxamine, TEPA : Tetraethlylenepentamine

≠ 'Inhibitor mixture' contained 390.10 µg sodium aurothiomalate, 75.60 µg N-phenylglycine and 186.10 µg EDTA.

* statistically significant, $p < 0.05$

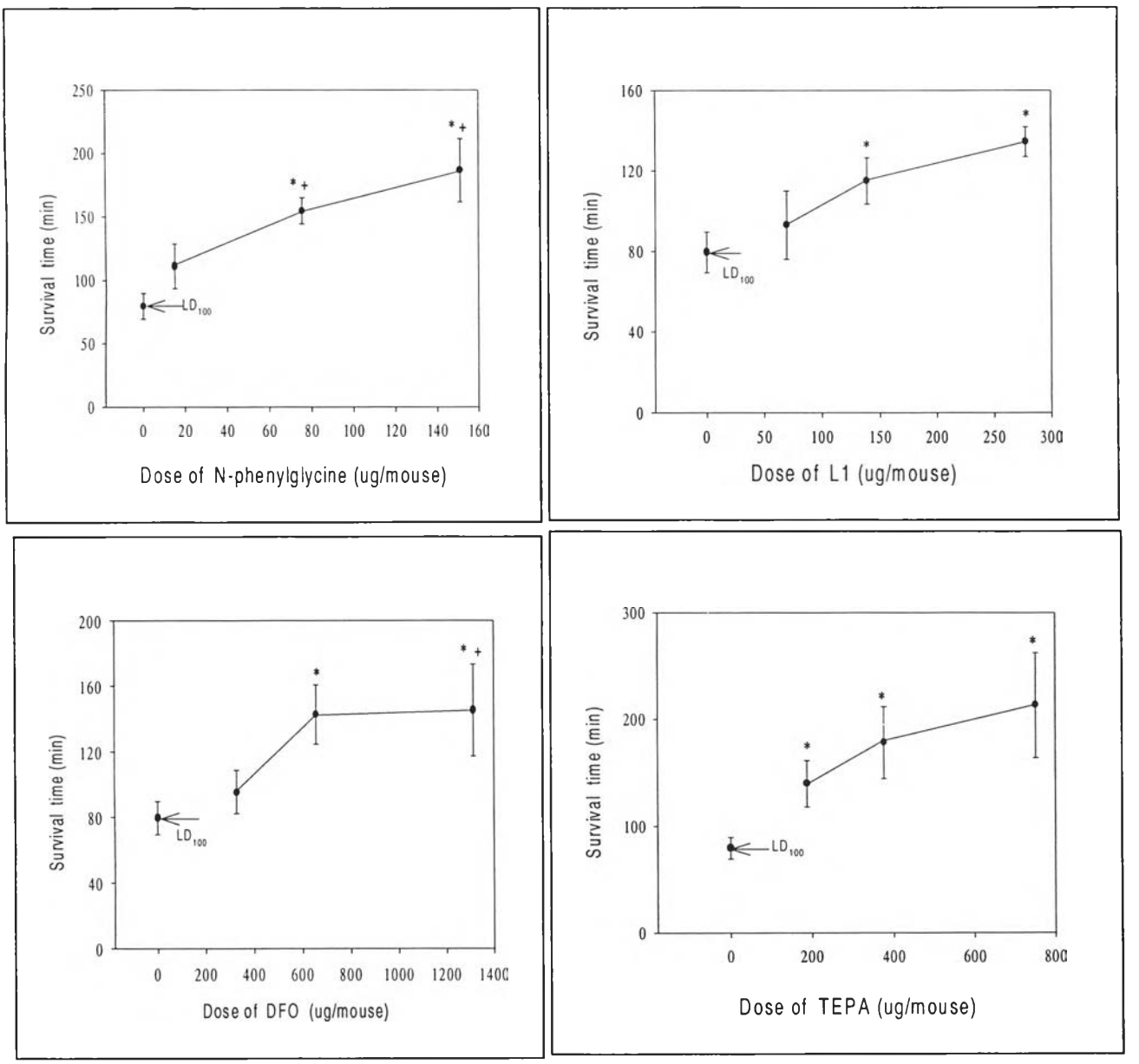


Figure 26. The effects of various metalloproteinase inhibitors on the survival time of mice injected with NK venom. Each point represented the mean ± S.E. (n= 5).

* statistically significant, $p < 0.05$

\+ 1 mouse survived

Table 44. Survival time of mice injected with *Naja kaouthia* (NK) venom in the presence and absence of various PLA_2 inhibitors.

Treatment +	Survival time (min) (Mean±S.E.)	Remark
NK venom 0.40 ug/gm mouse	79.50 ± 10.21	
NK venom 40 μg/gm mouse + DMSO	80.52 ± 11.30	
NK venom + 36.09 μg Quinine	70.40 ± 6.48	
NK venom + 180.45 μg Quinine	104.80 ± 15.86	
NK venom + 360.09 μg Quinine	127.50 ± 9.61 *	
NK venom + 3.47 μg p-BPB	140.25 ± 19.24 *	
NK venom + 6.94 μg p-PBP	181.66 ± 15.34 *	1 mouse survived
NK venom + 13.8 μg p-BPB	244.00 ± 16.00 *	1 mouse survived
NK venom + 5.30 μg Mefloquine	125.66 ± 21.32	
NK venom + 10.60 μg Mefloquine	153.20 ± 29.70 *	1 mouse survived
NK venom + 21.20 μg Mefloquine	170.50 ± 28.20 *	1 mouse survived
NK venom + 186.10 μg EDTA	85.50 ± 6.80	
NK venom + 372.20 μg EDTA	134.55 ± 7.64 *	
NK venom + 744.40 μg EDTA	144.00 ± 11.59 *	

+ p-BPB : para-bromophenacyl bromide, EDTA : Ethylenediamine tetraacetic acid,

* statistically significant, $p < 0.05$

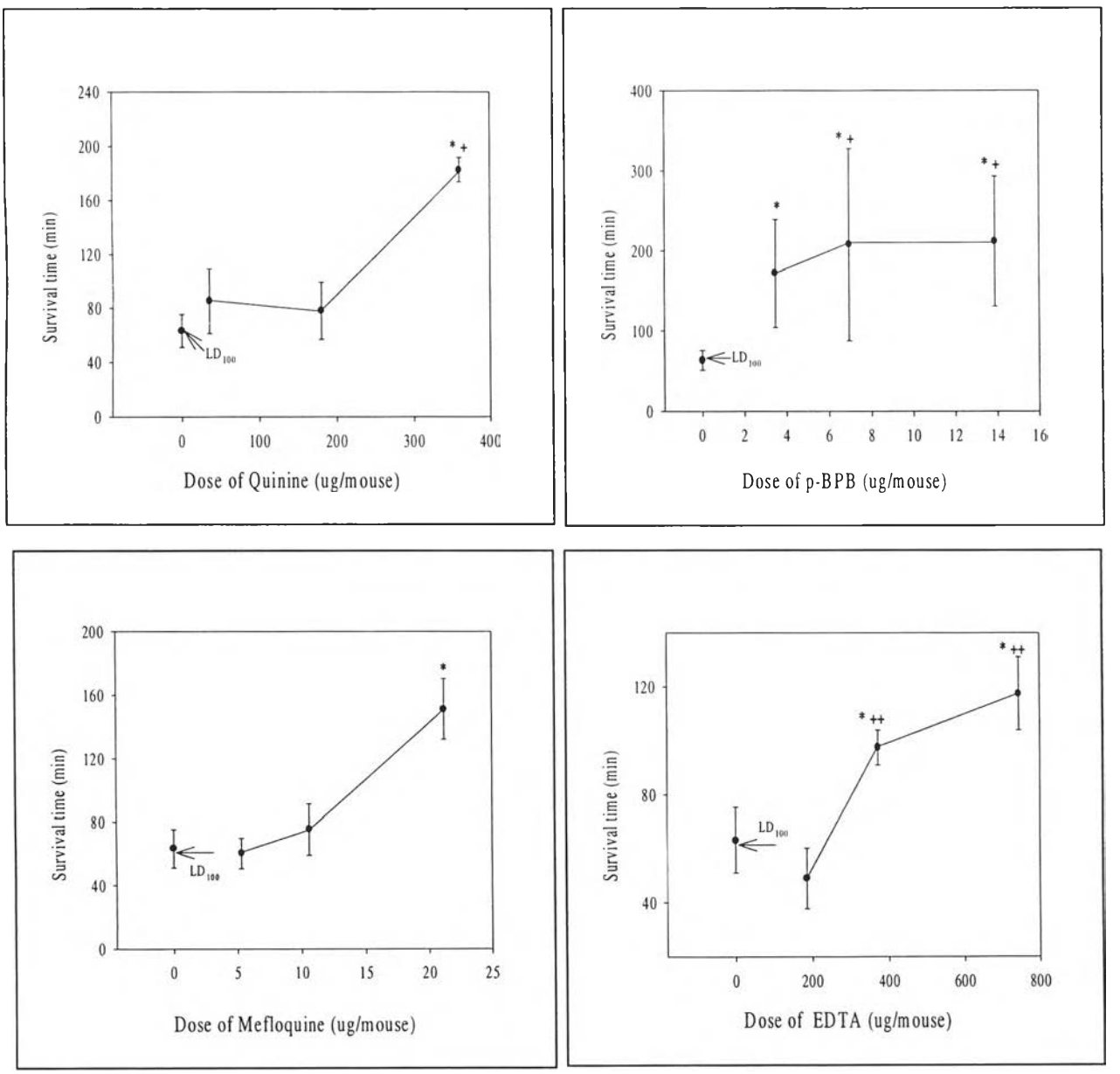


Figure 27. The effects of various phospholipase A_2 inhibitors on the survival time of mice injected with NK venom. Each point represented the mean ± S.E. (n= 5).

* statistically significant, $p < 0.05$

\+ 1 mouse survived

2.4. Pathological changes of the thigh muscle induced by *Calloselasma rhodostoma* (CR) venom

The histopathological changes of the thigh muscle induced by CR venom were observed under light microscope. The muscle sample was dehydrated in graded series of ethanol, embedded, in parafin, sectioned by using a microtome and the sections were stained with hematotoxylin and eosin as described in Materials and Methods.

The muscle injected with CR venom showed extensive hemorrhage and massive bleeding into interstitial spaces, accompanied by edematous swelling. The severe myonecrosis associated with hemorrhage was observed, ranging from circumscribed destruction of the muscle tissue to diffused degenerative myolytic events (Fig 28b).

The effect of inhibitors on the pathological changes of the muscle are shown in Figure 28c for EDTA, Figure 28d for TEPA and Figure 29e for N-phenylglycine. The photograph shows hemorrhage and bleeding into the intersitital spaces to a lesser degree than that observed in the absence of the inhibitors. Pathological changes do not appeared in the regions of damaged muscle cells because the Z-disks in these areas were observed.

Photomicrograph illustrating the typical changes in mouse thigh muscle caused by *Calloselasma rhodostoma* (CR) venom in the absence or presence of inhibitors (EDTA, N-phenylglycine or TEPA)

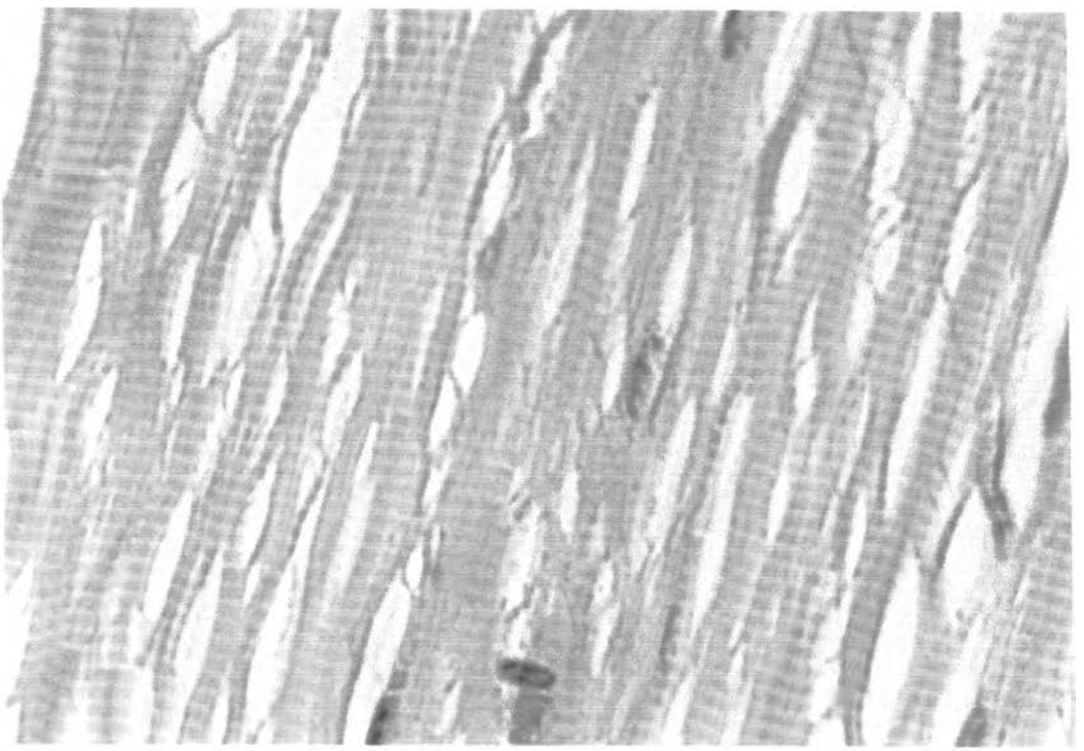
a) Normal thigh muscle : peripheral nuclei within skeletal muscle and the regular transversal striations of fiber are observed.

b) The muscle lesion caused by the injection of 25 μg/mouse of CR venom after 3 hours. This lesion shows severe interstitial hemorrhage and congestion. Skeletal muscle necrosis which is evident by the complete loss of cell structures.

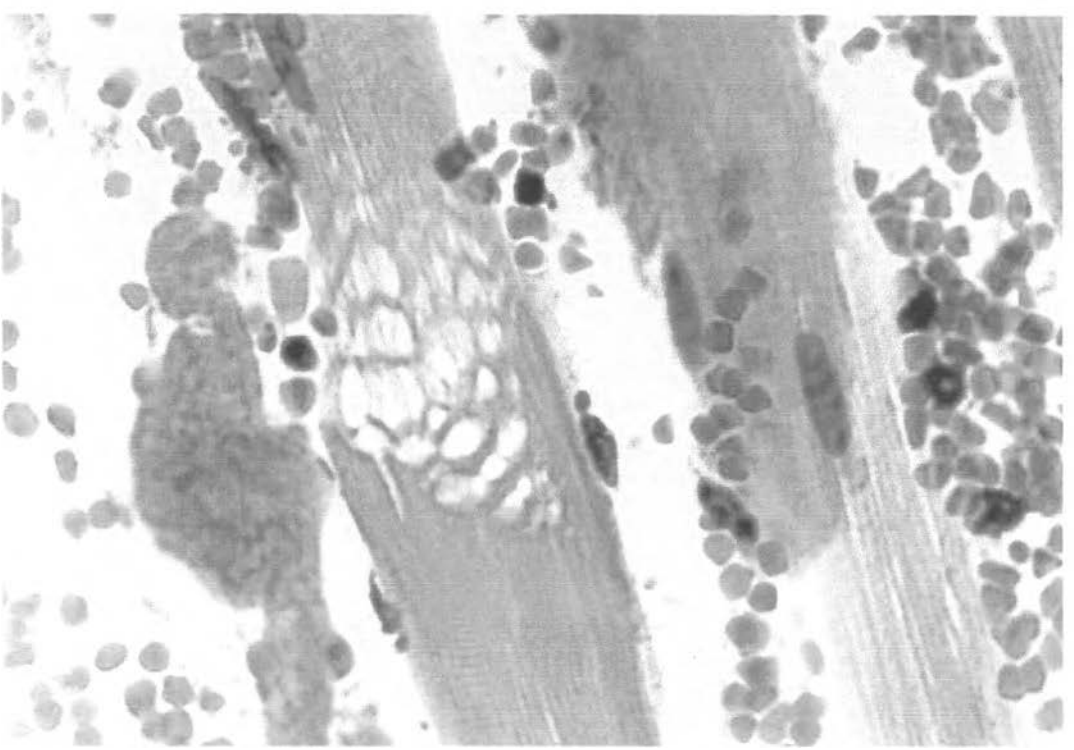
c) Thigh muscle injected with a mixture of 25 μg/mouse CR venom and 93.05 μg EDTA. The area of hemorrhage decreases inside the interstitial space of muscle is decrease.

d) Thigh muscle injected with a mixture of 25 μg/mouse CR venom and 92.90 μg TEPA. No necrotic skeletal muscle is observed, resulting in the Z-line appearance. The damage site is slightly hemorrhage inside the interstitial space of muscle.

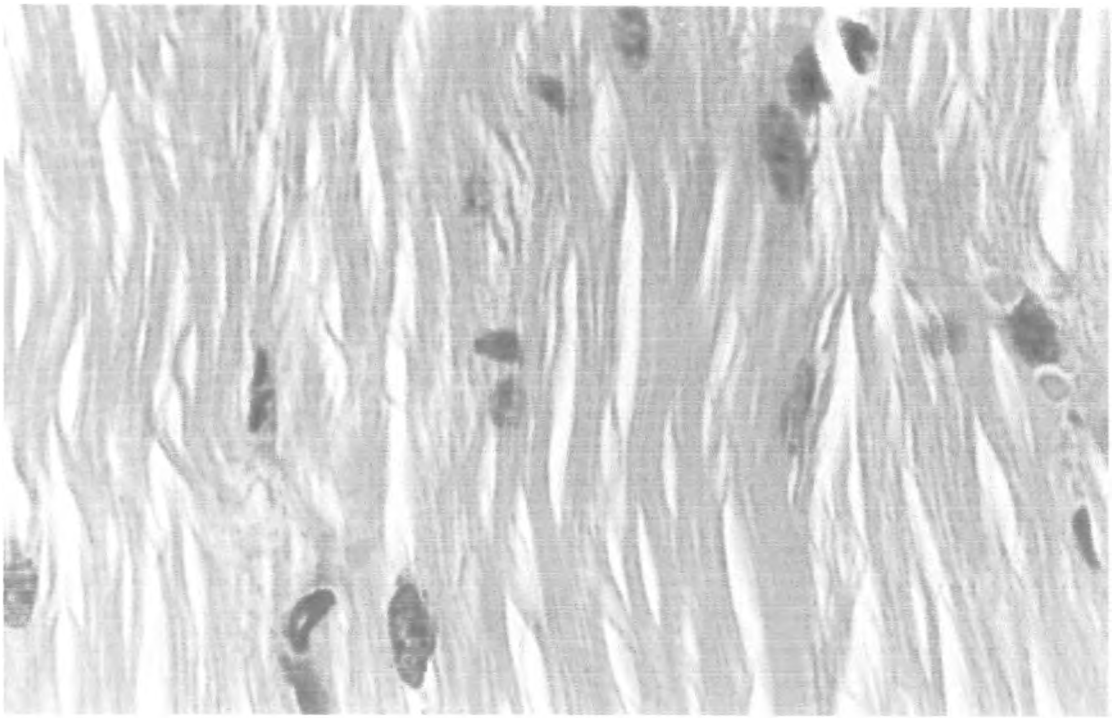
e) Thigh muscle injected with a mixture of 25 μg/mouse CR venom and 37.80 μg N-phenylglycine. The necrosis is not pronounced because the transverse striations of fibers are observed. The damage site is slightly hemorrhage inside the interstitial space of muscle.



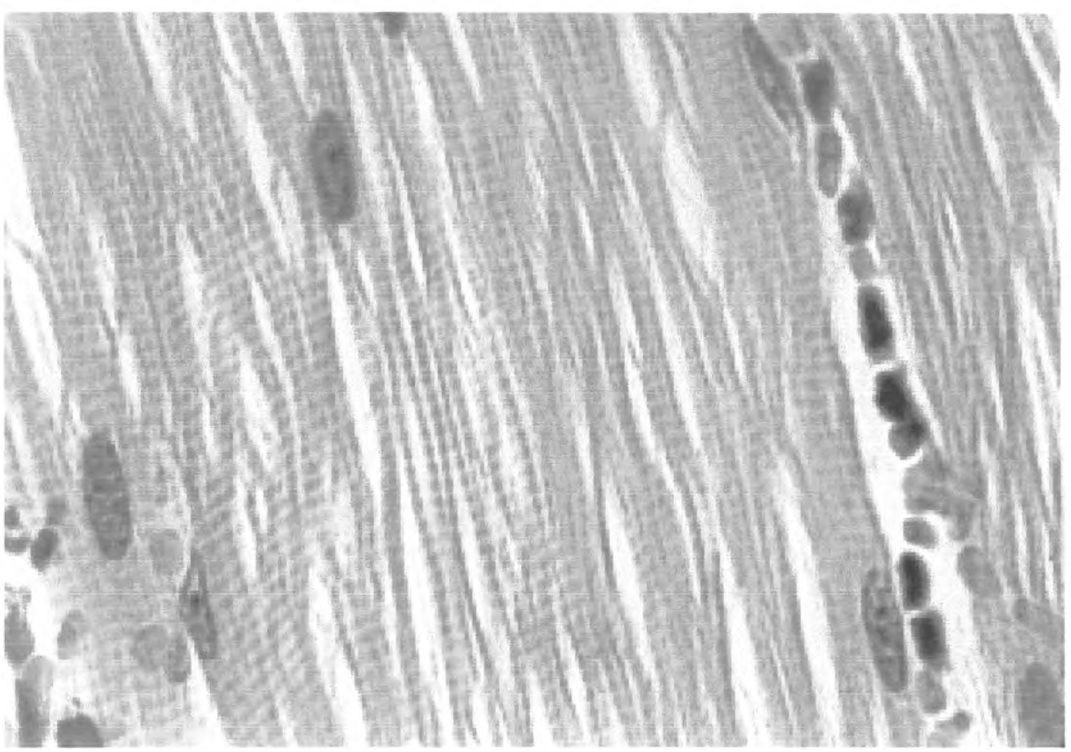
(a) Normal muscle



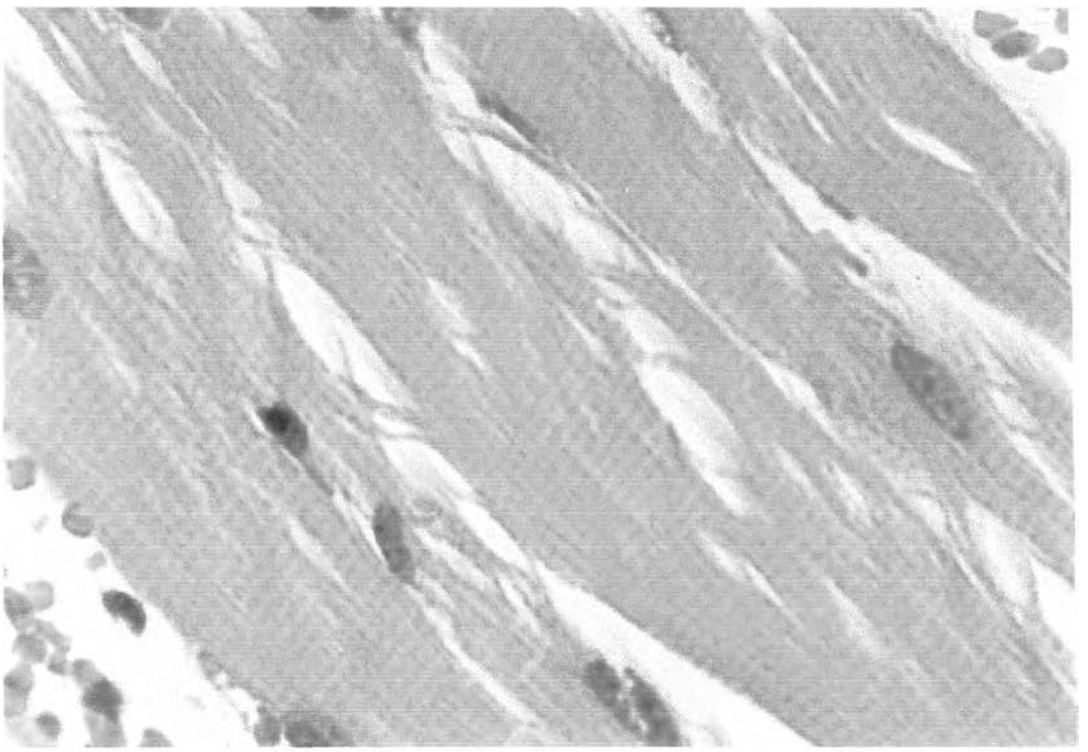
(b) 25 μg/mouse CR venom



(c) 25 μg/mouse CR venom + 93.05 μg EDTA



(d) 25 μg/mouse CR venom + 92.80 μg TEPA



(e) 25 μg/mouse CR venom + 37.80 μg N-phenylglycine