CHAPTER 4
RESULTS

1. Preparation of anhydrobarakol hydrochloride

The extraction of anhydrobarakol hydrochloride gave rise to 0.2% yield from three kilograms of fresh young leaves and flowers of *Cassia siamea*, Lamk. The physical and spectroscopic characteristics of anhydrobarakol hydrochloride were evaluated; UV λ_{max} (EtOH) nm (log ε): 241(4.8) and 398(4.54) (Figure 10); IR ν_{max} (KBr) 3445, 1670, and 1271 cm^{-1} (Figure 11); ^{1}H-NMR (D_2O): δ 6.94 (1H), 6.90 (1H), 6.78 (1H), 6.71 (1H) and 2.61, 2.38 (6H, 2s, 2Me) (Figure 12). Mass spectra; m/z (rel.): M+ 216 (3), 214 (100), 186 (158), 115 (11), 89 (11), 63 (9) and 51 (9) (Figure 13). These data were similar to those previous works of Bycroft (1970) and Kaokaew (1993) (Table 1).

2. Effect of anhydrobarakol hydrochloride on rat locomotor activity

Anhydrobarakol hydrochloride suppressed significantly the locomotion activity of rat after an intraperitoneal injection. The animals demonstrated a sedative condition with seldom movement. The effect was clearly observed at approximately 5 minutes after the injection and lasted more than 60 minutes.

At dose of 10 mg/kg body weight, the locomotive activity was observed to decreased (total number of counts) at 5 minutes after injection and gradually down to maximum decrease at 30 minutes. During the 60 minutes period after the injection, the mean activity of each group was different. The controlled group was 1755.00 compared to the treatment group (1067.25). The percentage of decrease was 39.19% (Table 2).

At dose of 20 mg/kg body weight, the locomotive activity was observed to decreased (total number of counts) at 5 minutes after injection and gradually down to maximum decrease at 30 minutes. During the 60 minutes period after the injection, the mean activity of each group was different. The controlled group was 1901.25 compared

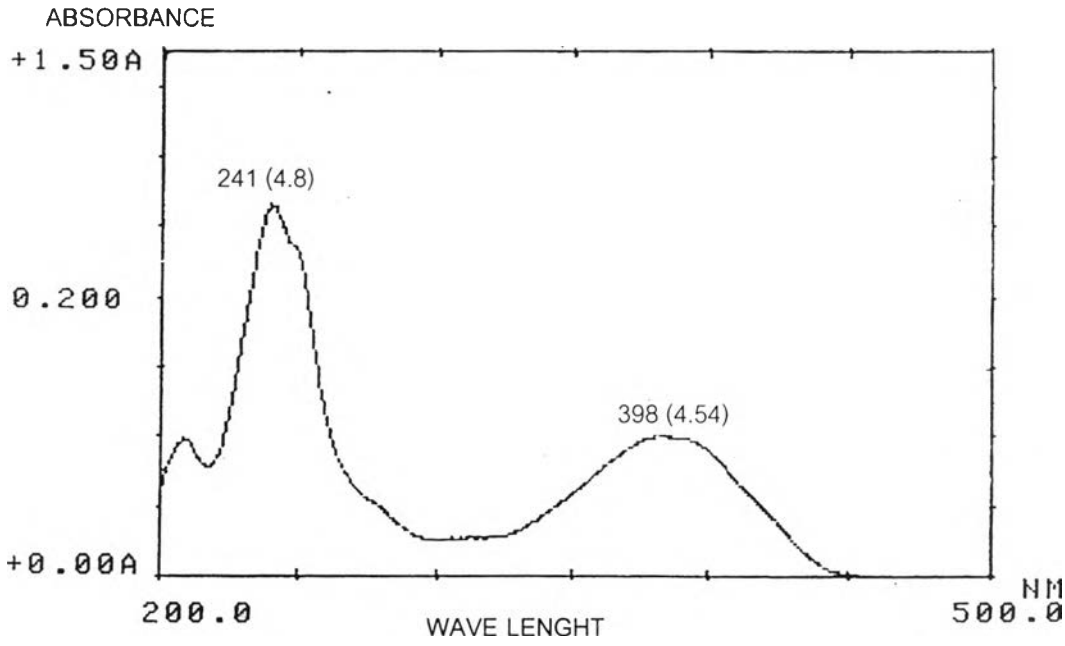


Figure 10 UV absorption spectrum of anhydrobarakol hydrochloride

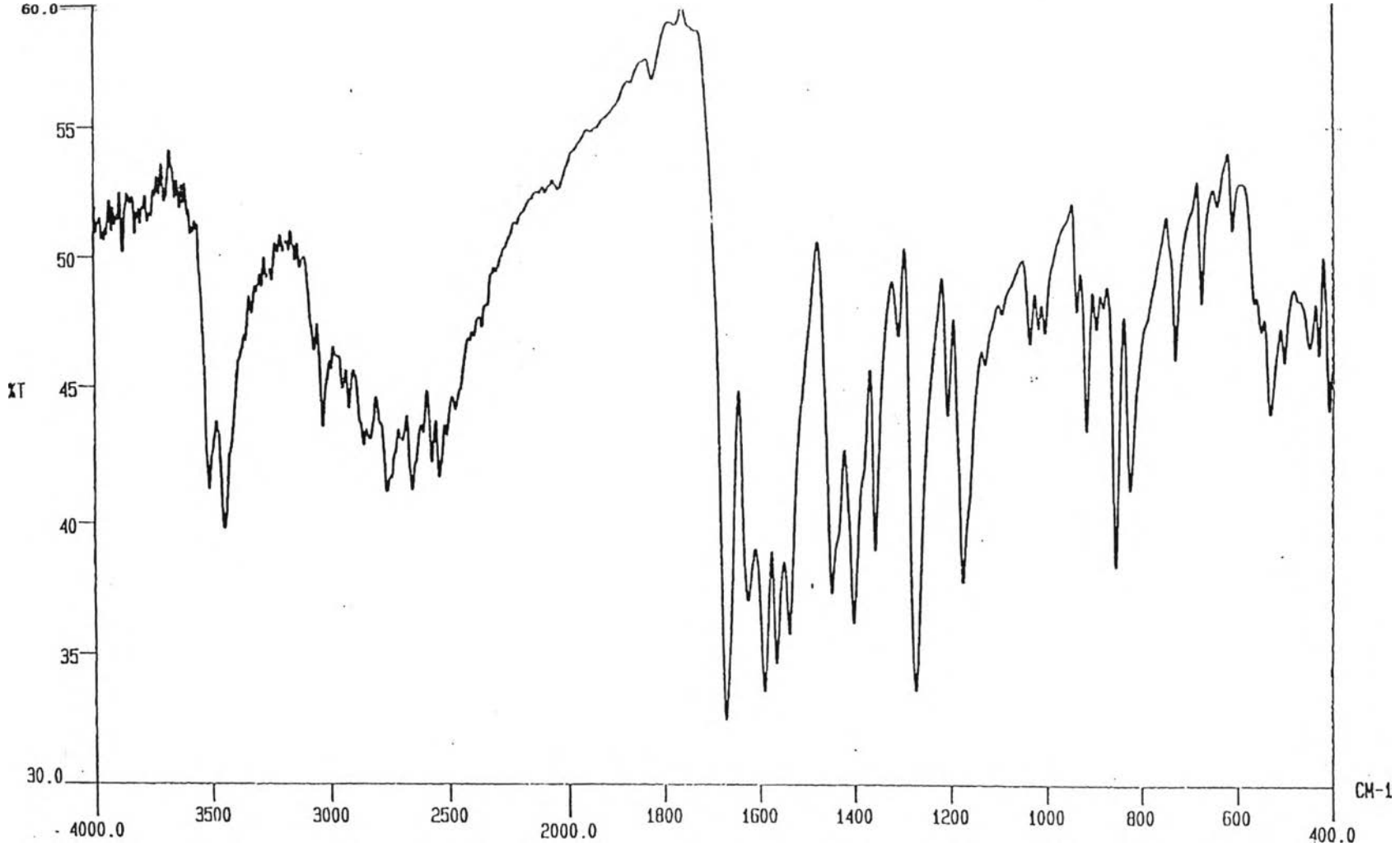


Figure 11 IR absorption spectrum of anhydrobarakol hydrochloride

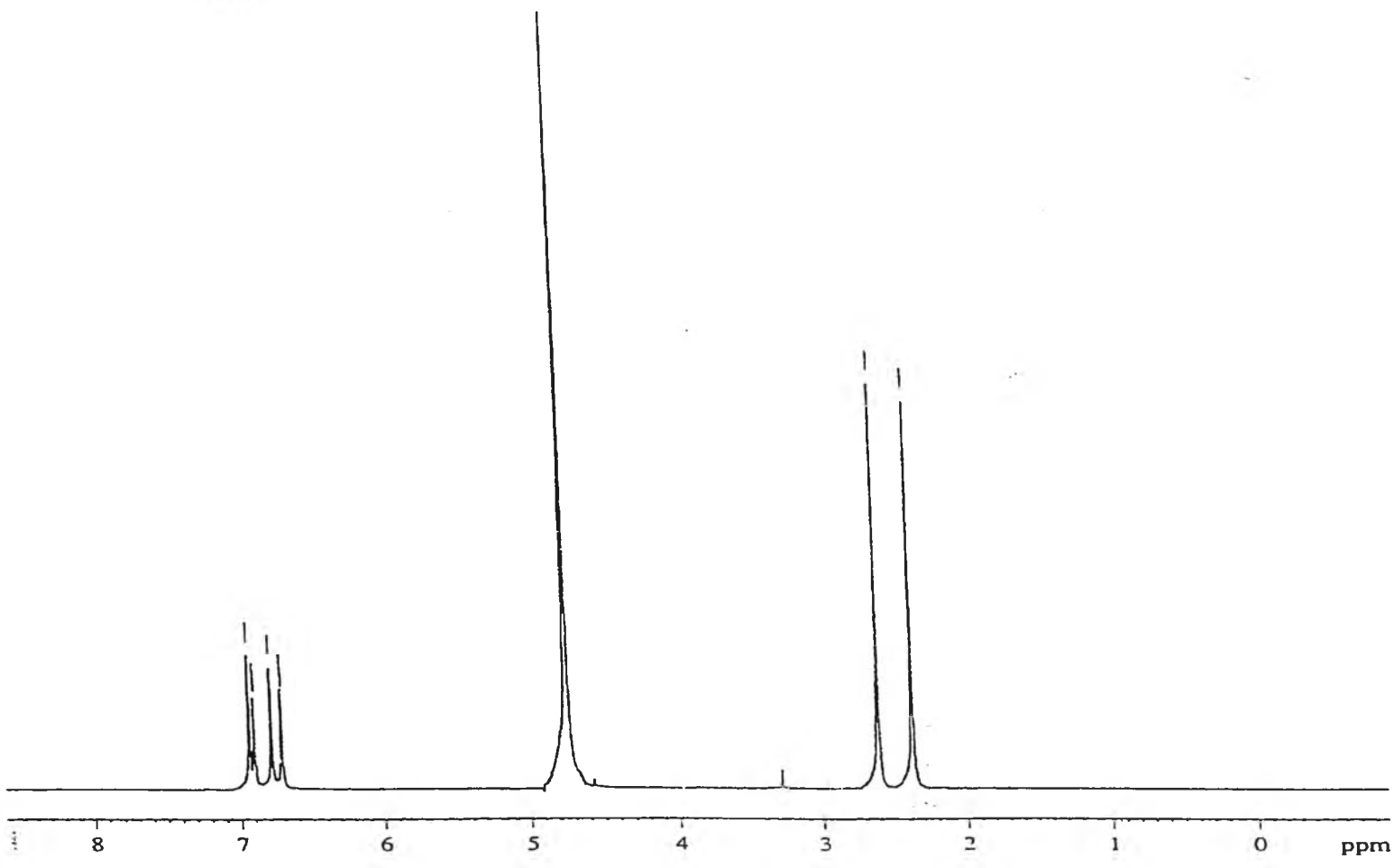


Figure 12 ^{1}H NMR spectrum of anhydrobarakol hydrochloride, in D_2O (at 200 MHz)

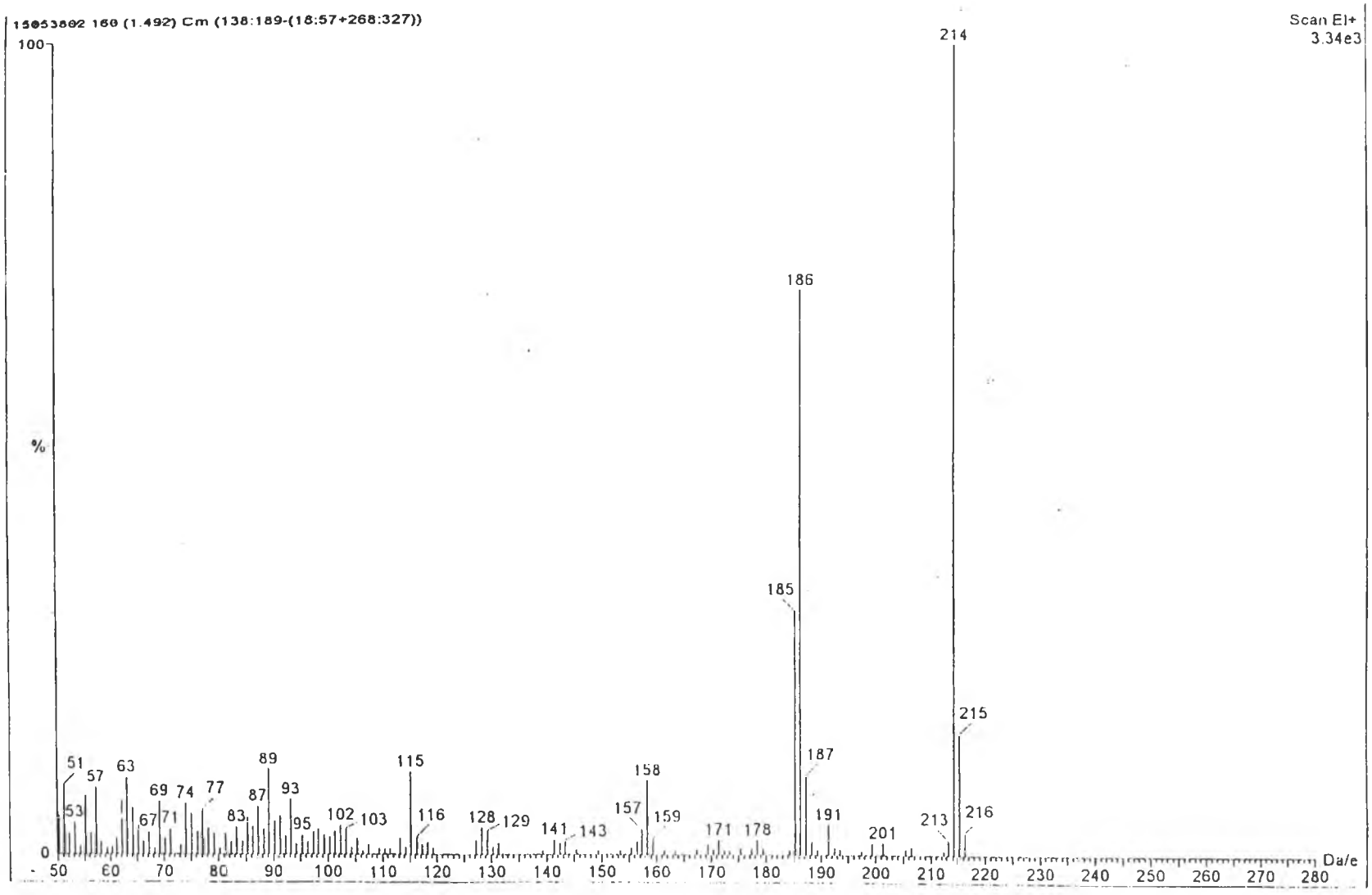


Figure 13 Mass spectra of anhydrobarakol hydrochloride

Table 1 Spectroscopic characteristics of anhydrobarakol hydrochloride

Type of spectrum	Anhydrobarakol hydrochloride (Bycroft, 1970)	Anhydrobarakol hydrochloride (Kaokeaw, 1993)	Anhydrobarakol hydrochloride (Present study)
UV λ_{max} nm (log ε):	In H_2O 241 and 472	in (EtOH) 240 (4.96), 384 (4.54)	in (EtOH) 241 (4.8), 398 (4.52)
IR ν_{max} (cm^{-1})	in Nujor 3500, 3440, 1660, 1620	in KBr 3500, 2740, 1600, 1442	in KBr 3445, 1670, 1589, 1271
NMR δ value (ppm)	in D_2O at 100 MHz 6.90 (4*1H), 2.48, 2.70 (6H, 2*s, 2*Me)	in D_2O at 200 MHz 6.8 (2*1H), 6.60 (1H), 6.40 (1H), 2.47, 2.33 (6H, 2*s, 2*Me)	in D_2O at 200 MHz 6.94 (1H), 6.90 (1H), 6.78 (1H), 6.71 (1H), 2.61, 2.38 (6H, 2s, 2Me)
Mass spectra (m/z)	M^+ 232 (2), 214 (100), 186 (78), 158 (23), 143 (6), 110 (17), 93 (10), 89 (10), 51 (13)	M^- 232 (>1), 214 (98), 186 (69), 158 (26), 143 (8), 115 (23), 93 (20), 89 (16), 51 (19), 43 (100)	M^+ 216 (3), 214 (100), 186 (69), 158 (32), 115 (11), 89 (11), 63 (9), 51 (9)

to the treatment group (997.75). The percentage of decrease is 47.52% (Table 2).

At dose of 40 mg/kg body weight, the locomotive activity was observed to decreased (total number of counts) at 5 minutes after injection and gradually down to maximum decrease at 30 minutes. During the 60 minutes period after the injection, the mean activity of each group was different. The controlled group was 1637.66 compared to the treatment group (754.50). The percentage of decrease is 53.92% (Table 2).

At dose of 60 mg/kg body weight, the locomotive activity was observed to decreased (total number of counts) at 5 minutes after injection and gradually down to maximum decrease at 30 minutes. During the 60 minutes period after the injection, the mean activity of each group was different. The controlled group was 1564.40 compared to the treatment group (624.40). The percentage of decrease is 60.08% (Table 2).

At dose of 80 mg/kg body weight, the locomotive activity was observed to decreased (total number of counts) at 5 minutes after injection and gradually down to maximum decrease at 30 minutes. During the 60 minutes period after the injection, the mean activity of each group was different. The controlled group was 1664.00 compared to the treatment group (573.16). The percentage of decrease is 65.55% (Table 2).

At dose of 100 mg/kg body weight, the locomotive activity was observed to decreased (total number of counts) at 5 minutes after injection and gradually down to maximum decrease at 30 minutes. During the 60 minutes period after the injection, the mean activity of each group was different. The controlled group was 1909.00 compared to the treatment group (546.16). The percentage of decrease is 71.39% (Table 2).

The dose-response curve clearly demonstrated that the effect of anhydrobarakol hydrochloride depend on the dosage (Figure 14). The effect gradually increased in relation to increase in the dosage.

Table 2 Effect of anhydrobarakol hydrochloride at various doses on rat locomotion activity.

Dose of Anhydrobarakol Hydrochloride (mg/kg body weight)	Normal Activity (Control group) (number of counts)	Test Activity (Treatment group) (number of counts)	% Decrease of Activity	Pair t-test (N=6, $P_{0.05}$)
10	1755.00±96.18	1067.25±70.04	39.19	0.016*
20	1901.25±74.29	997.75±8.29	47.52	0.000*
40	1637.66±93.16	754.5±23.09	53.92	0.003*
60	1564.40±33.11	624.40±25.36	60.08	0.000*
80	1664.00±99.39	573.16±26.65	65.55	0.001*
100	1909.00±92.33	546.16±35.61	71.39	0.000*

* Significant different when compare control group with treatment group

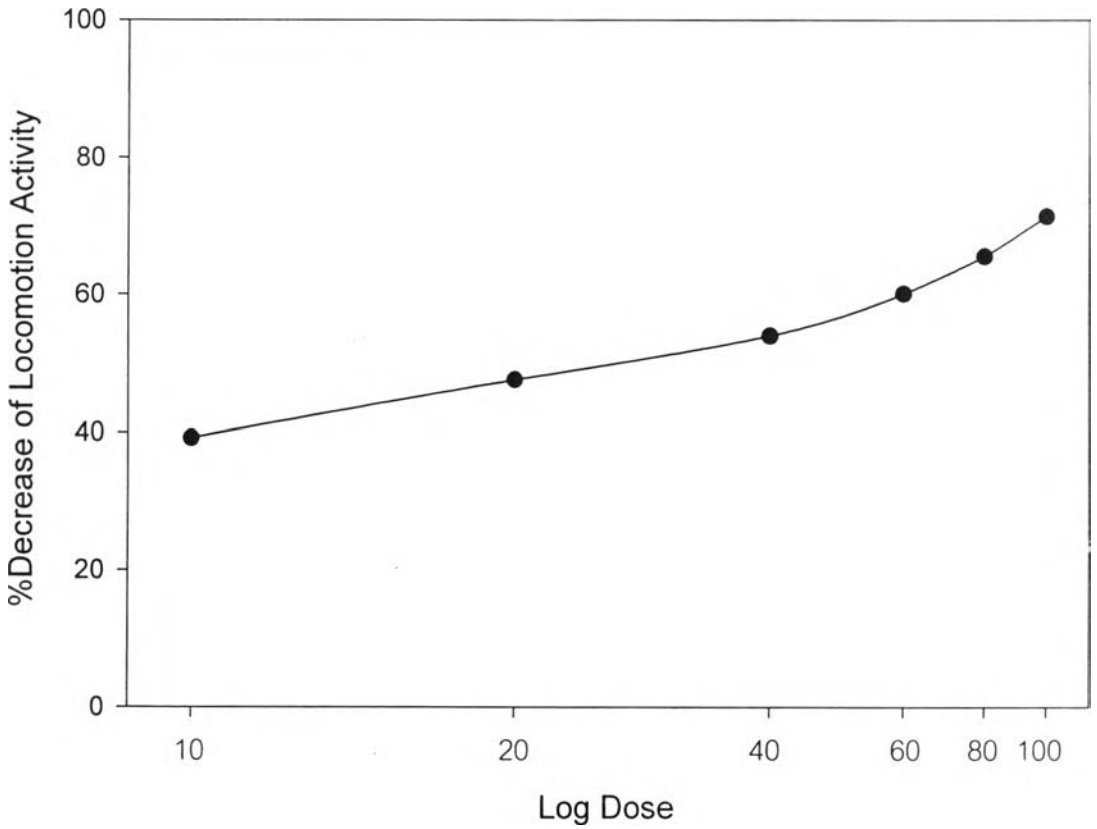


Figure 14 Dose response curve of percent decrease of locomotion activity after treatment with anhydrobarakol hydrochloride dose 10, 20, 40, 60, 80, 100 mg/kg body weight.

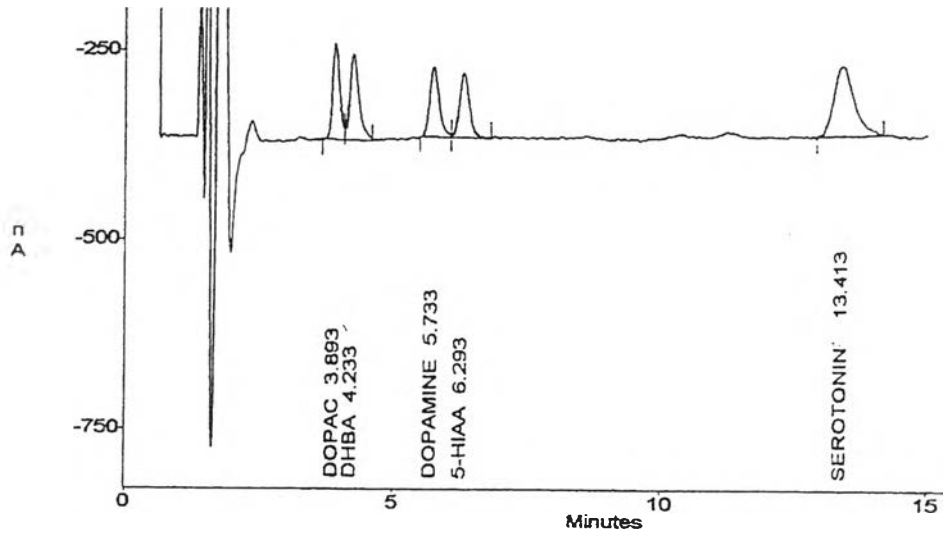
3. Determination of monoamines in the brain nuclei by high performance liquid chromatography with electrochemical detection

Representative chromatograms of the external standard and the internal standard in the supernatant of brain nuclei were shown in Figure 15. The effect of anhydrobarakol hydrochloride in any brain region on DA and 5-HT is described below.

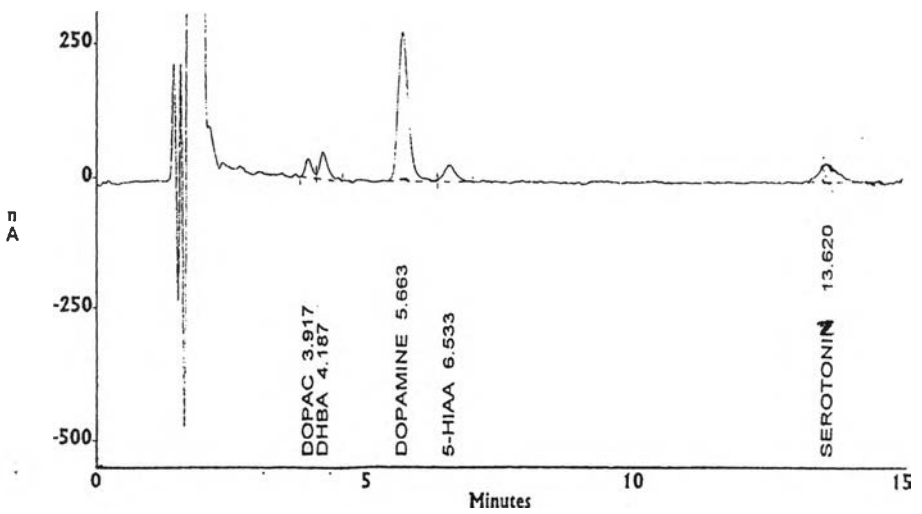
At dose of 10 mg/kg body weight of anhydrobarakol hydrochloride, the concentrations of dopamine were not changed in any brain regions of rat except at the head of caudate 2. Dopamine was low in concentration at this area when compared with control group (Table 3, Figure 16). On the other hand, concentration of other amines and their metabolites were not changed in any brain regions of rat when compared with control group (Tables 4, 5, 6, Figures 17, 18, 19).

At dose of 60 mg/kg body weight of anhydrobarakol hydrochloride, the concentration of dopamine were not changed in any brain regions of rat except at the head of caudate 2. Dopamine was low in concentration at this area when compared with control group (Table 3, Figure 16). On the other hand, concentration of other amines and their metabolites were not changed in any brain regions of rat when compared with control group (Tables 4, 5, 6, Figures 17, 18, 19).

Anhydrobarakol hydrochloride at the dose of 10 mg/kg did not produce significant change in dopamine concentration compared with the dose of 60 mg/kg in any brain regions of rat (Table 3, Figure 16). Concentrations of the other amines and their metabolites in rat brains receiving anhydrobarakol hydrochloride at the dose of 10 mg/kg were not significantly changed when compared with the dose 60 mg/kg in any brain regions of rat (Tables 4, 5, 6, Figures 17, 18, 19).



A



B

Figure 15 Chromatogram of external standard (A) and the supernatant from brain nuclei (Caudate tail 2) of rat (B).

Table 3 The effect of anhydrobarakol hydrochloride (B.HCl) at various doses on rat brain region dopamine.

Brain Area	Dopamine (pg/μg protein) Mean±SEM			ANOVA (N=10, $P_{0.05}$)
	Control group (Normal saline, i.p.)	Treatment group (B.HCl 10 mg/kg, i.p.)	Treatment group (B.HCl 60 mg/kg, i.p.)	
1. Caudate head 1	241.61±13.59	220.72±6.81	217.69±10.62	0.215
2. Caudate head 2	225.27±7.22	195.46±4.30*	197.65±9.78*	0.017**
3. Caudate Middle 1	134.66±12.35	116.87±4.82	114.44±3.85	0.295
4. Caudate Middle 2	116.68±10.39	106.60±1.91	113.21±2.96	0.620
5. Caudate tail 1	82.42±4.83	77.66±2.82	82.47±8.11	0.765
6. Caudate tail 2	76.71±5.41	70.02±3.97	63.59±1.86	0.128
7. Substantia nigra 1	25.11±1.11	28.34±4.63	29.11±2.27	0.551
8. Substantia nigra 2	30.10±1.75	30.67±3.76	29.81±1.95	0.974
9. Substantia nigra 3	30.69±2.11	25.72±2.74	31.38±2.32	0.298

*Different when compare with control group

**Significant ($P<0.05$)

Table 4 The effect of anhydrobarakol hydrochloride (B.HCl) at various doses on rat brain region serotonin.

Brain Area	Serotonin (pg/μg protein) Mean±SEM			ANOVA ($N=10$, $P_{0.05}$)
	Control group (Normal saline, i.p.)	Treatment group (B.HCl 10 mg/kg, i.p.)	Treatment group (B.HCl 60 mg/kg, i.p.)	
1. Caudate head 1	3.86±0.42	3.33±0.13	3.23±0.14	0.170
2. Caudate head 2	3.28±0.40	3.43±0.59	2.90±0.47	0.147
3. Caudate Middle 1	7.08±0.49	6.75±0.60	7.83±1.27	0.644
4. Caudate Middle 2	9.25±0.79	7.14±0.73	7.55±1.66	0.293
5. Caudate tail 1	10.61±0.74	9.03±0.95	10.16±1.68	0.512
6. Caudate tail 2	9.95±0.73	9.38±0.76	10.32±0.57	0.692
7. Hippocampus 1	7.89±0.79	6.84±0.64	8.74±1.19	0.362
8. Hippocampus 2	6.43±0.72	5.95±0.84	6.48±0.81	0.877
9. Hippocampus 3	9.37±1.04	7.44±0.42	9.02±1.15	0.316
10. Substantia nigra 1	17.16±0.71	17.25±1.57	18.95±1.12	0.459
11. Substantia nigra 2	17.87±0.70	19.74±0.65	18.70±0.54	0.140
12. Substantia nigra 3	18.21±0.97	19.68±1.63	19.49±1.39	0.701
13. Dorsal raphe nuclei	25.82±1.94	27.05±2.54	22.19±1.77	0.333
14. Median raphe nuclei 1	22.57±1.21	24.77±2.65	21.43±1.75	0.496
15. Median raphe nuclei 2	19.76±1.82	20.35±0.89	20.44±1.47	0.936
16. Raphe nucleus 1	7.22±0.56	8.52±0.77	8.06±0.69	0.362
17. Raphe nucleus 2	11.32±0.62	12.10±1.00	11.69±0.66	0.779

Table 5 The effect of anhydrobarakol hydrochloride (B.HCl) at various doses on rat brain region DOPAC.

Brain Area	DOPAC (pg/μg protein) Mean±SEM			ANOVA ($N=10$, $P_{0.05}$)
	Control group (Normal saline, i.p.)	Treatment group (B.HCl 10 mg/kg, i.p.)	Treatment group (B.HCl 60 mg/kg, i.p.)	
1. Caudate head 1	16.64±1.37	14.13±1.37	18.68±2.24	0.207
2. Caudate head 2	14.18±1.24	13.80±0.62	16.72±0.79	0.121
3. Caudate Middle 1	9.63±1.09	7.86±0.44	8.26±1.13	0.406
4. Caudate Middle 2	6.83±0.81	5.67±0.30	7.29±0.80	0.306
5. Caudate tail 1	4.62±0.49	4.07±0.39	4.99±0.45	0.409
6. Caudate tail 2	4.25±0.42	3.99±0.31	4.74±0.23	0.367
7. Substantia nigra 1	5.56±0.28	6.46±0.36	6.00±0.63	0.125
8. Substantia nigra 2	5.81±0.37	6.35±0.27	5.63±0.30	0.332
9. Substantia nigra 3	6.72±0.64	8.00±0.40	6.86±1.09	0.442

Table 6 The effect of anhydrobarakol hydrochloride (B.HCl) at various doses on rat brain region 5-HIAA.

Brain Area	5-HIAA (pg/μg protein) Mean±SEM			ANOVA (N=10, $P_{0.05}$)
	Control group (Normal saline, i.p.)	Treatment group (B.HCl 10 mg/kg, i.p.)	Treatment group (B.HCl 60 mg/kg, i.p.)	
1. Caudate head 1	11.77±0.52	11.04±0.69	11.84±0.64	0.610
2. Caudate head 2	11.34±0.54	11.69±0.57	11.41±0.55	0.894
3. Caudate Middle 1	11.18±0.58	10.29±0.44	11.42±0.71	0.383
4. Caudate Middle 2	12.21±0.46	11.52±0.65	11.67±0.99	0.785
5. Caudate tail 1	12.89±0.45	12.67±0.33	12.05±0.60	0.445
6. Caudate tail 2	12.86±0.61	11.91±0.31	13.46±0.91	0.272
7. Hippocampus 1	13.57±0.65	13.68±0.78	13.42±0.67	0.966
8. Hippocampus 2	11.62±0.73	11.16±0.61	11.21±0.54	0.854
9. Hippocampus 3	12.19±0.52	10.89±0.59	11.73±0.57	0.280
10. Substantia nigra 1	20.37±0.61	20.89±0.84	21.04±0.44	0.757
11. Substantia nigra 2	25.97±1.15	26.20±1.30	25.45±0.72	0.883
12. Substantia nigra 3	25.57±0.59	24.22±0.73	25.79±0.94	0.318
13. Dorsal raphe nuclei	28.70±1.42	28.95±2.23	28.96±1.95	0.994
14. Median raphe nuclei 1	32 08±0.86	33.89±1.01	31.05±1.21	0.176
15. Median raphe nuclei 2	35.13±1.09	34.09±1.25	34.02±1.61	0.808
16. Raphe nucleus 1	21.03±0.75	21.07±1.19	21.57±1.09	0.920
17. Raphe nucleus 2	30.05±1.24	31.21±1.40	30.59±0.90	0.797

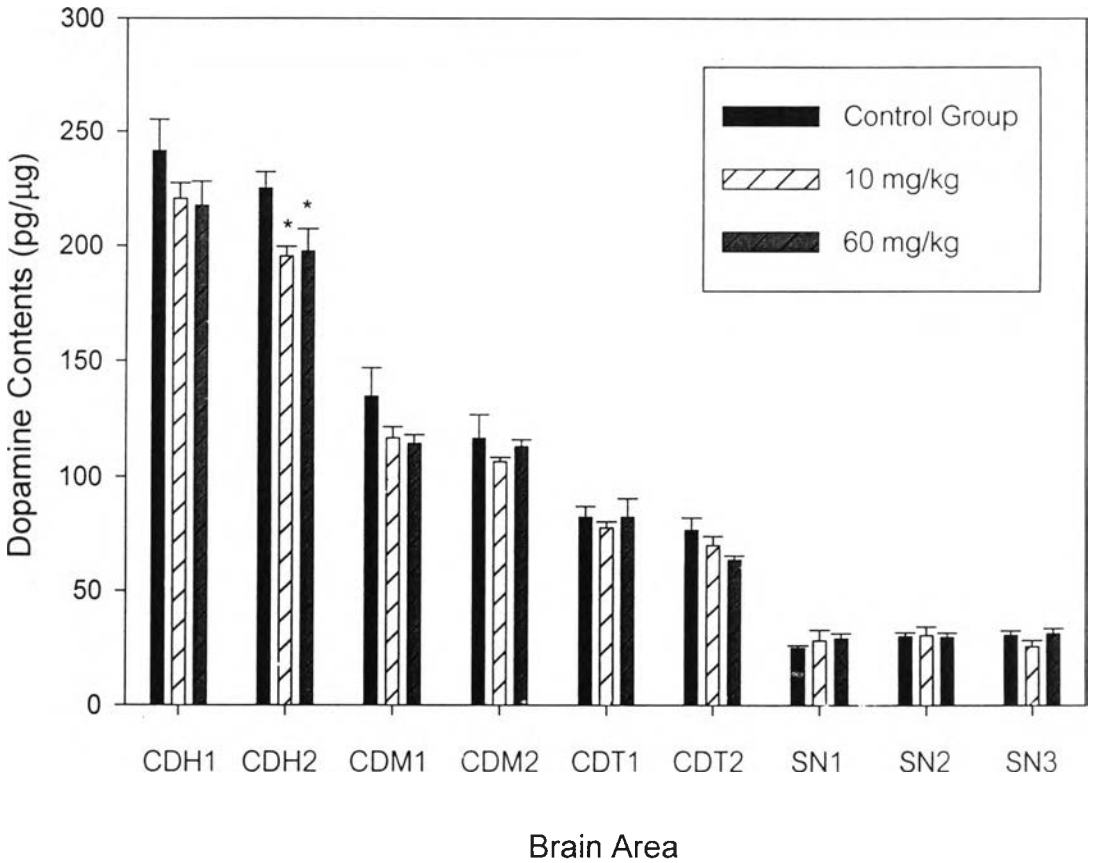


Figure 16 Effect of anhydrobarakol hydrochloride at 10-60 mg/kg bodyweight on rat brain region dopamine.

CDH1 (Caudate Head 1), CDH2 (Caudate Head 2), CDM1 (Caudate Middle 1), CDM2 (Caudate Middle 2), CDT1 (Caudate Tail 1), CDT2 (Caudate Tail 2), SN1 (Substantia nigra 1), SN2 (Substantia nigra 2), SN3 (Substantia nigra 3). Data are presented as mean±S.E.M., n=10. *P<0.05 compared with control group, using ANOVA with post hoc Duncan's multiple range test.

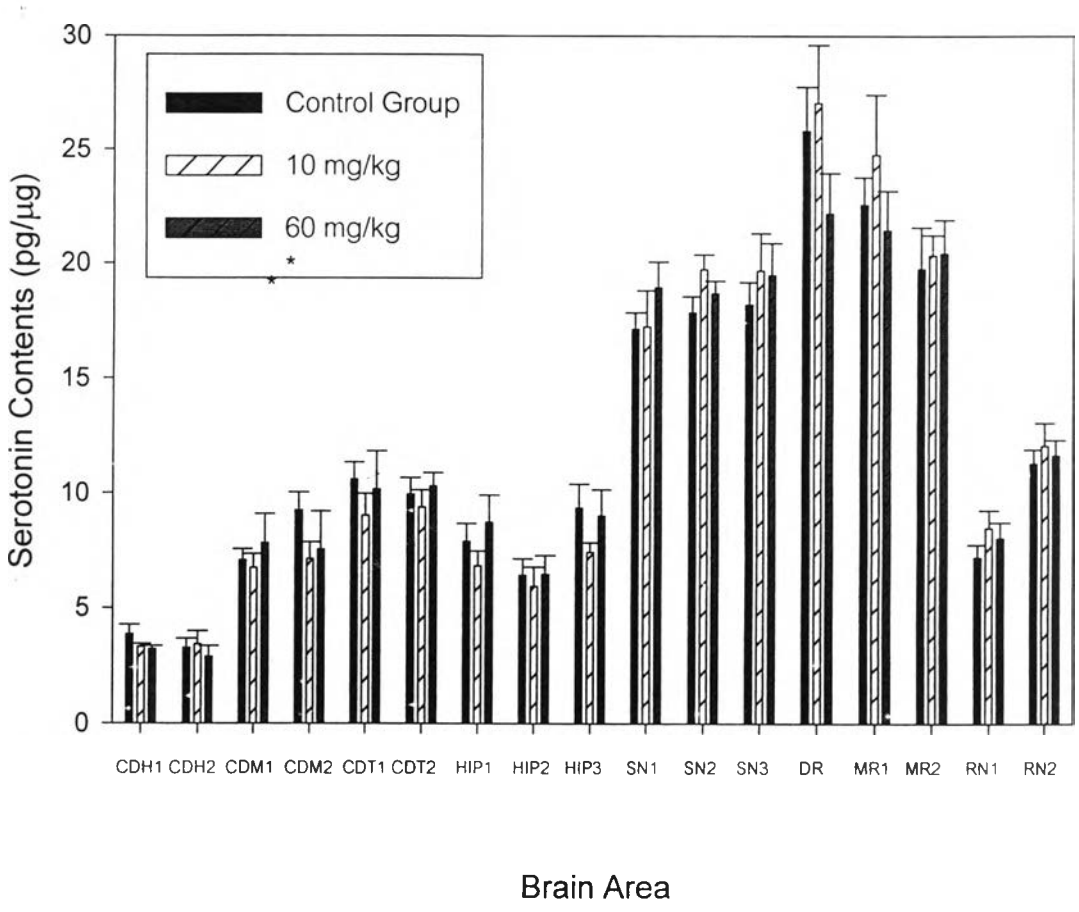


Figure 17 Effect of anhydrobarakol hydrochloride at 10-60 mg/kg bodyweight on rat brain region serotonin.

CDH1 (Caudate Head 1), CDH2 (Caudate Head 2), CDM1 (Caudate Middle 1), CDM2 (Caudate Middle 2), CDT1 (Caudate Tail 1), CDT2 (Caudate Tail 2), HIP1 (Hippocampus 1), HIP2 (Hippocampus 2), HIP3 (Hippocampus 3), SN1 (Substantia nigra 1), SN2 (Substantia nigra 2), SN3 (Substantia nigra 3), DR (Dorsal raphe nuclei), MR1 (Median raphe nuclei 1), MR2 (Median raphe nuclei 2), RN1 (Raphe nucleus 1), RN2 (Raphe nucleus 2). Data are presented as mean±S.E.M., n=10, using ANOVA with post hoc Duncan's multiple range test.

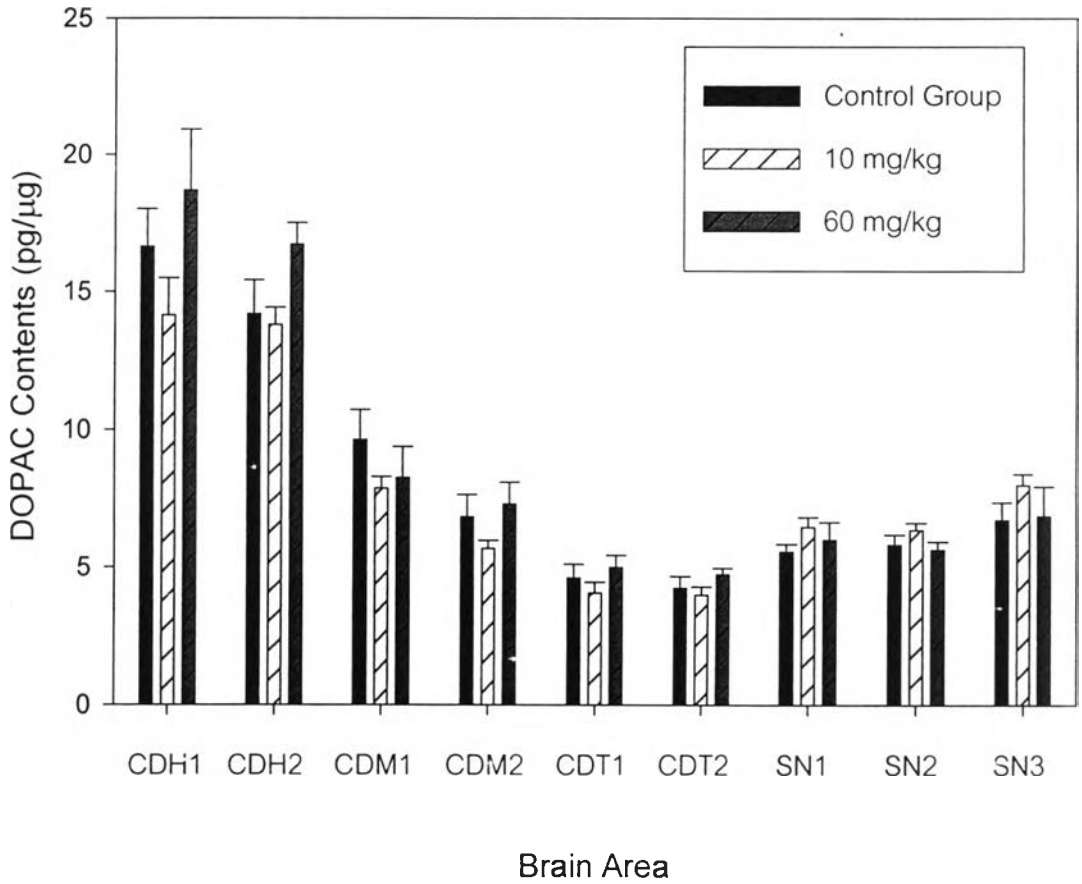


Figure 18 Effect of anhydrobarakol hydrochloride at 10-60 mg/kg bodyweight on rat brain region DOPAC.

CDH1 (Caudate Head 1), CDH2 (Caudate Head 2), CDM1 (Caudate Middle 1), CDM2 (Caudate Middle 2), CDT1 (Caudate Tail 1), CDT2 (Caudate Tail 2), SN1 (Substantia nigra 1), SN2 (Substantia nigra 2), SN3 (Substantia nigra 3). Data are presented as mean±S.E.M., n=10, using ANOVA with post hoc Duncan's multiple range test.

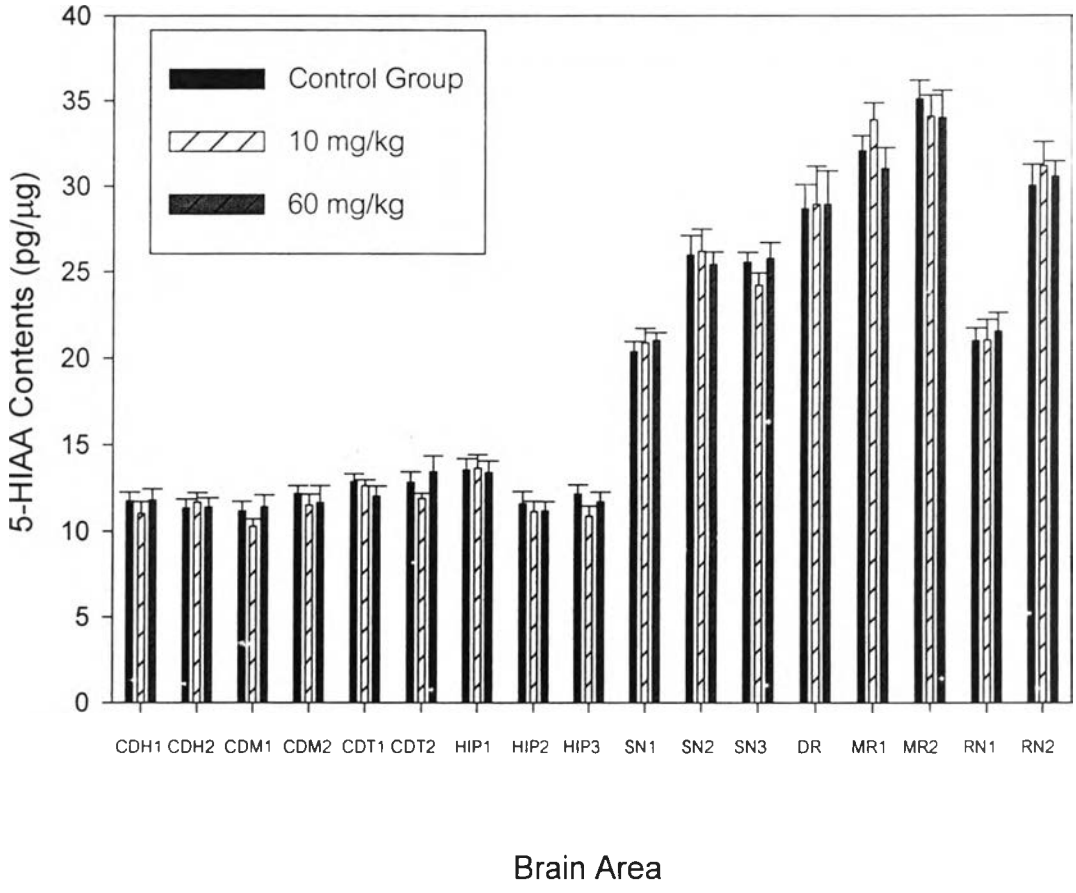


Figure 19 Effect of anhydrobarakol hydrochloride at 10-60 mg/kg bodyweight on rat brain region 5-HIAA.

CDH1 (Caudate Head 1), CDH2 (Caudate Head 2), CDM1 (Caudate Middle 1), CDM2 (Caudate Middle 2), CDT1 (Caudate Tail 1), CDT2 (Caudate Tail 2), HIP1 (Hippocampus 1), HIP2 (Hippocampus 2), HIP3 (Hippocampus 3), SN1 (Substantia nigra 1), SN2 (Substantia nigra 2), SN3 (Substantia nigra 3), DR (Dorsal raphe nuclei), MR1 (Median raphe nuclei 1), MR2 (Median raphe nuclei 2), RN1 (Raphe nucleus 1), RN2 (Raphe nucleus 2). Data are presented as mean$\pm$S.E.M., (n=10), using ANOVA with post hoc Duncan's multiple range test.