Chapter IV

Results

1. Anticonvulsant activity

None of the mice in control groups receiving either NSS or PEG400 (0.1 ml/25 g B.W. i.p. and 0.3 ml/g B.W. p.o.) was protected against any of the convulsive agents or stimuli used in the present study. In contrast, HMV given intraperitoneally or orally (Table 1) was effective in both MES and PTZ models while convulsion induced by strychnine or bicuculline was rather insensitive to HMV. Similar results but with comparatively lower efficacy were obtained for VPA (Table 1) in MES and PTZ models.

1.1 Anticonvulsant activity against MES

As illustrated in Figure 3, HMV i.p. demonstrated a dose related anticonvulsant activity against MES with the optimal pretreated time at 15 min giving the ED_{50} of 77 mg/kg B.W., the lowest value obtained from different pretreated times (Figure 3, 5). HMV was also orally active, exhibiting a higher ED_{50} of 168 mg/kg B.W. (Table 1) against MES.

Comparable results of VPA were demonstrated in Figure 4 and 5 and Table1. VPA displayed the optimal pretreated time at 30 min giving respective ED_{50} of 214 and 480 mg/kg B.W. when given intraperitoneally and orally.

1.2 Anticonvulsant activity against PTZ .

In line with the results in MES test, HMV which was given either intraperitoneally or orally, was effective against PTZ seizures. The ED_{50} was 35 and 51 mg/kg B.W. for intraperitoneally and oral route of administration respectively (Table 1).

Similar results were demonstrated by VPA. However a higher ED_{50} were noted, being 108 and 195 mg/kg B.W. (Table 1) for intraperitoneally and orally given VPA respectively.

1.3 Anticonvulsant activity against Strychnine or Bicuculline test.

HMV was found to be rather ineffective against seizures in these two models. When given intraperitoneally, none of HMV doses up to 400 mg/kg B.W. showed anticonvulsant activity against seizures induced by either strychnine or bicuculline in the test animals.

2. Toxicity

2.1 Acute toxicity test

The most frequent adverse signs observed in mice receiving high doses (400, 800, 1400 mg/kg B.W.) of HMV were sedation, ataxia, loss of righting reflex, hypnosis, respiratory tract secretion and death which occurred mostly within 24 hours. Lethality was noted after 72 hours and the median lethal doses (LD_{50}) of HMV and VPA were 722 and 717 mg/kg B.W., respectively. (Figure 6, 8; Table 2)

Though LD_{50} of HMV and VPA were almost identical, the relative safety margin (LD_{50}/ED_{50}) of HMV illustrated in Table 2, is about 3 times higher than that of VPA (9.37 vs. 3.35 in MES and 20.63 vs. 6.64 in PTZ).

2.2 Rotorod test

All control mice receiving NSS and PEG400 (0.1 ml/25 g B.W. i.p.) were able to maintain their equilibrium for at least 1 min on the rotating rod in 3 successive trials. Neural impairment indicated by an inability of the animals to maintain their equilibrium was exhibited by an i.p. administration of various doses of HMV and VPA in a dose related manner (Figure 7). The TD_{50} was 89 and 274 mg/kg B.W. for HMV and VPA, respectively (Figure 7 and 8; Table 2). However the protection index ($PI = TD_{50}/ED_{50}$, Table 2) of HMV is very closed to or identical with those of VPA (1.16 vs. 1.28 in MES and 2.54 in PTZ).

2.3 Locomotor activity

In comparison to NSS, an i.p. administration of, PEG400 (0.1 ml/25 g B.W.), HMV (35, 75 mg/kg B.W.) and VPA (100, 200 mg/kg B.W.) significantly depress the locomotor activity of mice (Figure 9 and 10). However, no statistically significant difference was noted among the effect of PEG400, HMV(35, 75 mg/kg B.W.) and VPA (100, 200 mg/kg B.W.).

2.4 Barbiturate potentiation test

As illustrated in Figure 11, PEG400 (0.1 ml/25 g B.W. i.p.) tended to slightly prolong barbiturate sleeping time but no statistically significance was noted between the effects of NSS and PEG400. HMV significantly lengthened the sleeping

time only when higher dose (75 mg/kg B.W. i.p.) was given, however, at lower dose (35 mg/kg B.W. i.p.) HMV is devoid of this effect. In contrast, the barbiturate sleeping time was significantly prolonged by both low and high doses of VPA i.p., The effect of higher dose of VPA (200 mg/kg B.W. i.p.) is statistically different from that elicited by NSS, PEG400 and low dose of VPA (100 mg/kg B.W. i.p.). The effect of VPA (100 mg/kg B.W.) i.p. was significantly deviated only from that of NSS but not PEG400.

3. Effect on the rat cortical GABA

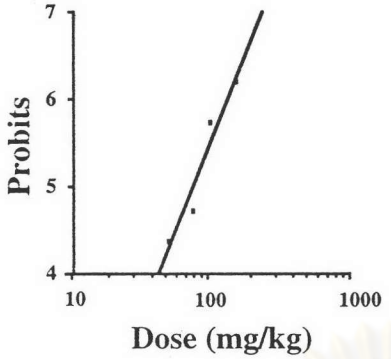
Alteration in GABA levels was expressed as a percent change from the basal value determined from three consecutive samples before the administration of the test substances.

Effects of HMV (75 and 150 mg/kg B.W. i.p.) and VPA (200 and 400 mg/kg B.W. i.p.) on cortical GABA were investigated in rats. Qualitative and quantitative analysis of GABA were accomplished by HPLC. Figure 12 and 13 illustrated the HPLC chromatograms of the standard amino acids and amino acids from rat cerebral dialysate.

In control groups, the spontaneous release of GABA was gradually decreased with time and appeared to be more prominent in NSS (0.1 ml/25 g B.W. i.p.) than that of PEG400 (0.1 ml/25 g B.W. i.p.) treated group (Figure 14 and 15). The changes of GABA level within the period of 180 min after the administration of test substances were shown in Figure 16. No statistical significance was observed between the effect of NSS and that of PEG400, though a slightly greater decrease in GABA was evident with the NSS group (Figure 14).

Being similar to the effect of NSS and PEG400, HMV75 mg/kg B.W. appeared not to have any major effect on GABA level. However, at higher dose of 150 mg/kg B.W., HMV exerted a significant increase in cortical GABA level (Figure 16).

With regards to NSS and PEG400, VPA 200 as well as 400 mg/kg B.W. i.p. significantly increased the amount of GABA. The effect of VPA 400 mg/kg B.W. seemed to be slightly stronger, but not of statistical significance from that elicited by VPA 200 mg/kg B.W. A clear difference was noted among the effect of VPA 400 mg/kg B.W. and HMV in the dose of 75 as well as 150 mg/kg B.W. (Figure 16).

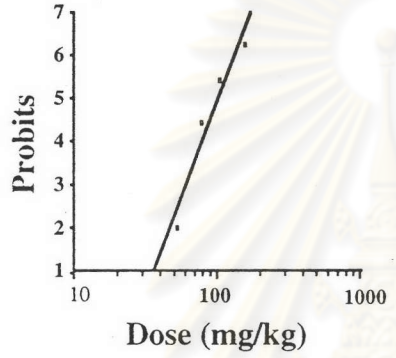


15 min.

Probits = - 2.7322 + 4.0998Log(dose)

$R^2 = 0.938$

ED_{50} = 77 (63-94) mg/kg

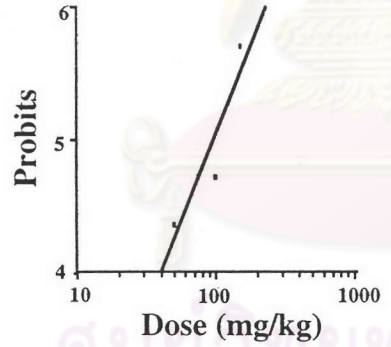


30 min.

Probits = - 12.753 + 8.8688Log(dose)

$R^2 = 0.939$

ED_{50} = 100 (91-110) mg/kg



60 min.

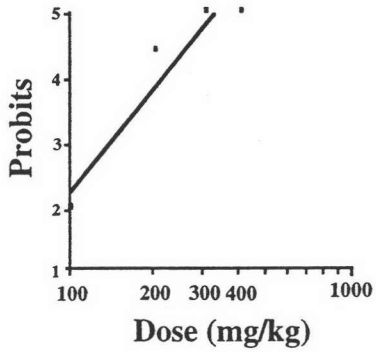
Probits = - 0.28589 + 2.6459Log(dose)

$R^2 = 0.84$

ED_{50} = 99 (73-134) mg/kg

Figure 3 Log dose response curves of HMV (i.p.) against MES at 15, 30 and 60 min pretreated times.

(Numbers in parentheses represent 95% confidence interval)

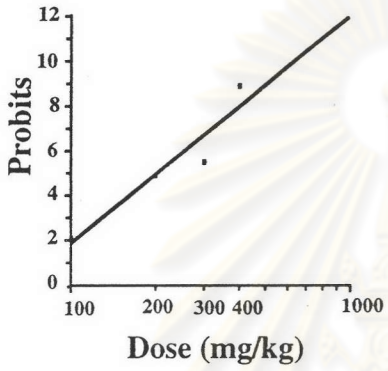


15 min.

Probits = - 8.5 094 +5.3594Log(dose)

$R^2 = 0.909$

ED_{50} = 332 (288-380) mg/kg

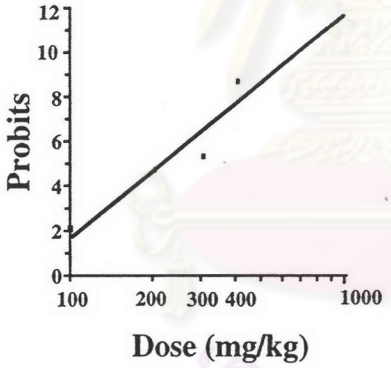


30 min.

Probits = - 18.675 +10.163Log(dose)

$R^2 = 0.899$

ED_{50} = 214 (198-231) mg/kg



60 min.

Probits = - 18.675 +10.163Log(dose)

$R^2 = 0.899$

ED_{50} = 214 (198-231) mg/kg

Figure 4 Log dose response curves of VPA (i.p.) against MES at 15, 30 and 60 min pretreated times.

(Numbers in parentheses represent 95% confidence interval)

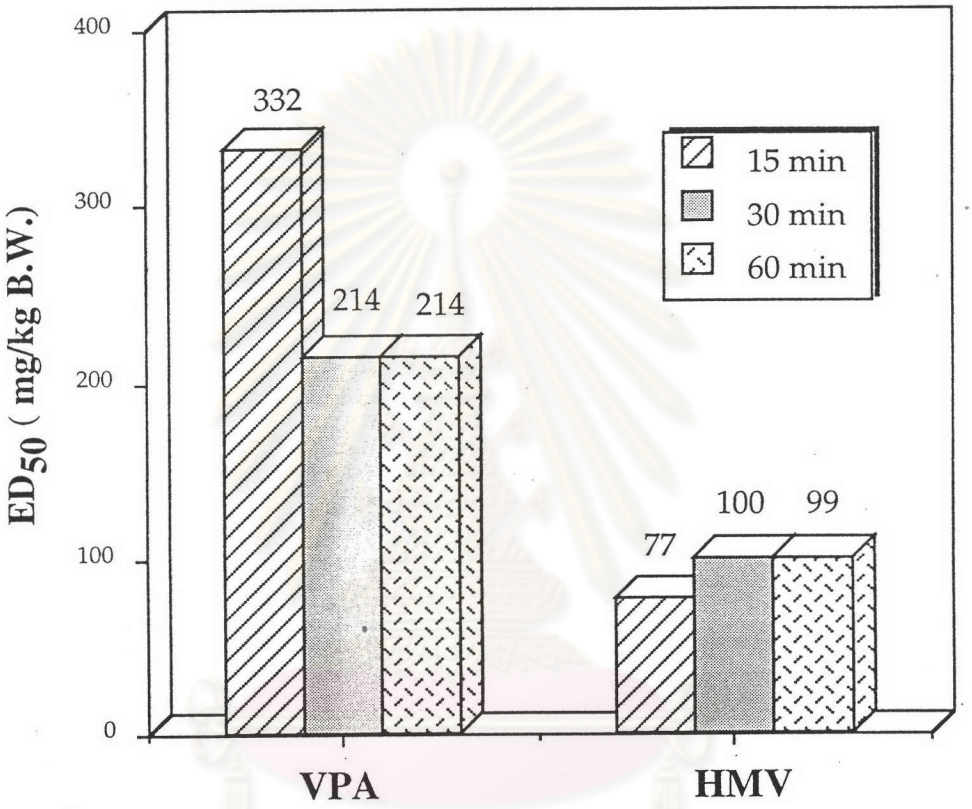


Figure 5 Effect of various pretreated times of intraperitoneally given HMV and VPA on anticonvulsant activity against MES in mice.

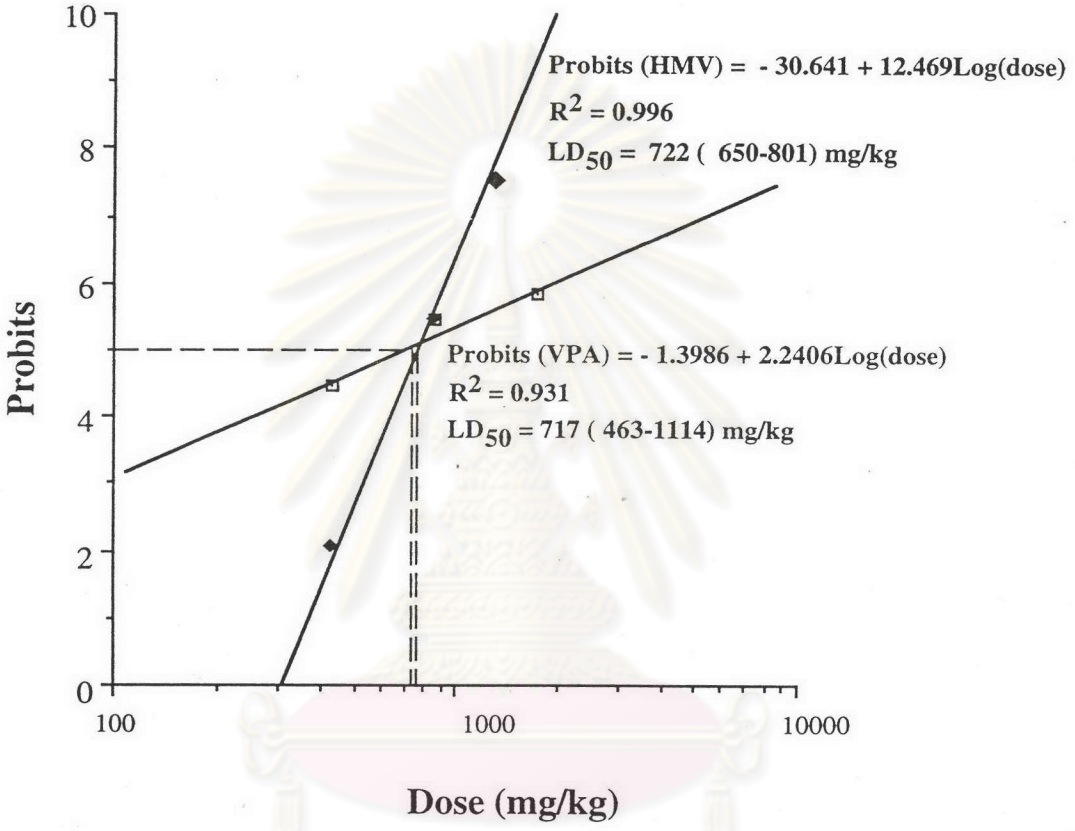


Figure 6 Log dose response curves of acute toxicity (lethality) of HMV and VPA (i.p.) in mice.

(Numbers in parentheses represent 95% confidence interval)

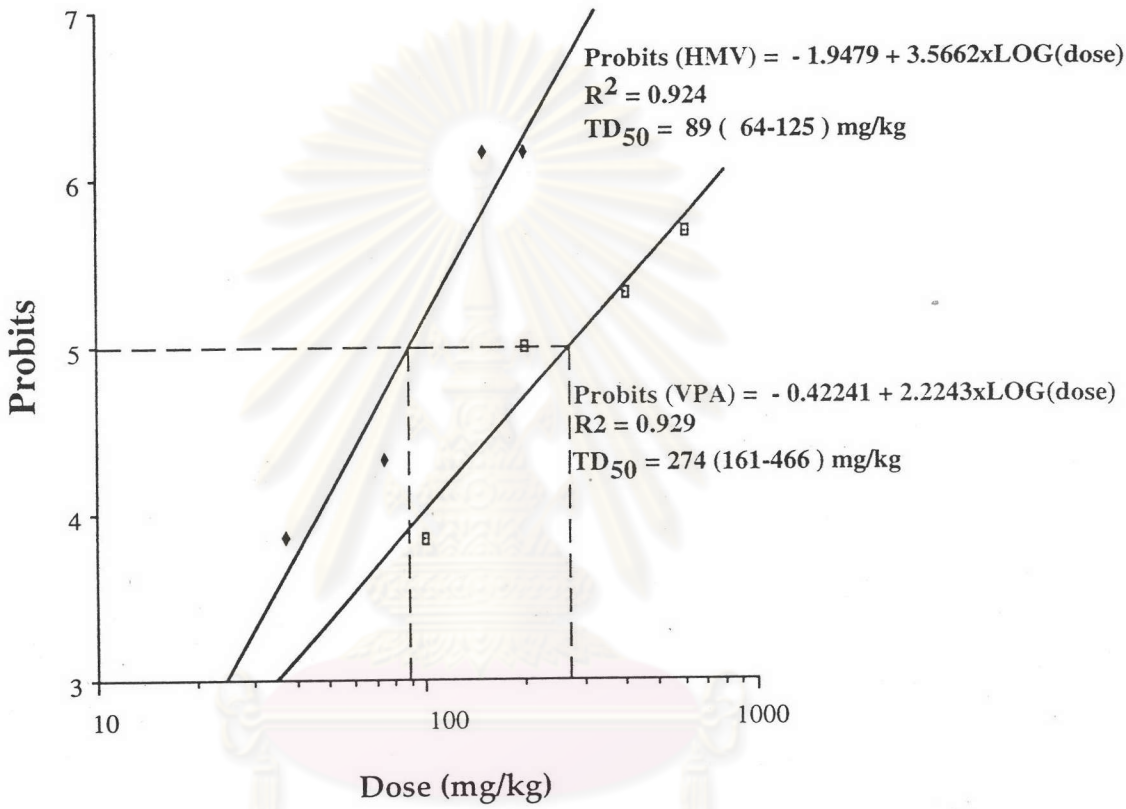


Figure 7 Log dose response curves of neurotoxicity exhibited by HMV and VPA (i.p.) in mice.

(Numbers in parentheses represent 95% confidence interval)

Table 1 Anticonvulsant activity of intraperitoneally or orally given HMV and VPA on different models of epilepsy in mice.

Animal models	Route	ED_{50} (mg/kg B.W.)	
		HMV	VPA
MES	i.p.	77 (91-110)	214 (198-231)
	p.o.	168 (77-364)	480 (367-630)
PTZ	i.p.	35 (23-65)	108 (83-140)
	p.o.	51 (44-59)	195 (141-271)

(Number in parentheses represent 95% confidence interval)

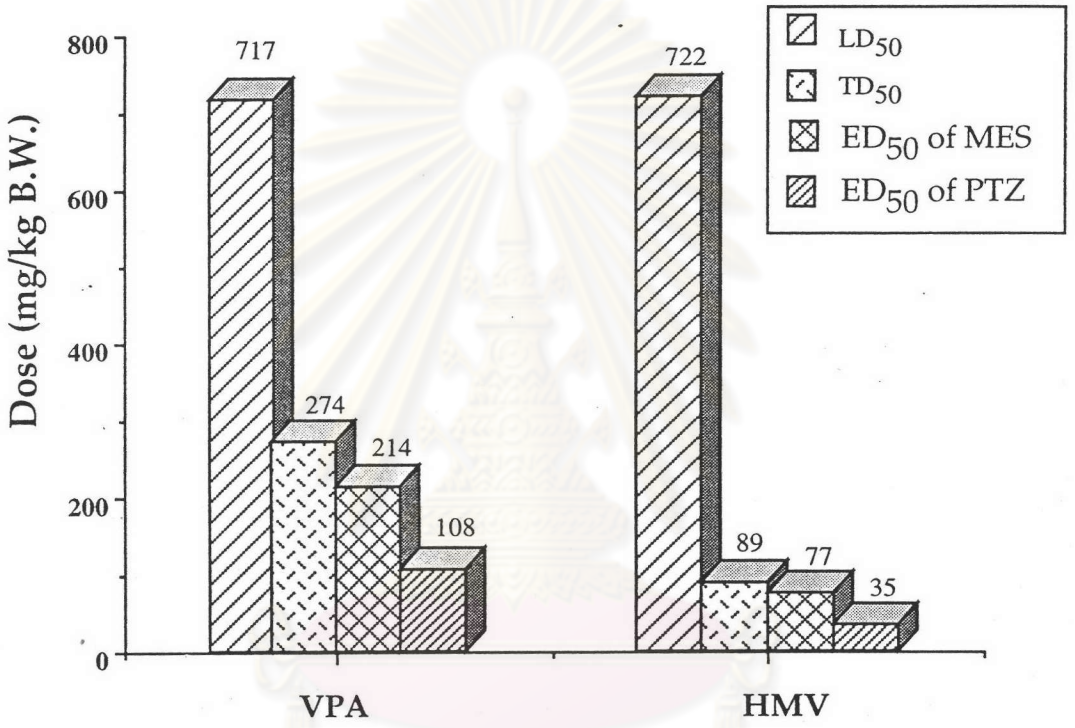
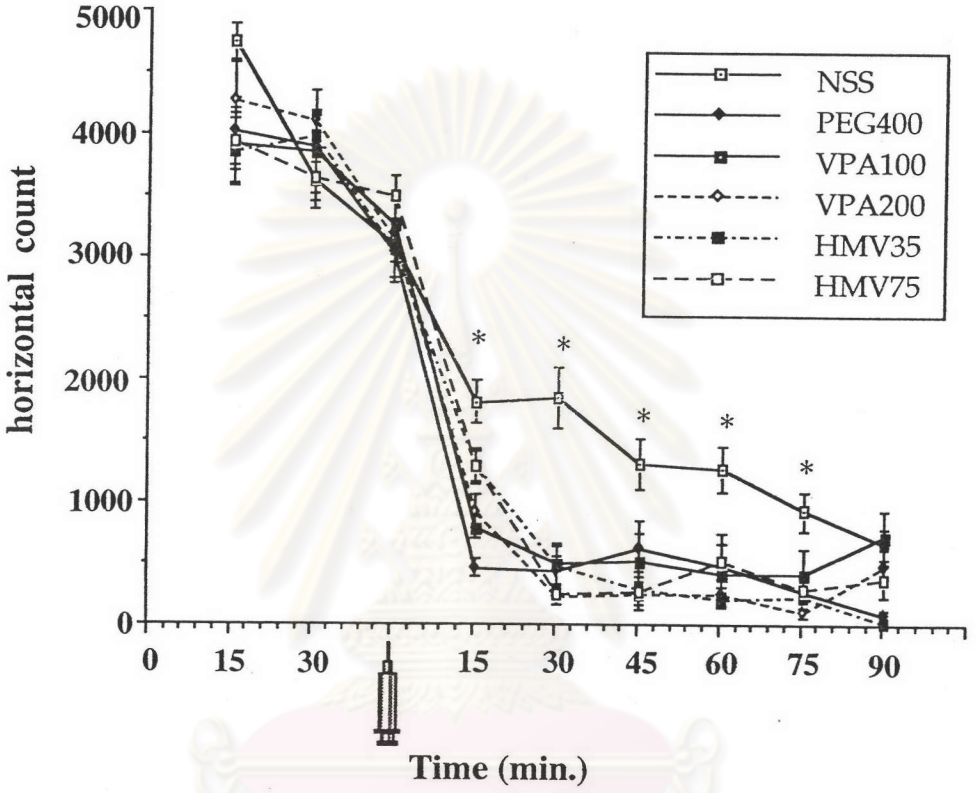


Figure 8 Illustration of the LD_{50}, TD_{50} and ED_{50} elicited by an intraperitoneal administration of HMV and VPA in MES and PTZ models

Table 2 ED_{50}, TD_{50}, LD_{50}, PI (TD_{50}/ED_{50}) and relative safety margin (LD_{50}/ED_{50}) of an intraperitoneal administration of HMV and VPA in MES and PTZ models.

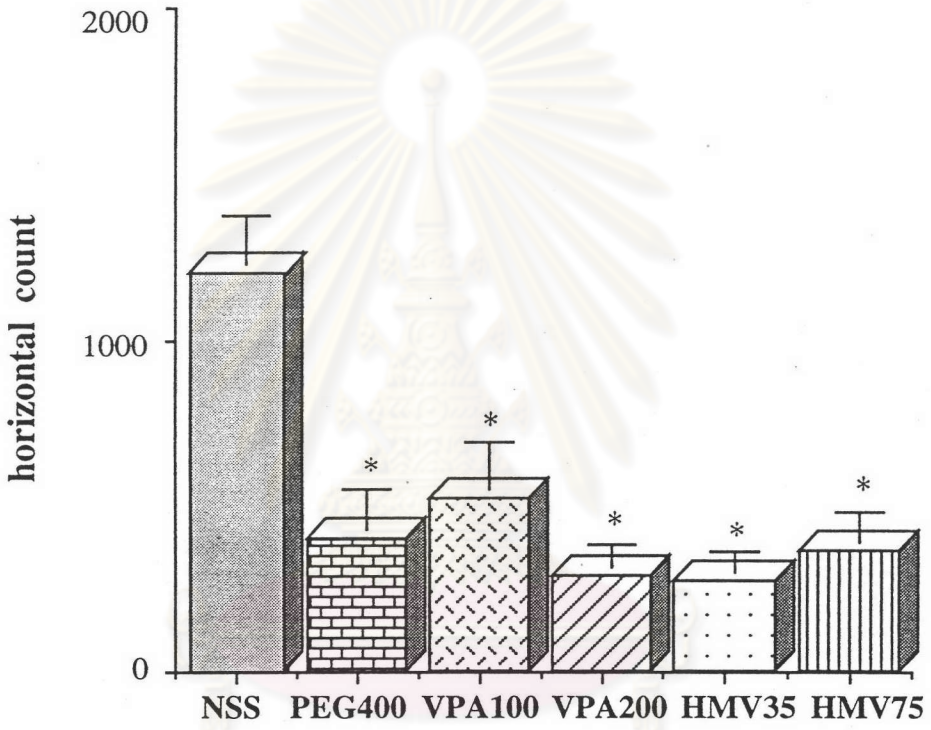
Parameter	Animal model	HMV	VPA
ED_{50} (mg/kg B.W.)	MES	77	214
	PTZ	35	108
TD_{50} (mg/kg B.W.)	Rotorod	89	274
PI	MES	1.16	1.28
	PTZ	2.54	2.54
LD_{50} (mg/kg B.W.)	-	722	717
Relative safety margin	MES	9.37	3.35
	PTZ	20.63	6.64



* $p < 0.01$ denotes statistially significant from PEG400 VPA100, VPA200, HMV35 and HMV75

n = 8

Figure 9 Effects of an intraperitoneal administration of VPA and HMV on horizontal counts (MEAN±SEM) of locomotor activity in mice at various times.



* $p < 0.01$ denotes statistically significant from NSS
n = 8

Figure 10. Effects of an intraperitoneal administration of VPA and HMV on total horizontal counts (MEAN±SEM) within 75 min after the injection of the test substances

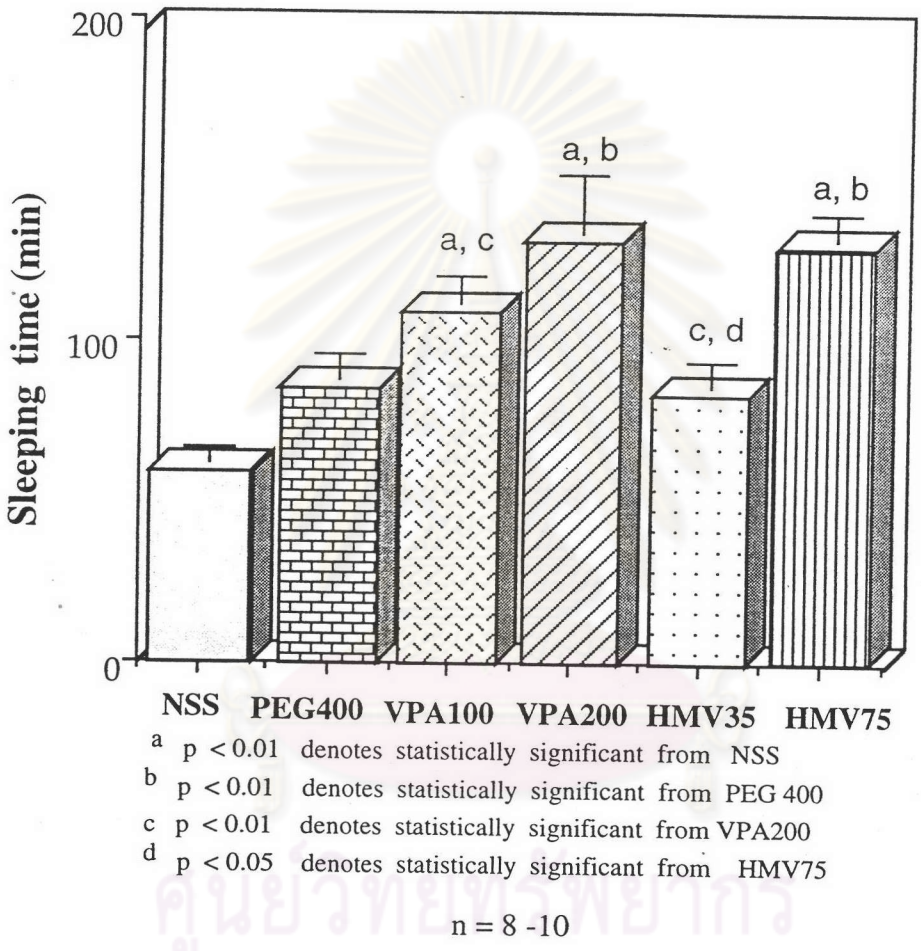


Figure 11. Effects of an intraperitoneal administration of VPA and HMV on barbiturate sleeping time (MEAN ± SEM) in mice.

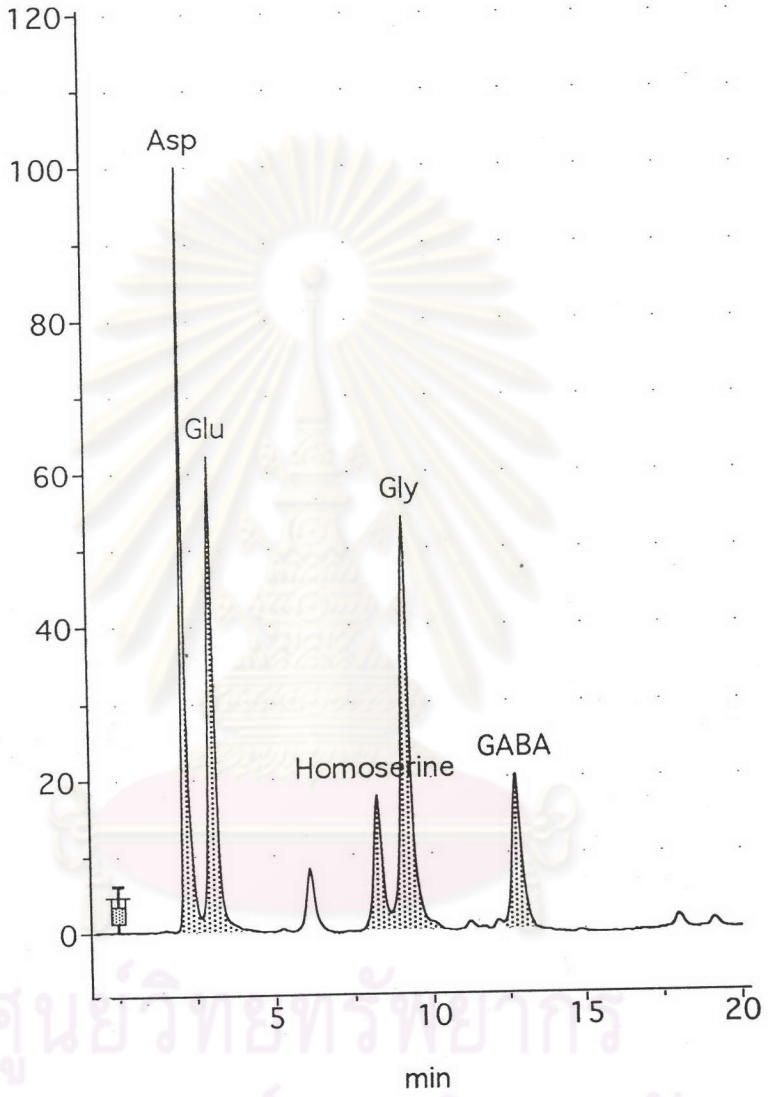


Figure 12. HPLC chromatogram of OPA-derivatized standard amino acids.

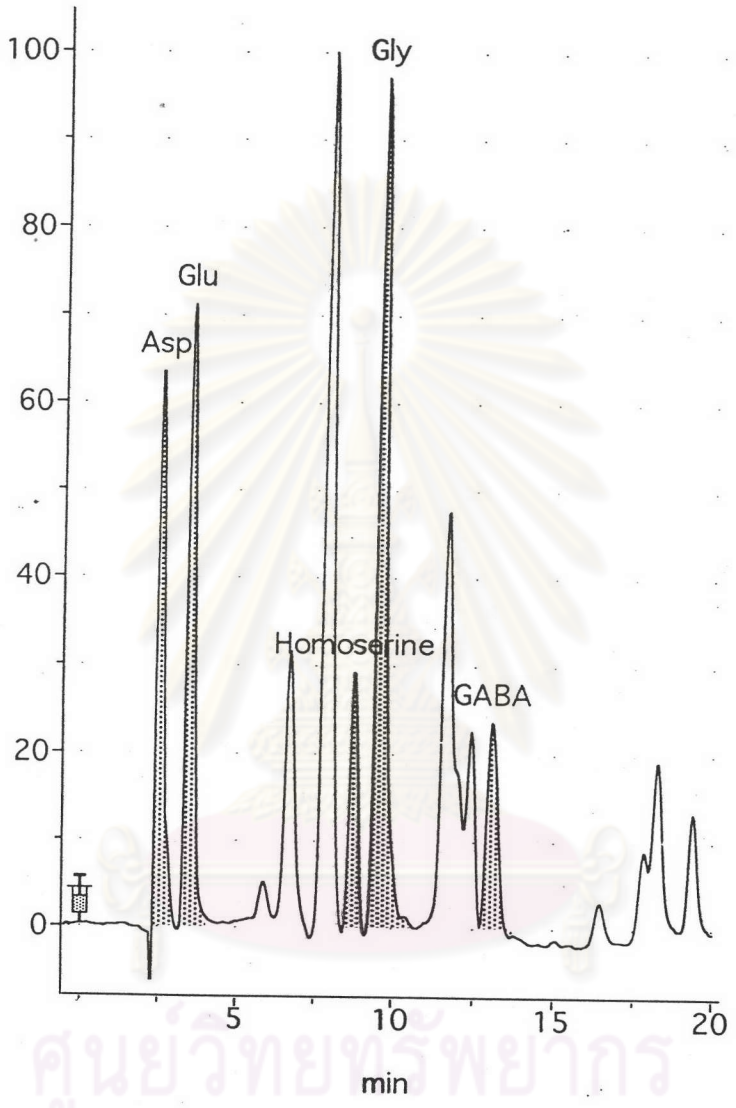
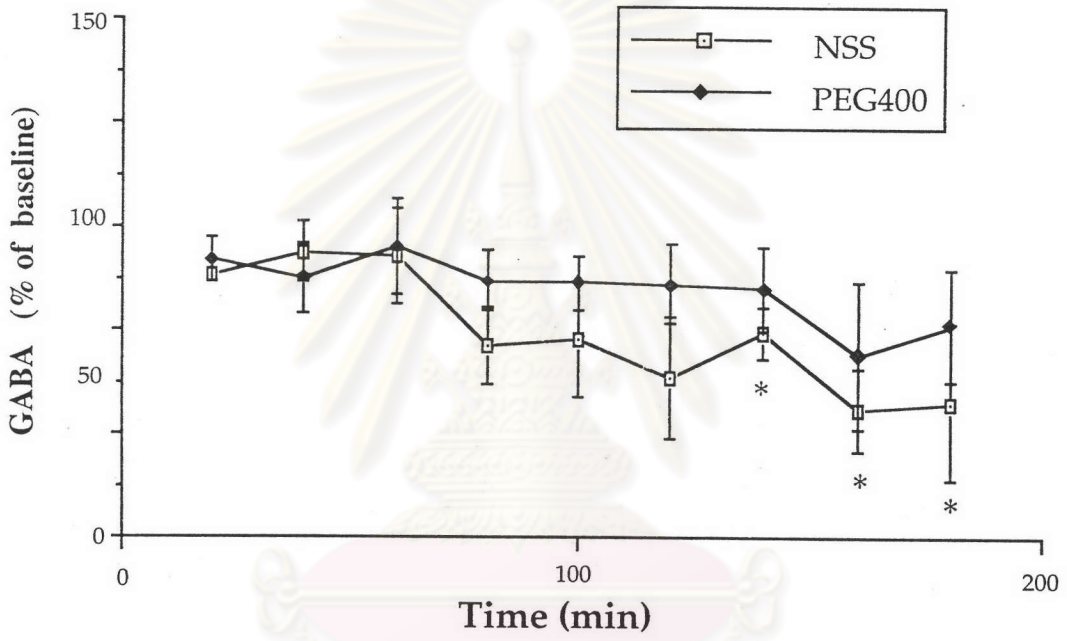


Figure 13. HPLC chromatogram of OPA-derivatized amino acids from the rat cerebral cortex.



* $p < 0.05$ denotes statistically significant from the first collection (Student paired t-test)

Figure 14 Changes in the rat cortical GABA levels at various times after an intraperitoneal administration of NSS and PEG400.

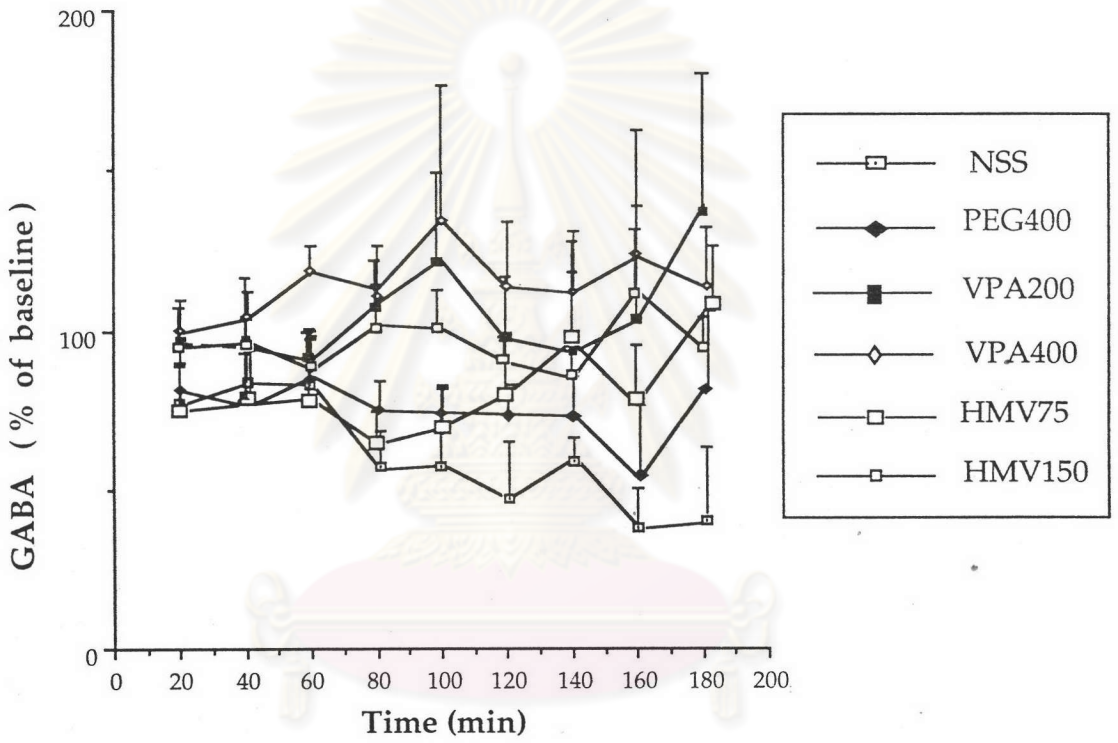
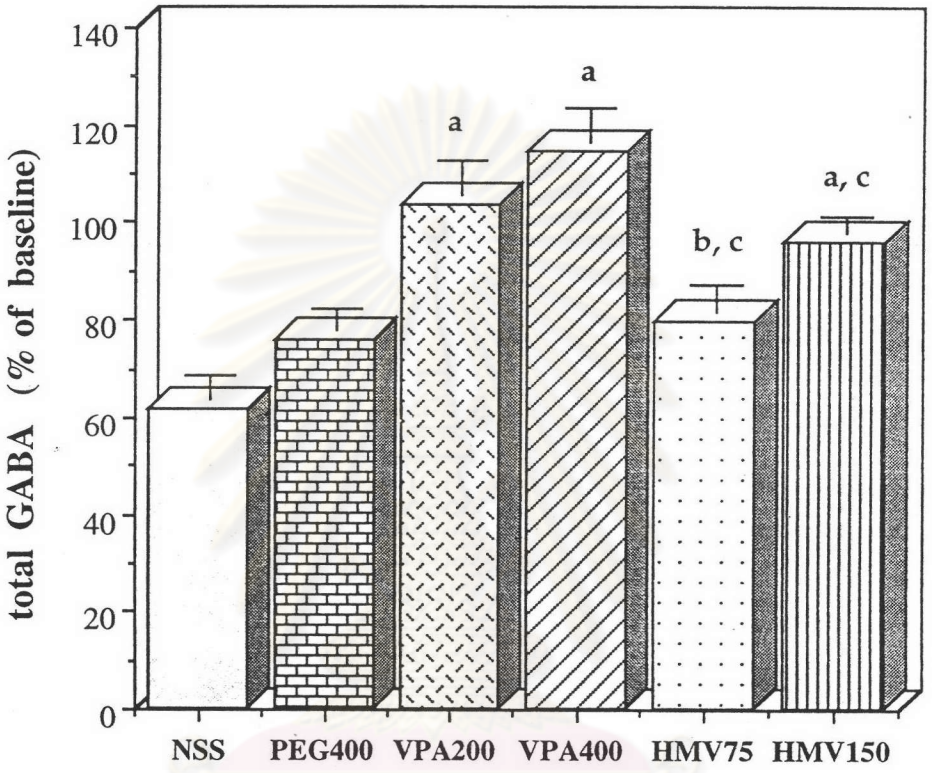


Figure 15 Effects of an intraperitoneal administration of VPA and HMV on the rat cortical GABA levels at various times.



[a] p< 0.05 denotes statistically significant from NSS and PEG400
[b] p< 0.05 denotes statistically significant from VPA200
[c] p< 0.05 denotes statistically significant from VPA400
n = 4-5

Figure 16 Effects of VPA and HMV on the total amount of the rat cortical GABA in the dialysates collected for 3 hours after an intraperitoneal administration of the test substances.