Chapter III

Results

1. Anticonvulsant activity

VHA, given intraperitoneally, demonstrated anticonvulsant activity in all animal models tested (MES, PTZ, bicuculiine induced convulsion and strychnine induced convulsion models). Either intracerebroventricularly or orally given VHA has demonstrated anticonvulsant activity in MES model. While PEG 400 (0.1 ml / 25 g B.W., i.p. and 0.3 ml / 25 g B.W. p.o.), which were given to control groups, exhibited no protection. In comparison, VHA produced higher potency than VPA about 2,1 and 2 times in MES, PTZ and bicuculline tests respectively. Furthermore, VHA was demonstrating peak effect at 15 min while it was 30 min for VPA. However, in MES test, duration of anticonvulsant activity of VPA was longer than those exhibited by VHA.

1.1 Anticonvulsant activity against MES test

1.1.1 Intraperitoneal route

As shown in Figure 5, 6 intraperitoneally given VHA and VPA demonstrated a protection against MES in mice in a dose dependent manner.

The ED_{50} of VHA were 114, 148 and 207 mg/kg B.W. at pretreated time of 15, 30 and 60 min, respectively, while corresponding values for VPA were 271, 211 and 218 mg/kg B.W.

The optimal pretreated time defined as the minimal time for the test substance to exert its highest anticonvulsant activity was found to be 15 min for VHA and 30 min for VPA. As shown in Figure 7, the ED_{50} of VHA and VPA at optimal pretreated time were 114 and 211 mg/kg B.W. respectively.

1.1.2 Duration of protection against MES

The ED_{50} of intraperitoneally given VPA and VHA were determined at 3 and 6 hours after dosing. The ED_{50} of both VPA and VHA increased as a function of time. At pretreated time of 1, 3 and 6 hours, the ED_{50} of VPA were 218, 210 and 336 mg/kg B.W. respectively, while corresponding values for VHA were 207, 466 and 832 mg/kg B.W. (Table 3 ; Figure 8).

1.1.3 Oral route

Like VPA, VHA was also orally active exhibited the ED_{50} which were about 2 times higher than the ED_{50} of the intraperitoneal route (Table 4). The ED_{50} of orally given VHA and VPA at optimal pretreated time, 15 min and 30 min, were 242 and 470 mg/kg B.W. respectively (Figure 9).

1.1.4 Intracerebroventricular route

VHA and VPA (sodium valproate), given intracerebroventricularly, demonstrated a protection against MES in a dose dependent manner exhibiting the ED_{50} at pretreated time of 15 min and 30 min, of 102 and 132 μM respectively (Figure 10). Whereas NSS, which was given to control group, exhibited no protection.

1.1.5 Tolerance

As shown in the Figure 11, the ED_{50} of Group 1 and Group 2 at optimal pretreated time (15 min) were 235 and 263 mg/kg B.W. respectively. Similar result were exhibited by VHA in 1.1.3.

Group 1, mice received PEG400 (0.3 ml / 25 g B.W., p.o.) for 5 days and group 2, mice received VHA of 242 mg / kg B.W., p.o. (ED_{50} of VHA, p.o., in MES

test at 15 min pretreated time) for 5 days. On day 6, both of them received VHA (p.o.) in various dose at 15 min pretreated time.

1.1.6 Effects of SKF-525A on anticonvulsant activity

The anticonvulsant potency of VPA and VHA were increased in MES model, when used in combination with SKF-525A.

As shown in the Figure 12, the ED_{50} of VPA and VHA (i.p.) at optimal pretreated time of 30 min and 15 min were 152 and 83 mg/kg B.W. respectively.

1.2 Chemically induced seizure tests

1.2.1 Anticonvulsant activity against PTZ seizure

In PTZ test, intraperitoneal injection of VHA and VPA in mice exhibited anticonvulsant against PTZ seizure in a dose dependent manner similar to MES test.

As shown in the Figure 13, the ED_{50} of VHA and VPA at optimal pretreated time were 97 and 99 mg/kg B.W. respectively.

1.2.2 Anticonvulsant activity against Bicuculline and Strychnine convulsion.

Both VHA and VPA exerted anticonvulsant activity against bicuculline induced convulsion. The ED_{50} of VHA and VPA were 153 and 382 mg/kg B.W. respectively (Figure 14). In strychnine test, VHA was effective in this model at the optimal pretreated time giving the ED_{50} of 441 mg/kg B.W., whereas VPA (600 mg/kg B.W.) was found to be ineffective (Table 4).

2. Toxicity test

2.1 Acute toxicity

The most frequent clinical signs observed in mice receiving high dose of test substance were ataxia, sedation, hypnosis and respiratory tract secretion.

The data of mortality, was evaluated by determination of the median lethal does (LD_{50}) following a single intraperitoneally administration of VHA and VPA in 72 hrs. However, most of death occurred in 24 hrs after dosing. As shown in Figure 15, the LD_{50} of VHA and VPA were 840 and 790 mg/kg B.W. respectively.

The relative safety margin (LD_{50} / ED_{50}) of VHA in MES and PTZ seizure test were 7.37 and 8.66, while the corresponding value for VPA were 3.74 and 7.98 respectively (Table 5).

2.2 Neurotoxicity

2.2.1 Rotorod test

In rotorod test, a control mice, receiving PEG 400, were able to maintain their equilibrium for at least 1 min on the rotating rod in 3 successive trials. The neurological impairment as indicated by an inability of the animal to maintain their equilibrium was exhibited by an intraperitoneal administration of various doses of VHA and VPA. As illustrated in Figure 16, both VHA and VPA inhibited the rotorod performance in a dose – dependent manner. The TD_{50} of VHA and VPA at optimal pretreated time, 15 min and 30 min, were 189 and 260 mg/kg B.W. respectively.

The protective index (PI, TD_{50} / ED_{50}), which were 1.66 and 1.23 in MES and 1.95 and 2.63 in PTZ test for VHA and VPA respectively (Table 5).

2.2.2 Duration of neurotoxicity

The effect of VHA and VPA on ability of mice to perform the rotorod test were followed for 6 hours. The TD_{50} of both VHA and VPA increased as a function of time. Apparently, the TD_{50} of VHA were always higher than those of VPA at any given time. At pretreated time of 1, 3 and 6 hours, the TD_{50} of VHA were 323, 608 and 1066 mg/kg B.W. respectively, while corresponding values for VPA were 219, 331 and 366 mg/kg B.W. (Table 6 ; Figure 17).

2.3 Locomotor activity

In comparison to NSS, an intraperitoneal administration of PEG 400 (0.1 ml/ 25 g. B.W.), VHA (90, 120 mg/kg B.W.) and VPA (100, 200 mg/kg B.W.) significantly depress the locomotor activity of mice (Figure 18). However, no statistically significant difference was noted among the effect of PEG 400, VHA (90 and 120 mg/kg B.W.) and VPA (100 and 200 mg/kg B.W.)

2.4 Barbiturate potentiation test

As shown in Figure 19, in comparison to NSS, PEG 400 (0.1 ml / 25 g B.W., i.p.) tended to significantly prolong barbiturate sleeping time. Though, in higher dose, both VHA (120 mg/kg B.W.) and VPA (200 mg/kg B.W.) significantly prolonged barbiturate sleeping time in comparison to PEG 400, the effect of low dose of VHA (90 mg/kg B.W.) and VPA (100 mg/kg B.W.) did not differ from PEG 400.

3. In vitro degradation of VHA by liver and brain homogenate

Degradation of VHA to VPA was not demonstrated by an incubation of VHA with either brain or liver homogenates. No statistically significant difference in VPA level between control and treated group was observed in either brain or liver homogenate preparation (Figure 21 and 22).

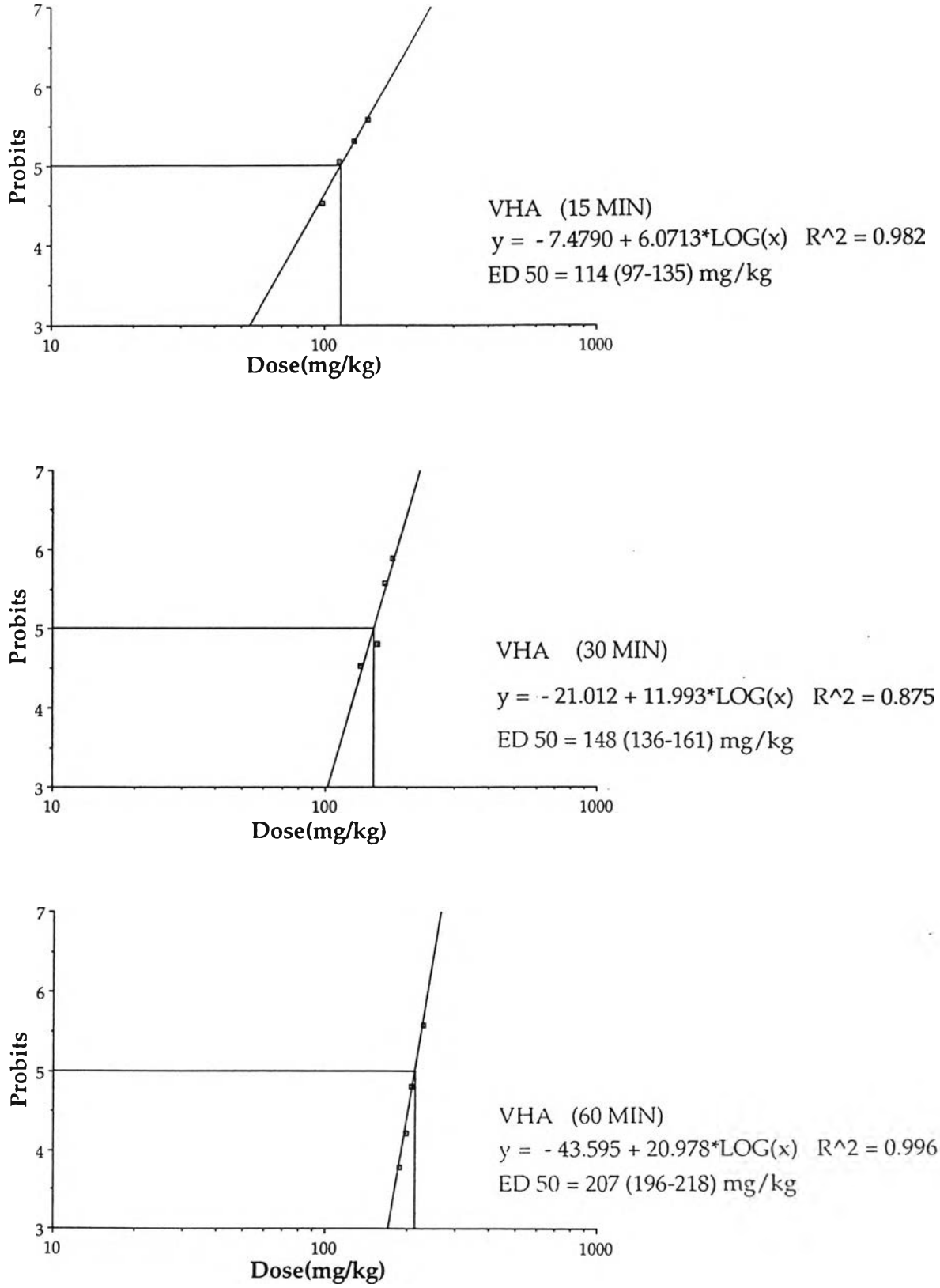


Figure 5. Log dose – response curves of VHA (i.p.) in MES test at pretreated times of 15, 30 and 60 min

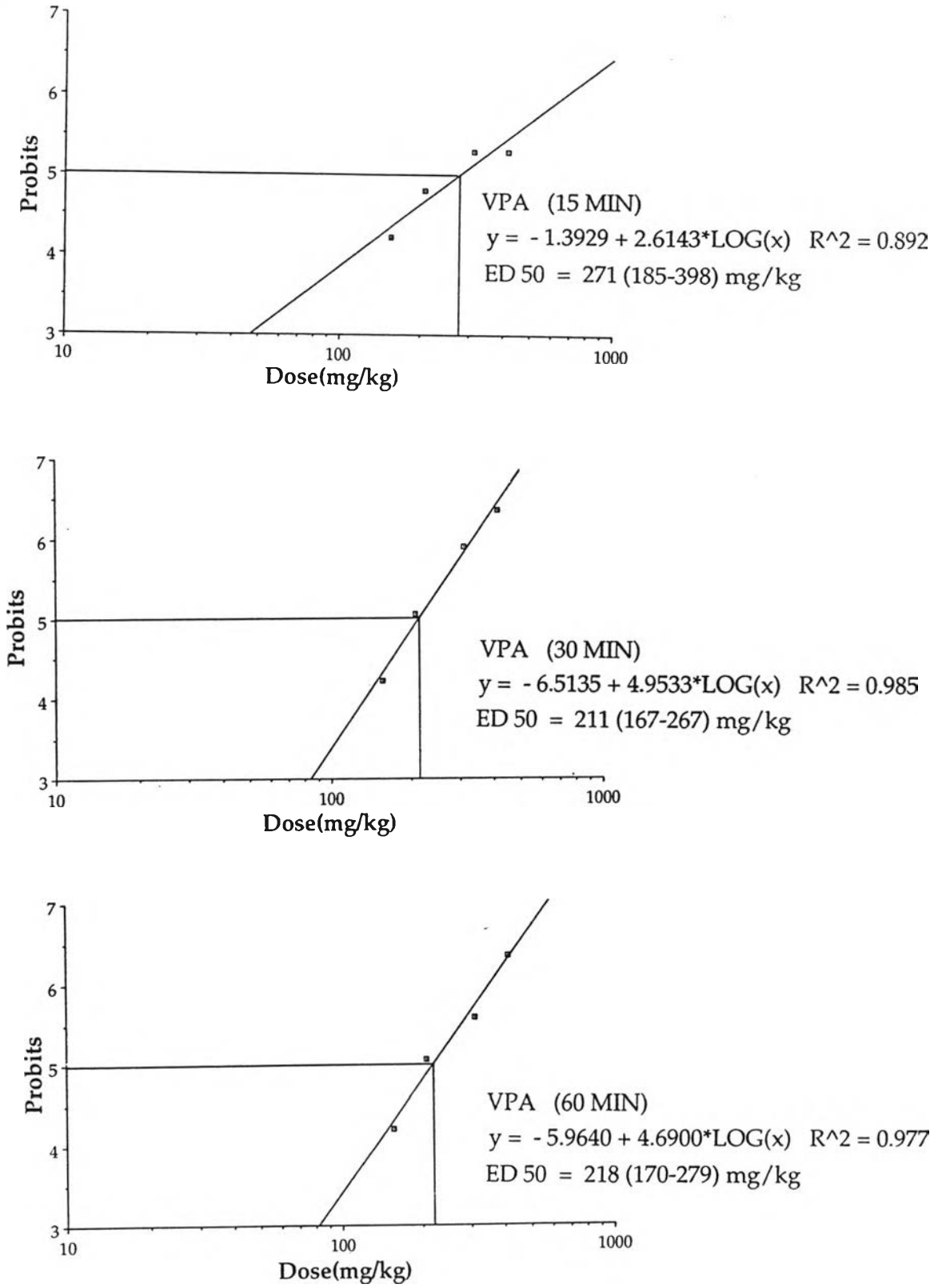


Figure 6. Log dose – response curves of VPA (i.p.) in MES test at pretreated times of 15, 30 and 60 min.

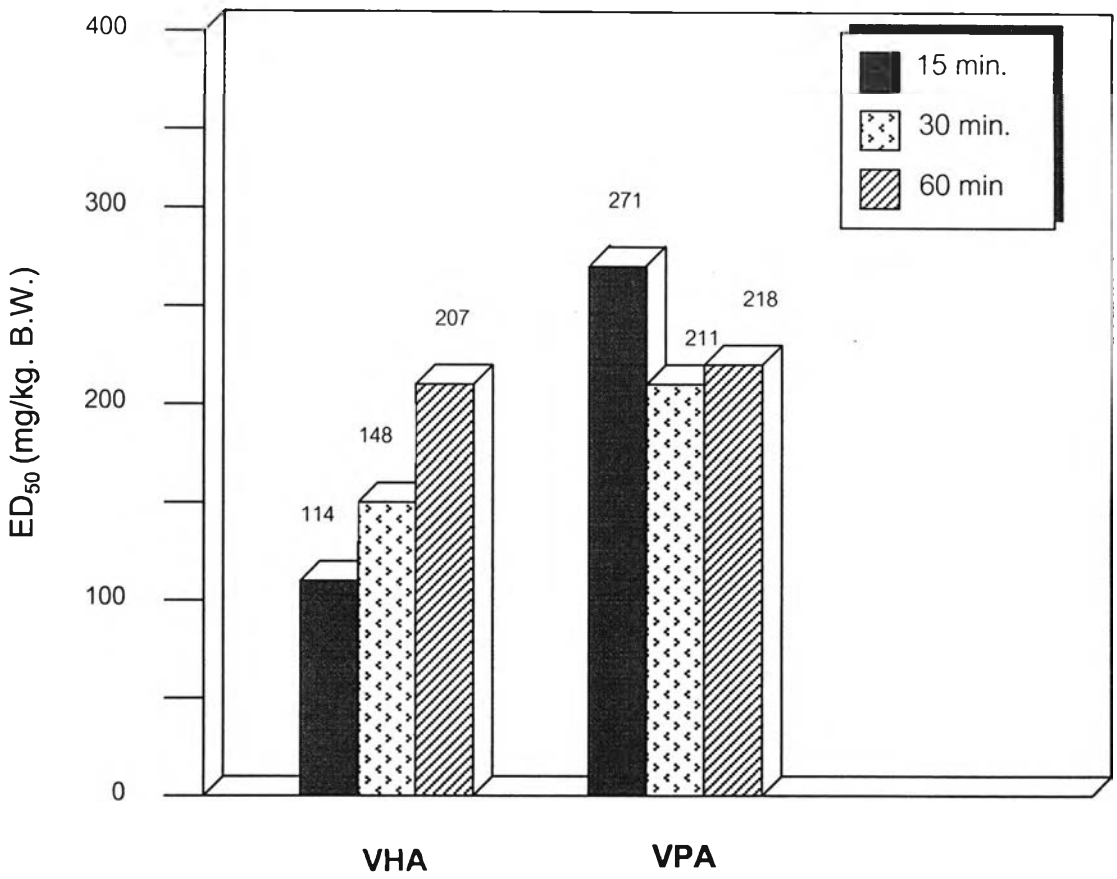


Figure 7. Comparison of ED_{50} at various pretreated times of intraperitoneally given VHA and VPA in MES test in mice.

Table 3. ED_{50} and relative safety margin (LD_{50}/ED_{50}) of an intraperitoneal administration of VHA and VPA at different time after dosing.

Time (hr)	ED_{50} (mg/kg B.W.)		Relative safety margin (LD_{50}/ED_{50})	
	VHA	VPA	VHA	VPA
1	207	218	4.06	3.62
3	466	210	1.80	3.76
6	832	336	1.01	2.16

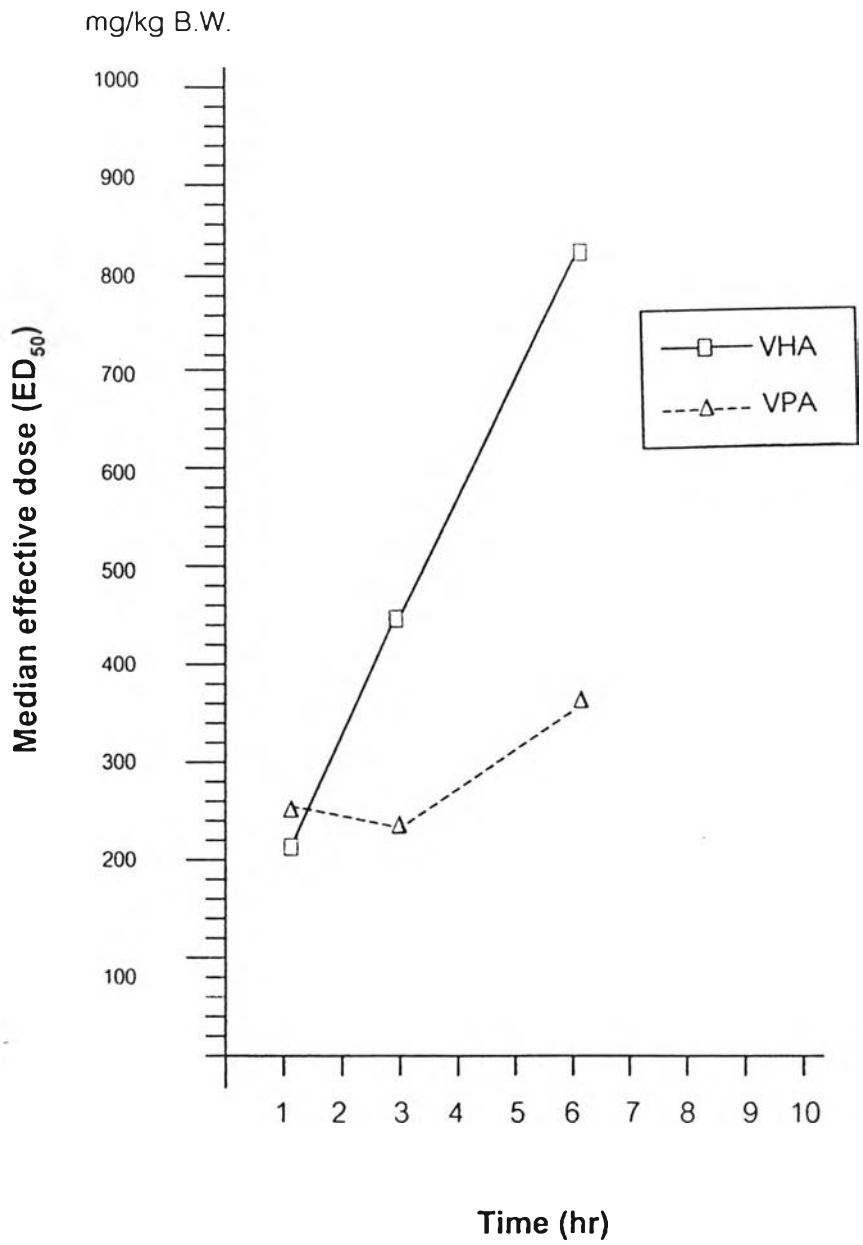
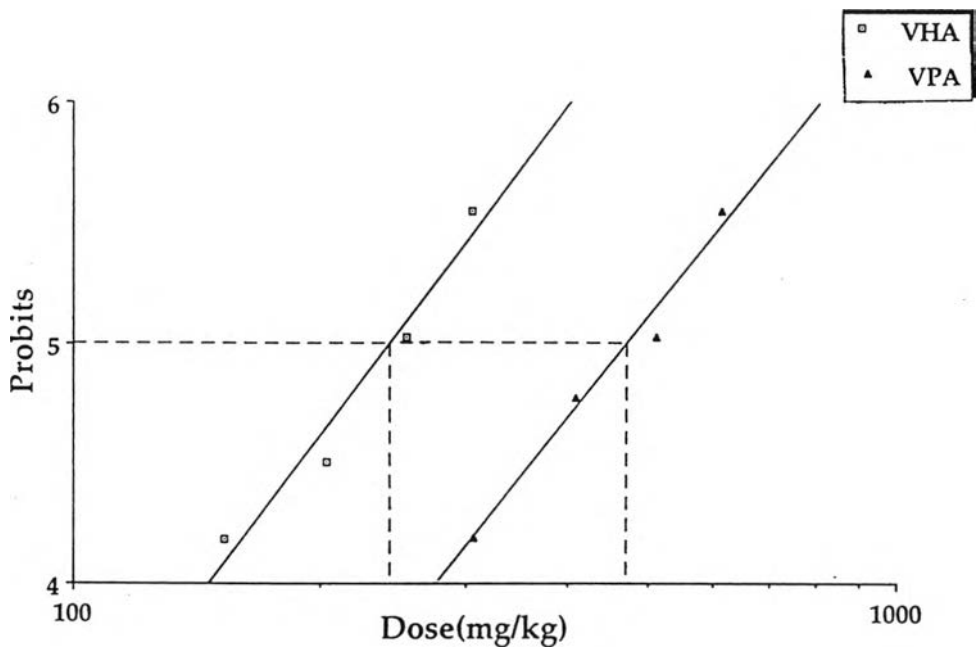


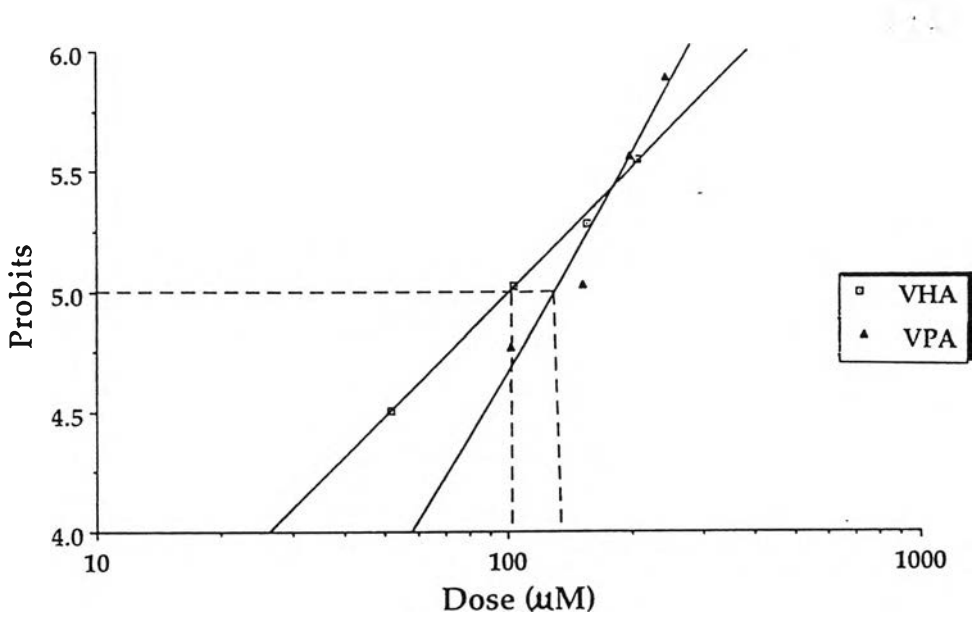
Figure 8. Protection against MES exhibited by VHA and VPA at various pretreated time in mice.



Probits (VHA) = -5.7901+4.5250*LOG(x), R^2 = 0.957
ED 50 = 242 (194-303) mg/kg

Probits (VPA)= -6.5604+4.3264*LOG(x), R^2 = 0.979
ED 50 = 470 (373-592) mg/kg

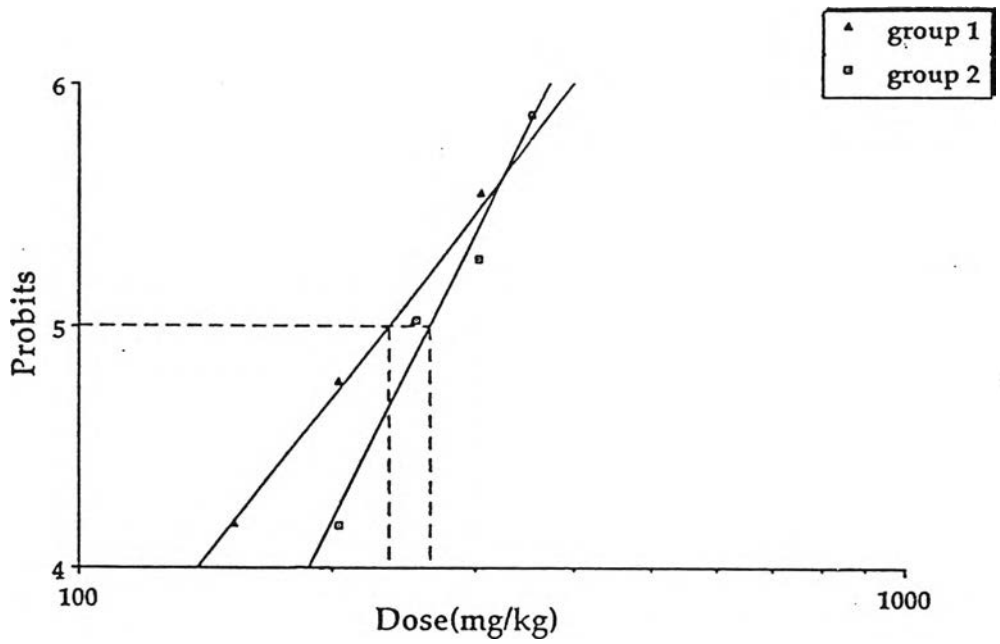
Figure 9. Log dose – response curves of VHA and VPA (p.o.) in MES test at their respective optimal pretreated time.



Probits (VHA) = 1.5759+1.7061*LOG(x), R^2 = 0.997
ED50 = 102 (57-184) μM

Probits (VPA) = -0.96702+2.8146*LOG(x), R^2 = 0.951
ED50 = 132 (92-189) μM

Figure 10. Log dose – response curves of VHA and VPA (i.c.v.) in MES test at their respective optimal pretreated time.



Tolerance (group 1)

y = -5.2580+4.3264*LOG(x), R^2 = 0.979

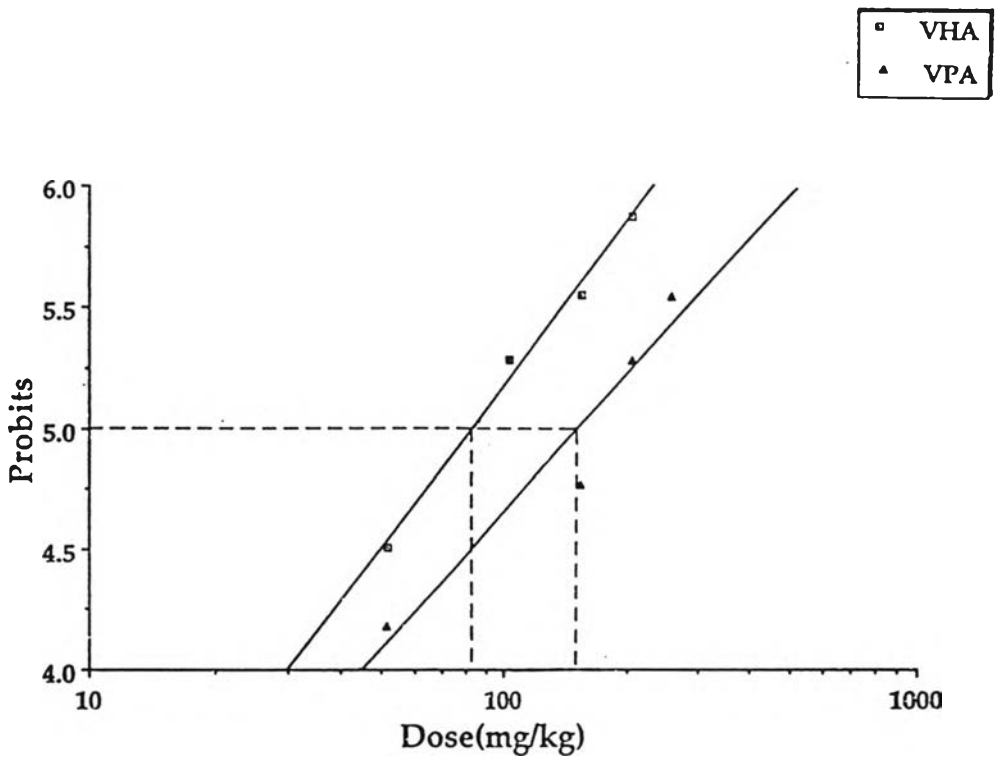
ED50 = 235 (186-296) mg/kg

Tolerance (group 2)

y = -10.929+6.5809*LOG(x), R^2 = 0.973

ED 50 = 263 (225-308) mg/kg

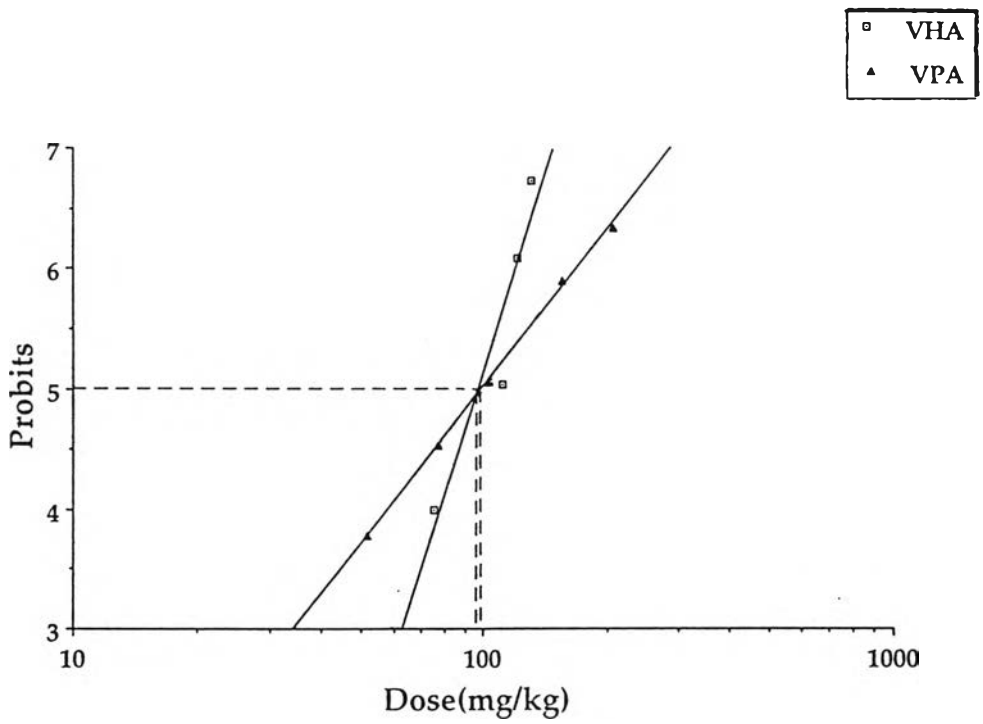
Figure 11. Log dose-response curves on tolerance in MES test of group 1, mice received PEG 400 (p.o.) for 5 days, and group 2, mice received VHA of 242 mg / kg B.W., p.o. for 5 days. On day 6, both of them received VHA (p.o.) in various dose and MES was performed at 15 min pretreated time and ED_{50} of both groups were calculated.



Probits(VHA) = 0.72306+2.2263*LOG(x), R^2 = 0.991
ED50 = 83 (53-130) mg/kg

Probits(VPA) = 0.91026+1.8709*LOG(x), R^2 = 0.929
ED50 = 152 (89-260) mg/kg

Figure 12. Log dose – response curves of VHA and VPA (i.p.) in combination with SKF – 525A in MES test at their respective optimal pretreated time.



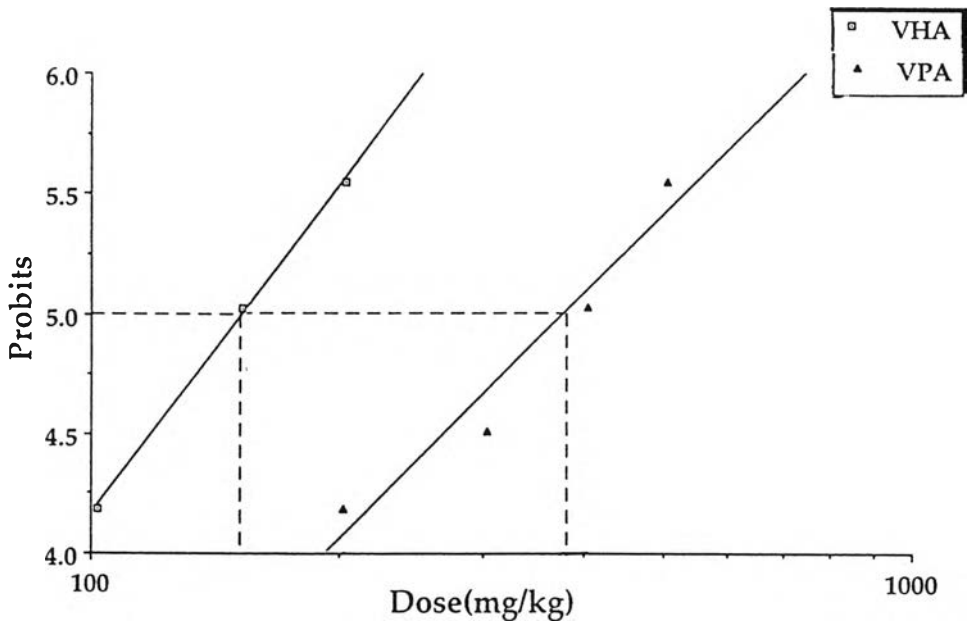
Probits (VHA) = -5.6518+5.3577*LOG(x), R^2 = 0.891

ED 50 = 97 (80 - 118) mg/kg

Probits (VPA) = -3.6193+4.3192*LOG(x), R^2 = 0.999

ED 50 = 99 (76-129) mg/kg

Figure 13. Log dose – response curves of VHA and VPA in PTZ test at their respective optimal pretreated time.



Probits (VHA) = -4.9346+4.5524*LOG(x), R^2 = 0.999

ED 50 = 153 (119-198) mg/kg

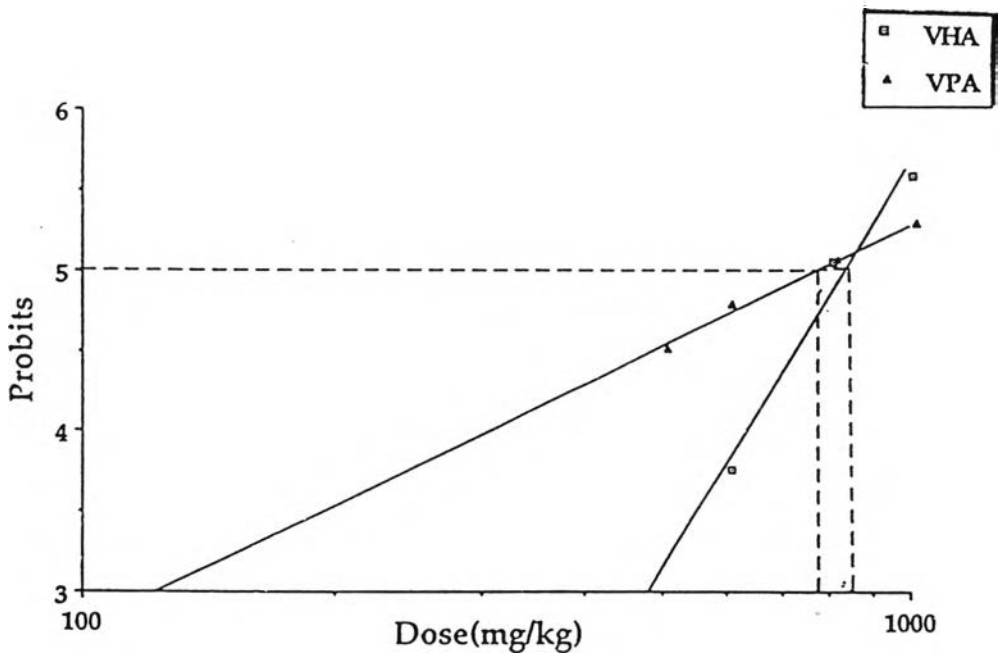
Probits (VPA) = -3.7575+3.3920*LOG(x), R^2 = 0.943

ED 50 = 382 (285-513) mg/kg

Figure 14. Log dose – response curves of VHA and VPA against bicuculline-induced convulsion in mice.

Table 4 Anticonvulsant activity of intraperitoneally or orally given VHA and VPA in various animal models at optimal pretreated time of 15 and 30 min respectively.

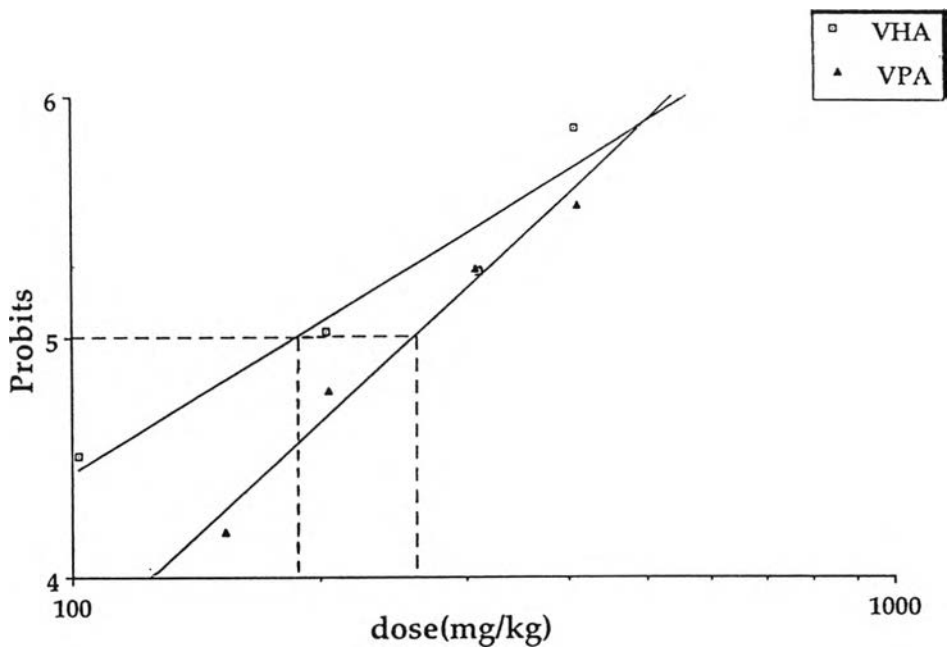
Seizure models	Route	ED_{50} of test substances (mg/kg B.W.)	
		VHA	VPA
MES	i.p.	114	211
	p.o.	242	470
PTZ	i.p.	97	99
Bicuculline	i.p.	153	382
Strychnine	i.p.	441	>600



Probits (VHA) = -19.099 + 8.2406*LOG(x), R^2 = 0.973
LD 50 = 840 (706-1000) mg/kg

Probits (VPA)= -2.2340+2.4966*LOG(x), R^2 = 0.991
LD 50 = 790 (530-1177) mg/kg

Figure 15 Log dose-response curves of VHA and VPA on acute toxicity (lethality) in mice.



Probits (VHA) = 0.20359+2.1062*LOG(x), R^2 = 0.939
TD 50 = 189 (117 - 304) mg/kg

Probits (VPA)= -2.6256+3.1587*LOG(x), R^2 = 0.976
TD 50 = 260 (190 - 356) mg/kg

Figure 16. Log dose – response curves of VHA and VPA in rotorod test in mice.

Table 5 ED_{50}, TD_{50}, LD_{50}, PI and relative safety margin of intraperitoneal administrations of VHA and VPA at optimal pretreated time in MES and PTZ seizure tests in mice.

Parameters (mg/kg.B.W.)	Tests	Substances	
		VHA	VPA
TD_{50}	Rotorod	189	260
LD_{50}	-	840	790
ED_{50}	MES	114	211
	PTZ	97	99
$PI(TD_{50}/ED_{50})$	MES	1.66	1.23
	PTZ	1.95	2.63
Relative Safety Margin (LD_{50}/ED_{50})	MES	7.37	3.74
	PTZ	8.66	7.98

Table 6 TD_{50} and protective index (PI = TD_{50} / ED_{50}) of an intraperitoneal administration of VHA and VPA at different times after dosing

Time (hr)	TD_{50} (mg/kg B.W.)		Protective index (PI = TD_{50}/ED_{50})	
	VHA	VPA	VHA	VPA
1	323	219	1.56	1.00
3	608	331	1.30	1.58
6	1066	366	1.28	1.09

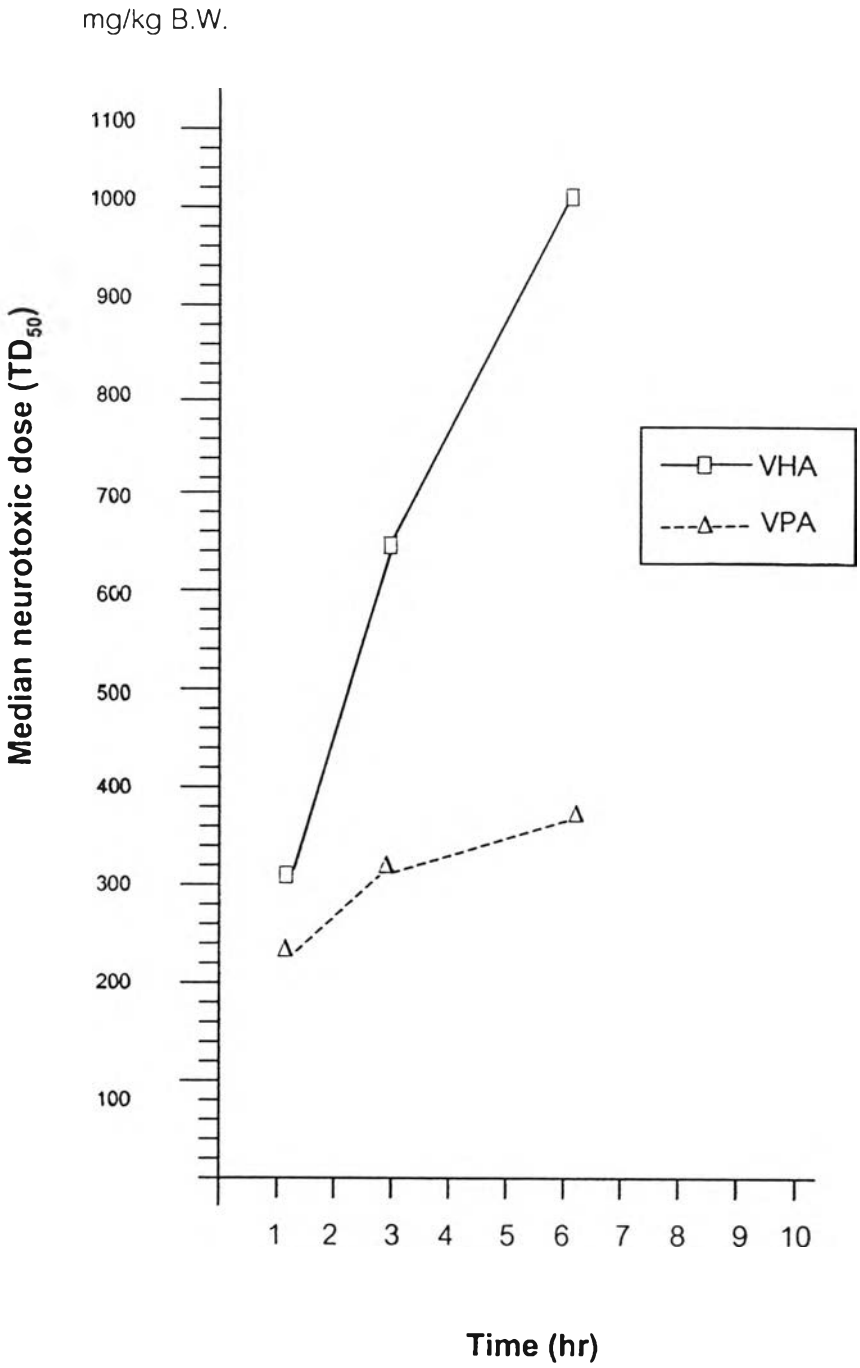
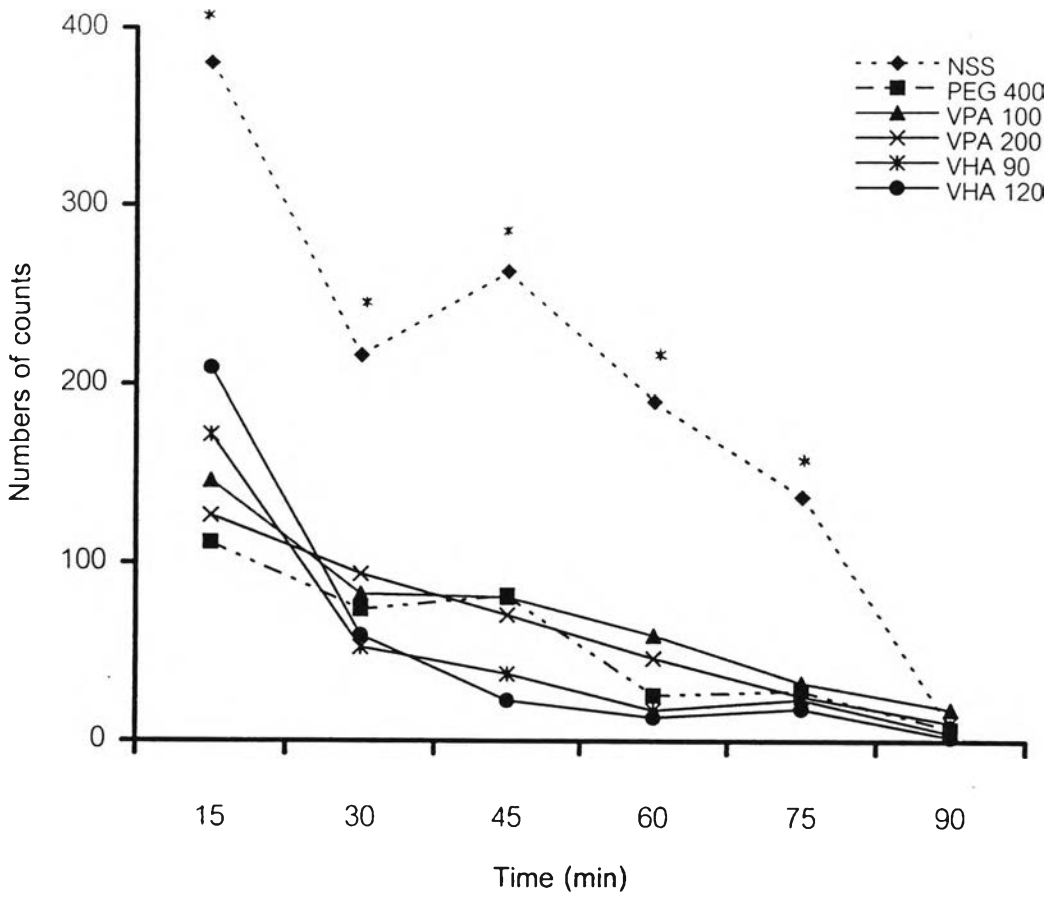
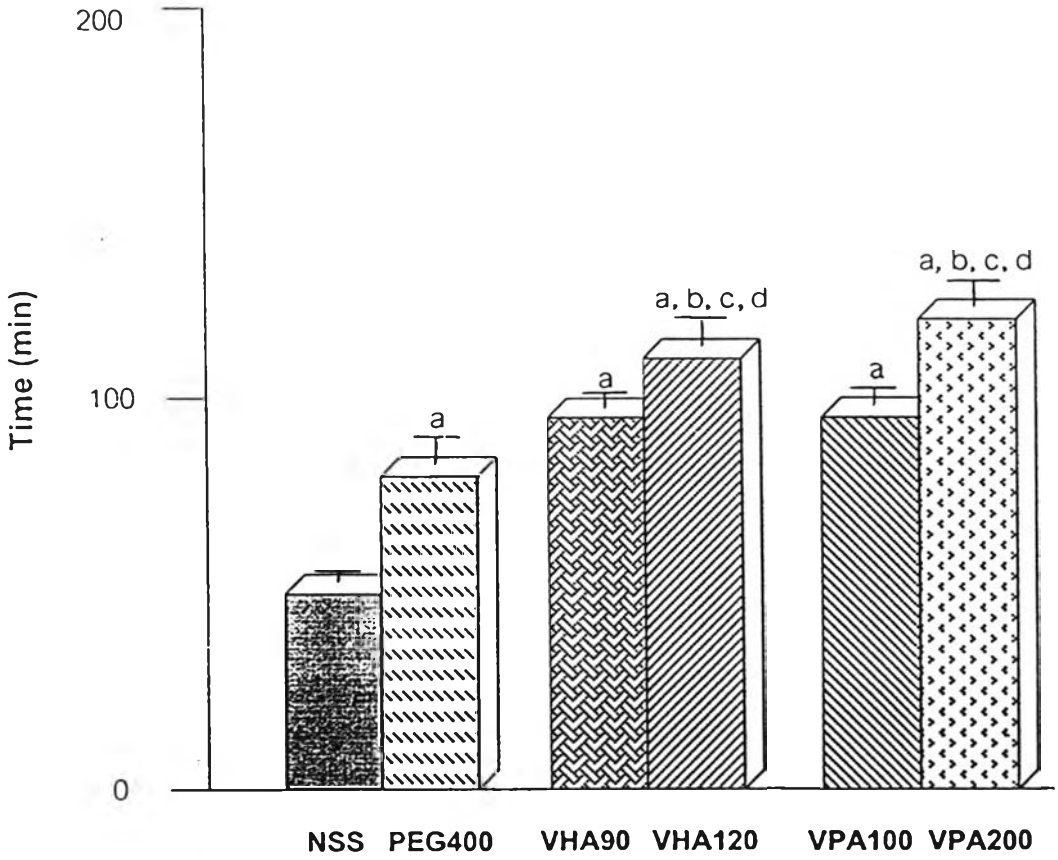


Figure 17. Neurotoxicity of VHA and VPA by rotorod test at various pretreated time in mice.



* $p < 0.05$ denotes statistically significant difference from PEG 400, VHA 90, VHA 120, VPA 100, and VPA 200

Figure 18. Effects of intraperitoneal administration of VHA and VPA on number of counts (Mean) of locomotor activity in mice at various times.



[a] $p < 0.05$ denotes statistically significant difference from NSS

[b] $p < 0.05$ denotes statistically significant difference from PEG 400

[c] $p < 0.05$ denotes statistically significant difference from VPA 100

[d] $p < 0.05$ denotes statistically significant difference from VHA 90

Figure 19. Effect of intraperitoneal administration of VHA and VPA on barbiturate sleeping time (Mean ± S.E.M.) in mice.

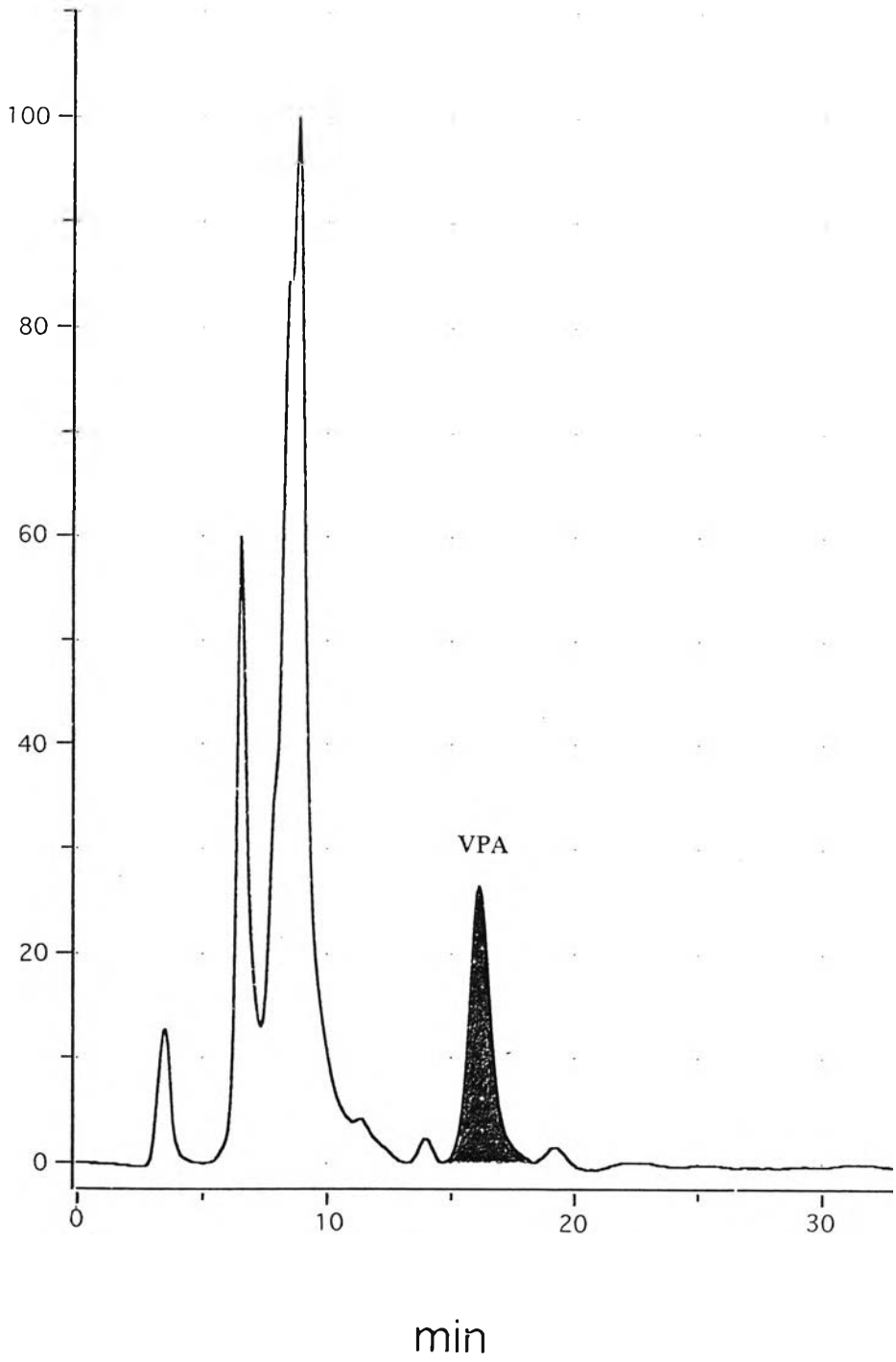


Figure 20. HPLC chromatogram of ADAM – derivatized standard VPA.

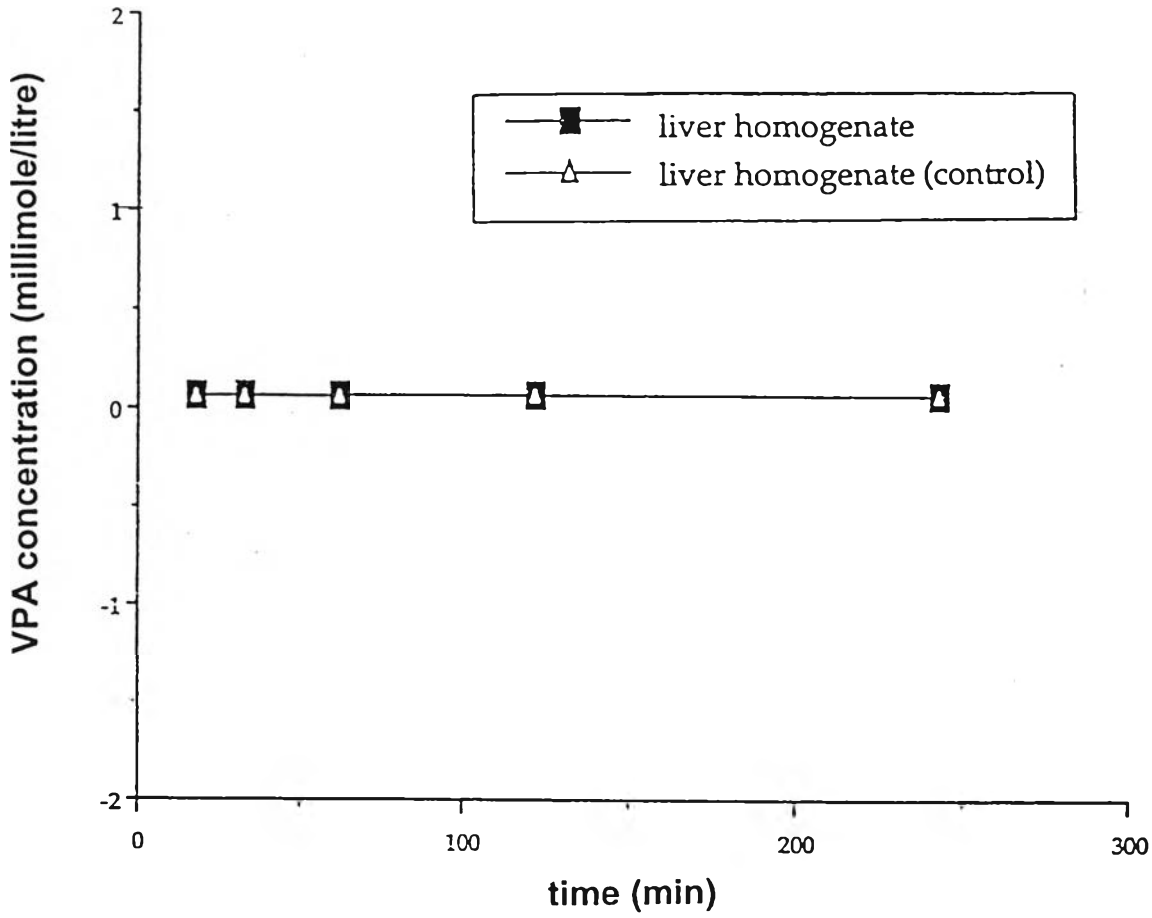


Figure 21. VPA concentration in rat's liver homogenate at various incubation times after the administration of VHA at time 0.

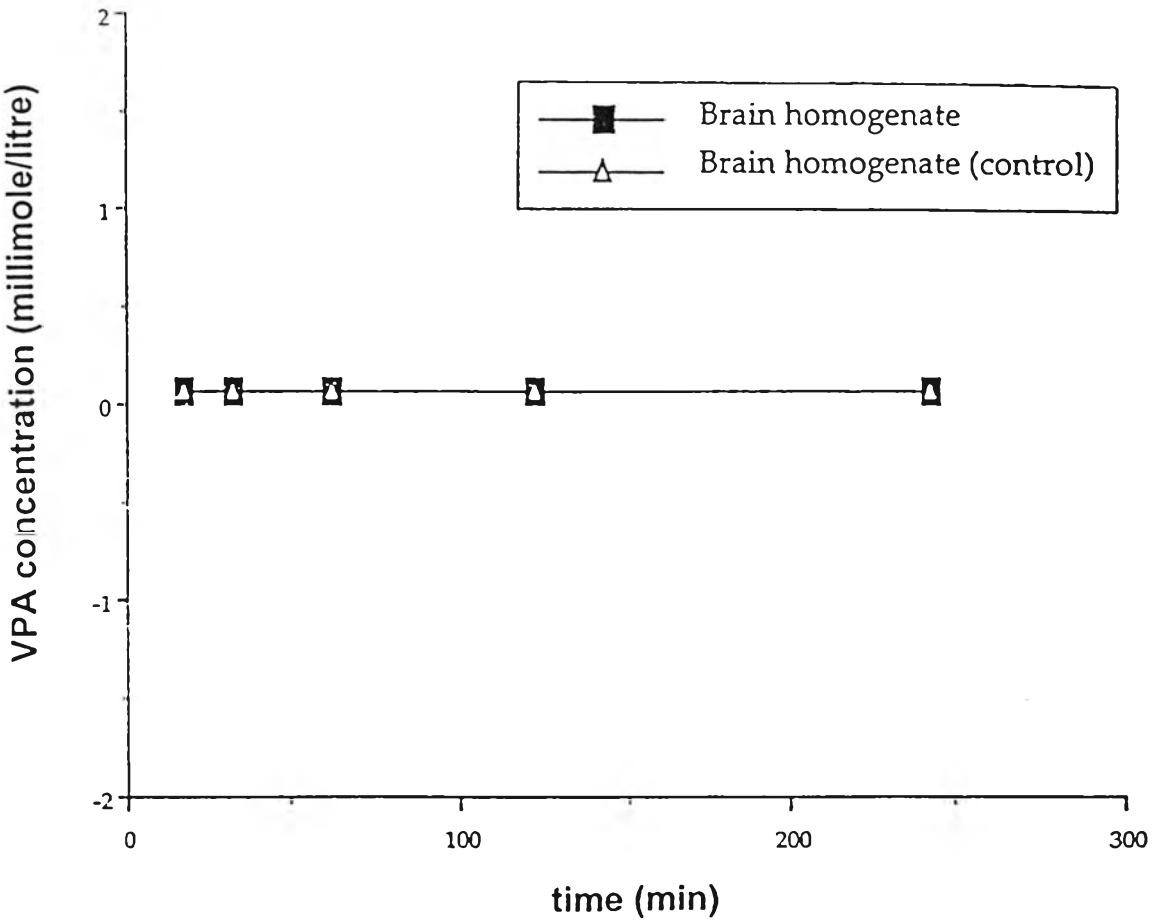


Figure 22. VPA concentration in rat's brain homogenate at various incubation times after the administration of VHA at time 0.