CHAPTER III

RESULTS

The resuts were divided into 17 parts on these following topics :-

1. Chronic effect of paracetamol treatment for 15 days on central 5-HT_{2A} serotonin receptors in rat frontal cortex
2. Chronic effect of paracetamol treatment for 15 days on central 5-HT_{2A} serotonin receptors in rat brain stem
3. Chronic effect of paracetamol treatment for 15 days on 5-HT uptake sites in rat frontal cortex
4. Chronic effect of paracetamol treatment for 15 days on 5-HT uptake sites in rat brain stem
5. Chronic effect of paracetamol treatment for 15 days on the levels of platelets 5-HT and its metabolite, 5-HIAA
6. Chronic effect of paracetamol treatment for 30 days on central 5-HT_{2A} serotonin receptors in rat frontal cortex
7. Chronic effect of paracetamol treatment for 30 days on central 5-HT_{2A} serotonin receptors in rat brain stem
8. Chronic effect of paracetamol treatment for 30 days on 5-HT uptake sites in rat frontal cortex

9. Chronic effect of paracetamol treatment for 30 days on 5-HT uptake sites in rat brain stem.
10. Chronic effect of paracetamol treatment for 30 days on the levels of platelets 5-HT and its metabolite, 5-HIAA
11. Acute effect of paracetamol treatment on central 5-HT_{2A} serotonin receptors in rat frontal cortex
12. Acute effect of paracetamol treatment on central 5-HT_{2A} serotonin receptors in rat brain stem
13. Acute effect of paracetamol treatment on 5-HT uptake sites in rat frontal cortex
14. Acute effect of paracetamol treatment on 5-HT uptake sites in rat brain stem
15. Acute and chronic effect of paracetamol treatment on antinociceptive activity
16. Chronic effect of paracetamol treatment for 15 days on rat body weight
17. Chronic effect of paracetamol treatment for 30 days on rat body weight

5-HT_{2A} Receptor Sites in Rat Frontal Cortex and Brain Stem Membranes

The specific [^{3}H]-spiperone binding to membranes of frontal cortex and brain stem from control and treated rats was saturable and Scatchard analysis of binding data yielded a straight line indicating the existence of a single population of binding sites within the concentration range of [^{3}H]-spiperone used. (Fig. 14-17, 20-22, 36-39, 42-44, 62-65, 68-71). The nonspecific binding was defined by 100 μM of ketanserin.

The dissociation equilibrium constants, K_d (given in nM) and the maximum number of binding sites, B_{max} (given in pmol/mg protein) were obtained from Scatchard plots.

5-HT Uptake Sites in Rat Frontal Cortex and Brain Stem Membranes

The specific [^{3}H]-imipramine binding to membranes of frontal cortex and brain stem from control and treated rats was saturable and Scatchard analysis of binding data yielded a straight line indicating the existence of a single population of binding sites within the concentration range of [^{3}H]-imipramine used. (Fig. 25-27, 30-32, 47-49, 52-54, 74-76, 79-81). The nonspecific binding was defined by 100 μM of fluoxetine.

The dissociation equilibrium constants, K_d (given in nM) and the maximum number of binding sites, B_{max} (given in pmol/mg protein) were obtained from Scatchard plots.

1. Chronic Effect of Paracetamol Treatment for 15 Days on Central 5-HT_{2A} Serotonin Receptors in Rat Frontal Cortex

After 15 days of drug administration, the B_{max} values in the frontal cortex for control and treated groups with paracetamol 200, 300 and 400 mg/kg/day were 2.18 ± 0.15, 1.93 ± 0.31, 1.34 ± 0.11 and 0.94 ± 0.01 pmol/mg protein, respectively. The K_d values for these four groups were 1.43 ± 0.15, 1.57 ± 0.34, 1.08 ± 0.08 and 1.24 ± 0.10 nM, respectively. The difference of the B_{max} values between control and treated groups with the two higher doses of paracetamol in this area was statistically significant ($p < 0.05$ and $p < 0.001$), whereas the K_d values remained unchanged (Table 3 and 4, Fig. 12 and 13).

Table 3. Comparison of the binding characteristics of [^{3}H]spiperone to membranes of frontal cortex between control and 15-day paracetamol-treated rats

Groups	K_d (nM)	B_{max} (pmol/mg protein)
Control	1.43 ± 0.15	2.18 ± 0.15
Para 200	1.57 ± 0.34	1.93 ± 0.31
Para 300	1.08 ± 0.08	1.34 ± 0.11*
Para 400	1.24 ± 0.10	0.94 ± 0.01***

The rats were i.p. injected with vehicle (control) and paracetamol (Para) 200, 300 and 400 mg/kg/day. Data were expressed as means ± S.E.M. of 5-6 rats per group. * indicate significant difference from control ($p < 0.05$). *** indicate significant difference from control ($p < 0.001$, the non-paired Student's *t*-test).

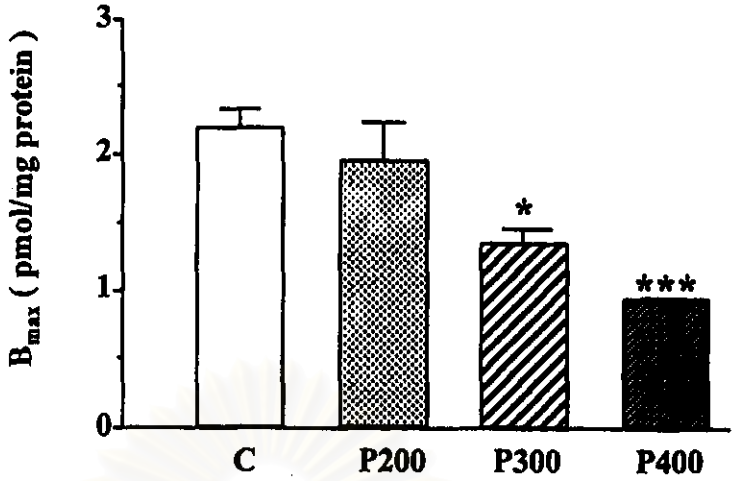

Figure 12. Comparison of the maximum number of binding sites (B_{max}) of [^{3}H]-spiperone in frontal cortex of control (C) and 15-day treated-rats with paracetamol 200 (P200), 300 (P300) and 400 (P400) mg/kg/day. B_{max} values were expressed as means ± S.E.M of 5-6 rats per group.

* indicate significant difference from control group ($p < 0.05$)

*** indicate significant difference from control group ($p < 0.001$).

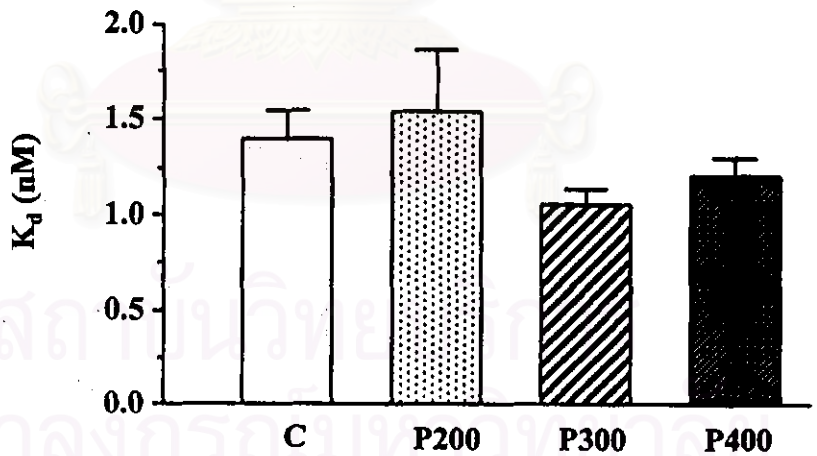

Figure 13. Comparison of the dissociation equilibrium constants (K_d) of [^{3}H]-spiperone binding sites in frontal cortex of control (C) and 15-day treated rats with paracetamol 200 (P200), 300 (P300) and 400 (P400) mg/kg/day. K_d values were expressed as means ± S.E.M. of 5-6 rats per group.

Table 4. Binding characteristics of [^{3}H]spiperone to frontal cortex membranes in control and 15-day paracetamol-treated rats

Groups	Rat No.	K_d (nM)	B_{max} (pmol/mg protein)
Control	1	1.9	2.24
	2	1.3	2.26
	3	0.8	1.49
	4	1.3	2.28
	5	1.7	2.69
	6	1.6	2.14
	means ± S.E.M.	**1.43 ± 0.15**	**2.18 ± 0.15**
Para 200	7	2.5	2.01
	8	0.9	1.38
	9	1.2	1.55
	10	1.7	2.76
	means ± S.E.M.	**1.57 ± 0.34**	**1.93 ± 0.31**
Para 300	11	1.2	1.54
	12	1.2	1.42
	13	1.1	1.17
	14	0.7	0.99
	15	1.2	1.59
	means ± S.E.M.	**1.08 ± 0.08**	**1.34 ± 0.11***
Para 400	16	1.0	0.96
	17	1.5	0.88
	18	1.4	0.95
	19	1.3	0.94
	20	1.0	0.96
	means ± S.E.M.	**1.24 ± 0.10**	**0.94 ± 0.01*****

Data were expressed as means ± S.E.M. from 5-6 rats per group. * indicate significant difference from control group ($p < 0.05$) *** indicate significant difference from control group ($p < 0.001$, non-paired Student's t-test). The saturation curve and Scatchard analysis of these data were shown in Fig. 14-17 and Fig. 87-90.

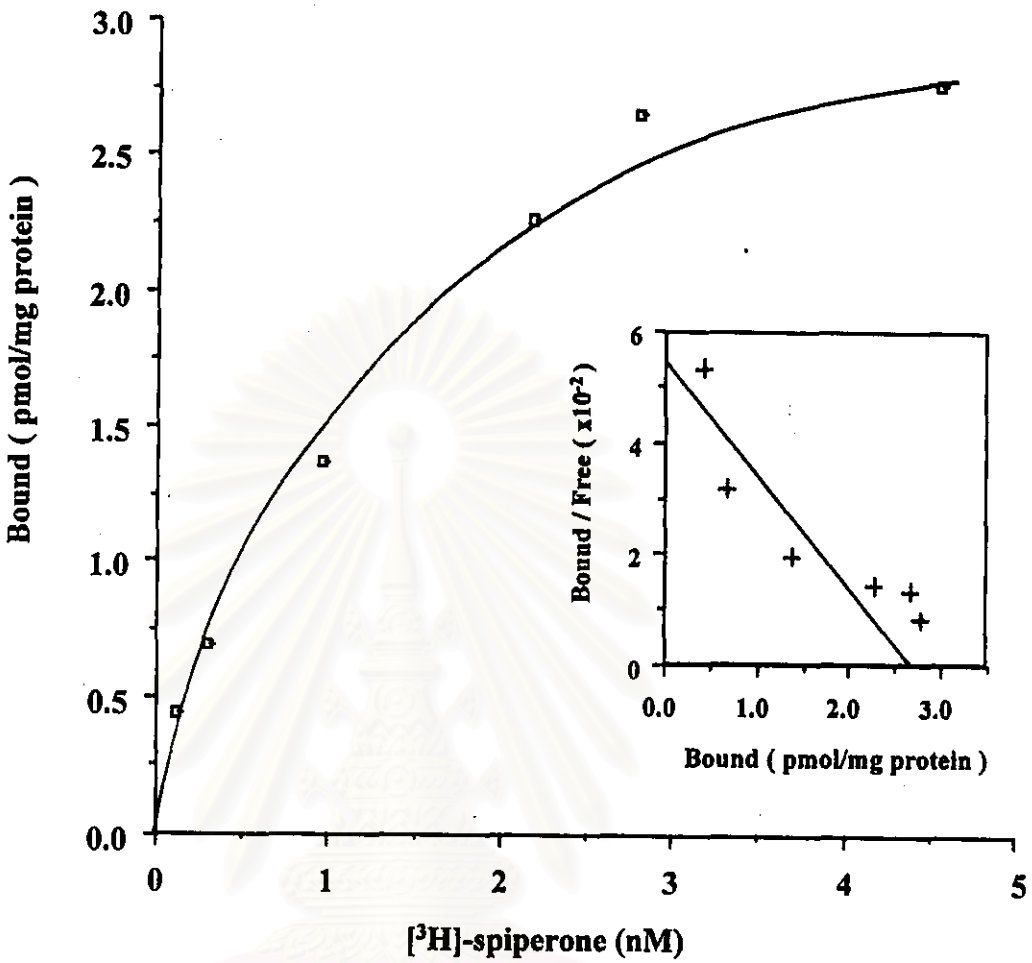

Figure 14. Saturation curve and Scatchard analysis (in the inset) of [^{3}H]-spiperone binding on frontal cortex membrane of control rat number 5, treated with vehicle i.p. once daily for 15 days. The binding was carried out in six concentrations of [^{3}H]-spiperone, ranging from 0.05 - 5 nM. The plots were obtained from duplicate determinations and represented the specific binding of [^{3}H]-spiperone. The line of best fit was analysed by the LIGAND computer program The result of this experiment was shown and provided a K_d value of 1.7 nM and B_{max} value of 2.69 pmol/mg protein.

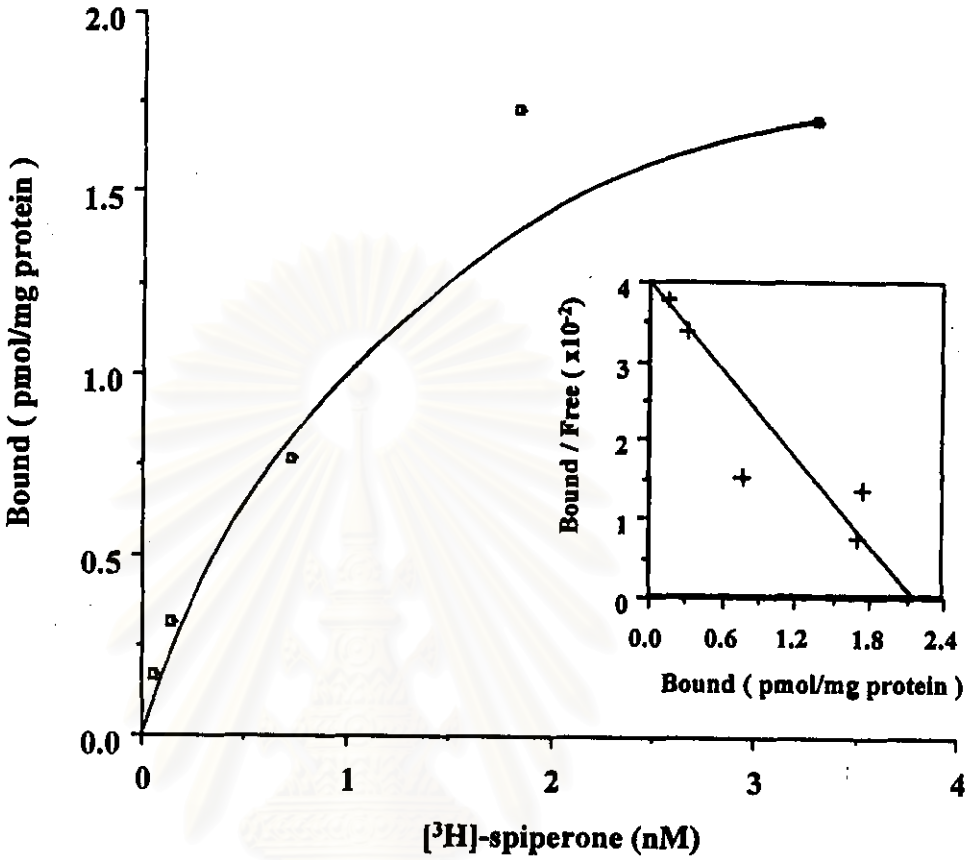

Figure 15. Saturation curve and Scatchard analysis (in the inset) of [^{3}H]-spiperone binding on frontal cortex membrane of control rat number 6, treated with vehicle i.p. once daily for 15 days. The binding was carried out in six concentrations of [^{3}H]-spiperone, ranging from 0.02 - 4 nM. The plots were obtained from duplicate determinations and represented the specific binding of [^{3}H]-spiperone. The line of best fit was analysed by the LIGAND computer program The result of this experiment was shown and provided a K_d value of 1.6 nM and B_{max} value of 2.14 pmol/mg protein.

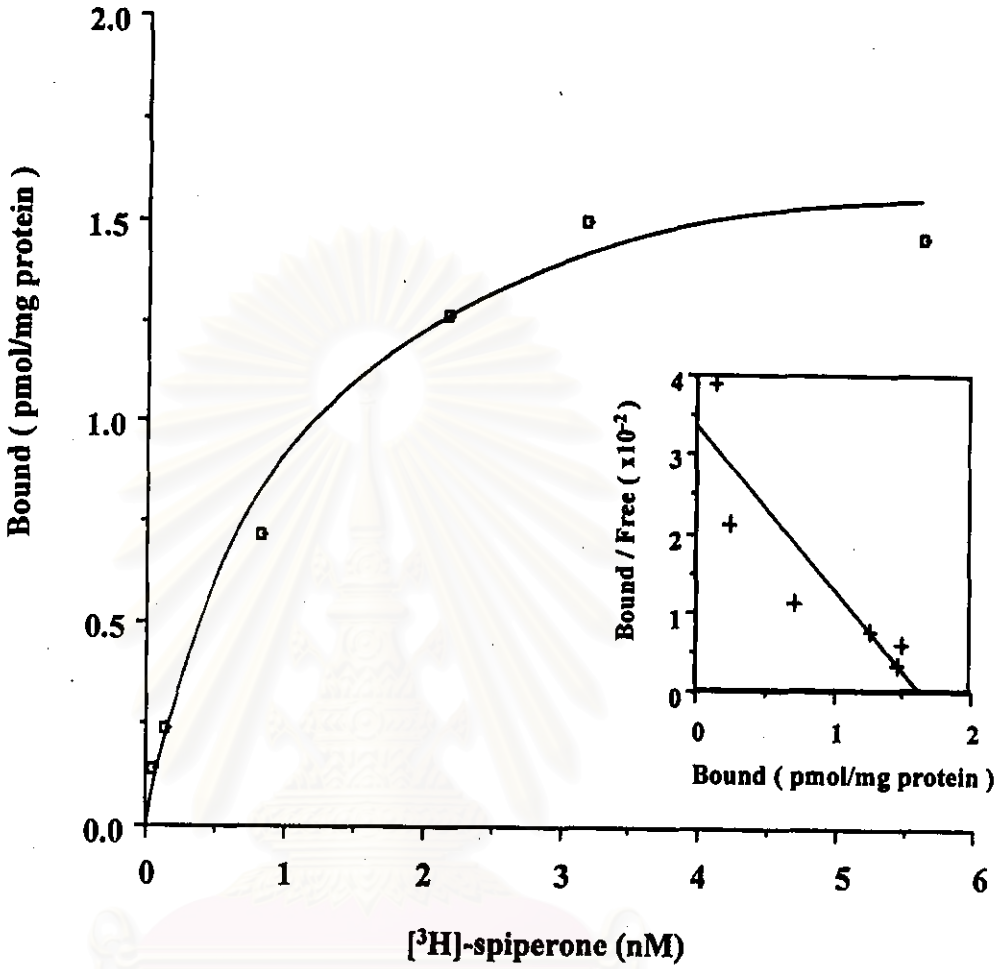

Figure 16. Saturation curve and Scatchard analysis (in the inset) of [^{3}H]-spiperone binding on frontal cortex membrane of rat number 15, treated with paracetamol 300 mg/kg/day i.p. for 15 days. The binding was carried out in six concentrations of [^{3}H]-spiperone, ranging from 0.03 - 6 nM. The plots were obtained from duplicate determinations and represented the specific binding of [^{3}H]-spiperone. The line of best fit was analysed by the LIGAND computer program. The result of this experiment was shown and provided a K_d value of 1.2 nM and B_{max} value of 1.59 pmol/mg protein.

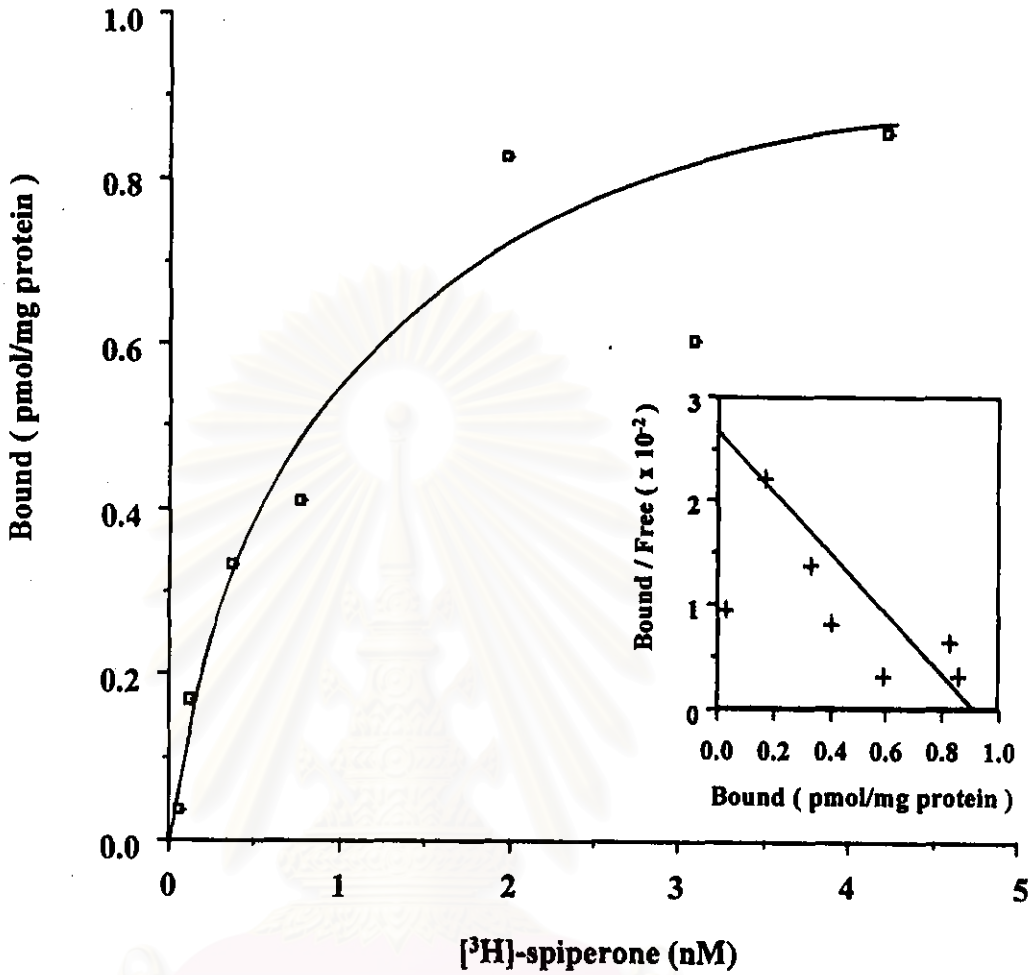

Figure 17. Saturation curve and Scatchard analysis (in the inset) of [^{3}H]-spiperone binding on frontal cortex membrane of rat number 20, treated with paracetamol 400 mg/kg/day i.p. for 15 days. The binding was carried out in seven concentrations of [^{3}H]-spiperone, ranging from 0.05 -5 nM. The plots were obtained from duplicate determinations and represented the specific binding of [^{3}H]-spiperone. The line of best fit was analysed by the LIGAND computer program. The result of this experiment was shown and provided a K_d value of 1.0 nM and B_{max} value of 0.96 pmol/mg protein.

2. Chronic Effect of Paracetamol Treatment for 15 Days on Central 5-HT_{2A} Serotonin Receptors in Rat Brain Stem

After 15 days of drug administration, the B_{max} values in the brain stem for control and treated groups with paracetamol 200, 300 and 400 mg/kg/day were 1.05 ± 0.14, 1.02 ± 0.08, 1.14 ± 0.29 and 1.27 ± 0.23 pmol/mg protein, respectively. The K_d values for these four groups were 0.88 ± 0.11, 0.83 ± 0.13, 0.87 ± 0.24 and 1.00 ± 0.11 nM, respectively. The B_{max} and K_d values between control and treated groups were not different (Table 5 and 6, Fig 18 and 19).

Table 5. Comparison of the binding characteristics of [^{3}H]spiperone to membranes of brain stem between control and 15-day paracetamol-treated rats

Groups	K_d (nM)	B_{max} (pmol/mg protein)
Control	0.88 ± 0.11	1.05 ± 0.14
Para 200	0.83 ± 0.13	1.02 ± 0.08
Para 300	0.87 ± 0.24	1.14 ± 0.29
Para 400	1.00 ± 0.11	1.27 ± 0.23

The rats were i.p. injected with vehicle (control) and paracetamol (Para) 200, 300 and 400 mg/kg/day. Data were expressed as means ± S.E.M. of 5-6 rats per group. Statistical comparisons were made using the non-paired Student's *t*-test.

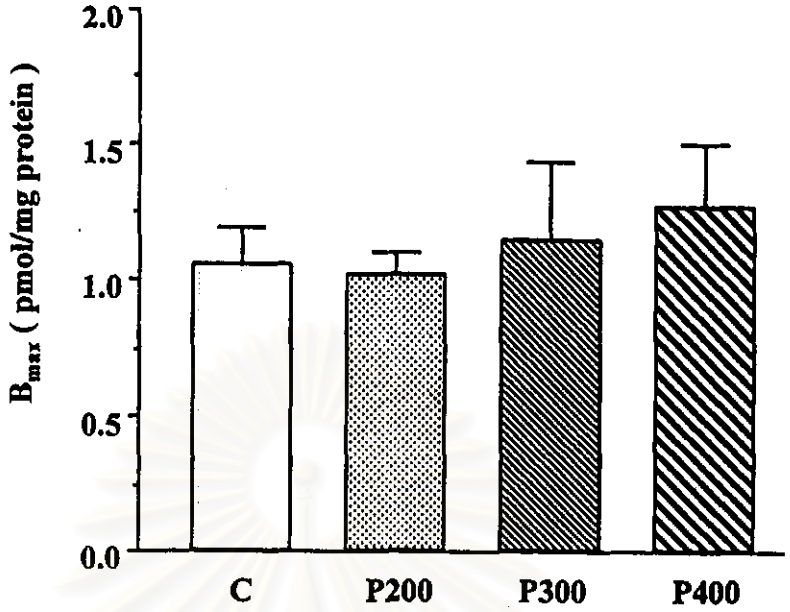

Figure 18. Comparison of the maximum number of binding sites (B_{max}) of [^{3}H]-spiperone in brain stem of control (C) and 15-day treated rats with paracetamol 200 (P200), 300 (P300) and 400 (P400) mg/kg/day. Bmax values were expressed as means ± S.E.M. of 5-6 rats per group.

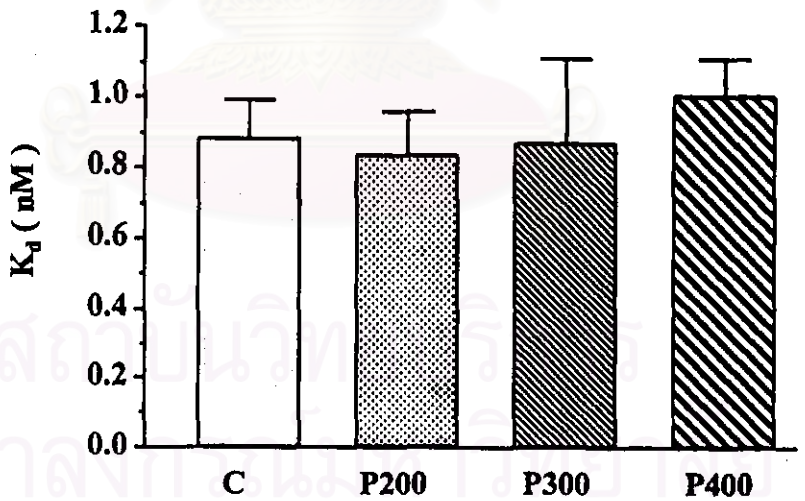

Figure 19. Comparison of the dissociation equilibrium constants (K_d) of [^{3}H]-spiperone binding sites in brain stem of control (C) and 15-day treated rats with paracetamol 200 (P200), 300 (P300) and 400 (P400) mg/kg/day. K_d values were expressed as means ± S.E.M. of 5-6 rats per group.

Table 6. Binding characteristics of [^{3}H]spiperone to brain stem membranes in control and 15-day paracetamol-treated rats

Groups	Rat No.	K_d (nM)	B_{max} (pmol/mg protein)
Control	1	1.2	1.03
	2	0.8	0.99
	3	0.6	1.12
	4	0.9	0.86
	5	1.2	1.68
	6	0.6	0.62
	means ± S.E.M.	**0.88 ± 0.11**	**1.05 ± 0.14**
Para 200	7	0.6	0.91
	8	0.7	1.23
	9	1.2	0.88
	10	0.8	1.05
	means ± S.E.M.	**0.83 ± 0.13**	**1.02 ± 0.08**
Para 300	11	1.0	1.28
	12	0.5	0.71
	13	1.6	1.79
	14	0.8	1.49
	means ± S.E.M.	**0.87 ± 0.24**	**1.14 ± 0.29**
Para 400	15	1.0	1.02
	16	1.0	1.57
	17	0.9	0.74
	18	0.7	0.99
	19	1.4	2.07
	means ± S.E.M.	**1.24 ± 0.10**	**0.94 ± 0.01**

Statistical comparisons were made using the non-paired Student's *t*-test. The saturation curve and Scatchard analysis of these data were shown in Fig. 20-22 and Fig. 91-94.

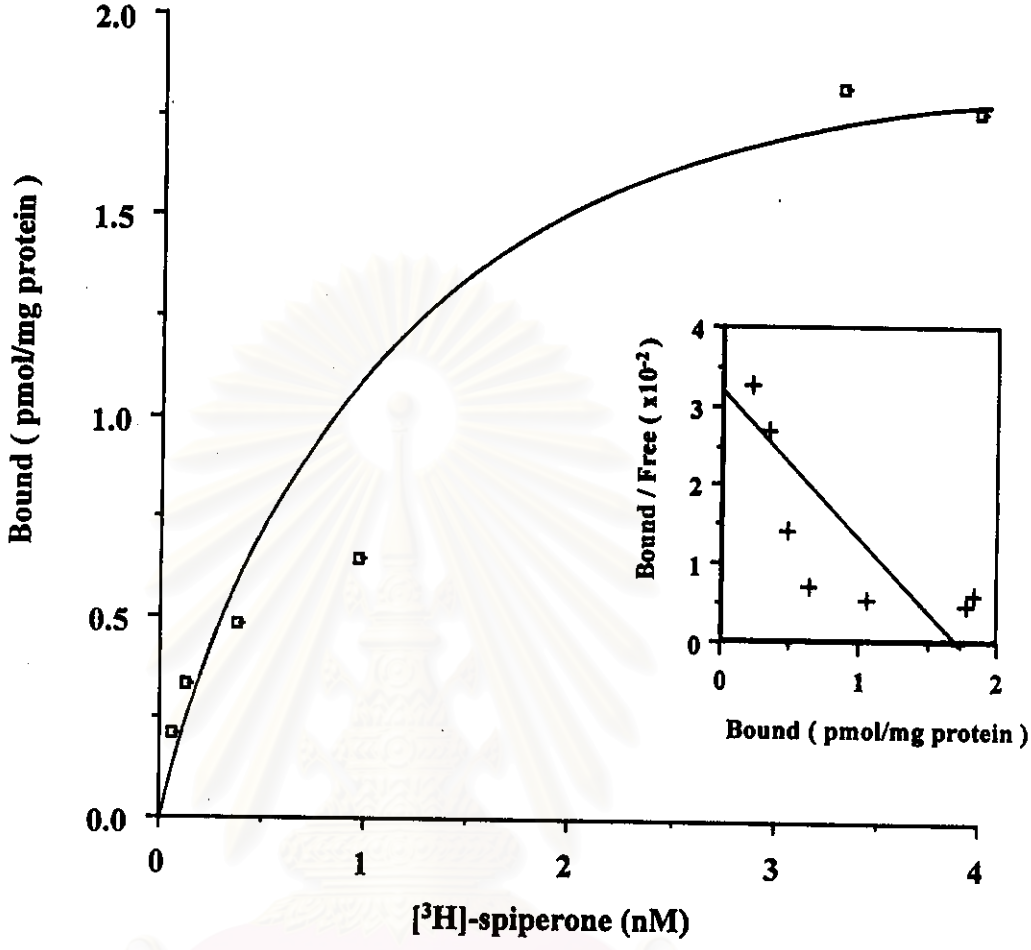

Figure 20. Saturation curve and Scatchard analysis (in the inset) of [^{3}H]-spiperone binding on brain stem membrane of control rat number 5, treated with vehicle i.p. once daily for 15 days. The binding was carried out in seven concentrations of [^{3}H]-spiperone, ranging from 0.04 - 4 nM. The plots were obtained from duplicate determinations and represented the specific binding of [^{3}H]-spiperone. The line of best fit was analysed by the LIGAND computer program. The result of this experiment was shown and provided a K_d value of 1.2 nM and B_{max} value of 1.68 pmol/mg protein.

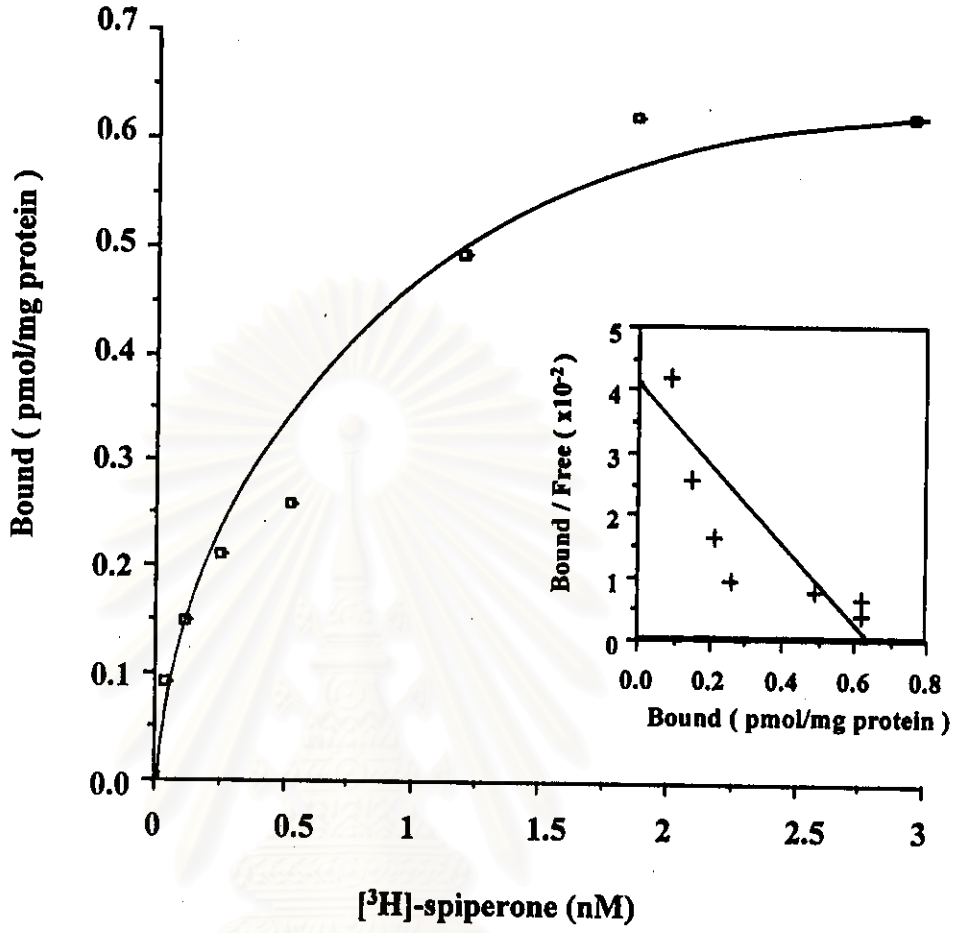

Figure 21. Saturation curve and Scatchard analysis (in the inset) of [^{3}H]-spiperone binding on brain stem membrane of control rat number 6, treated with vehicle i.p. once daily for 15 days. The binding was carried out in seven concentrations of [^{3}H]-spiperone, ranging from 0.02 - 3 nM. The plots were obtained from duplicate determinations and represented the specific binding of [^{3}H]-spiperone. The line of best fit was analysed by the LIGAND computer program. The result of this experiment was shown and provided a K_d value of 0.6 nM and B_{max} value of 0.62 pmol/mg protein.

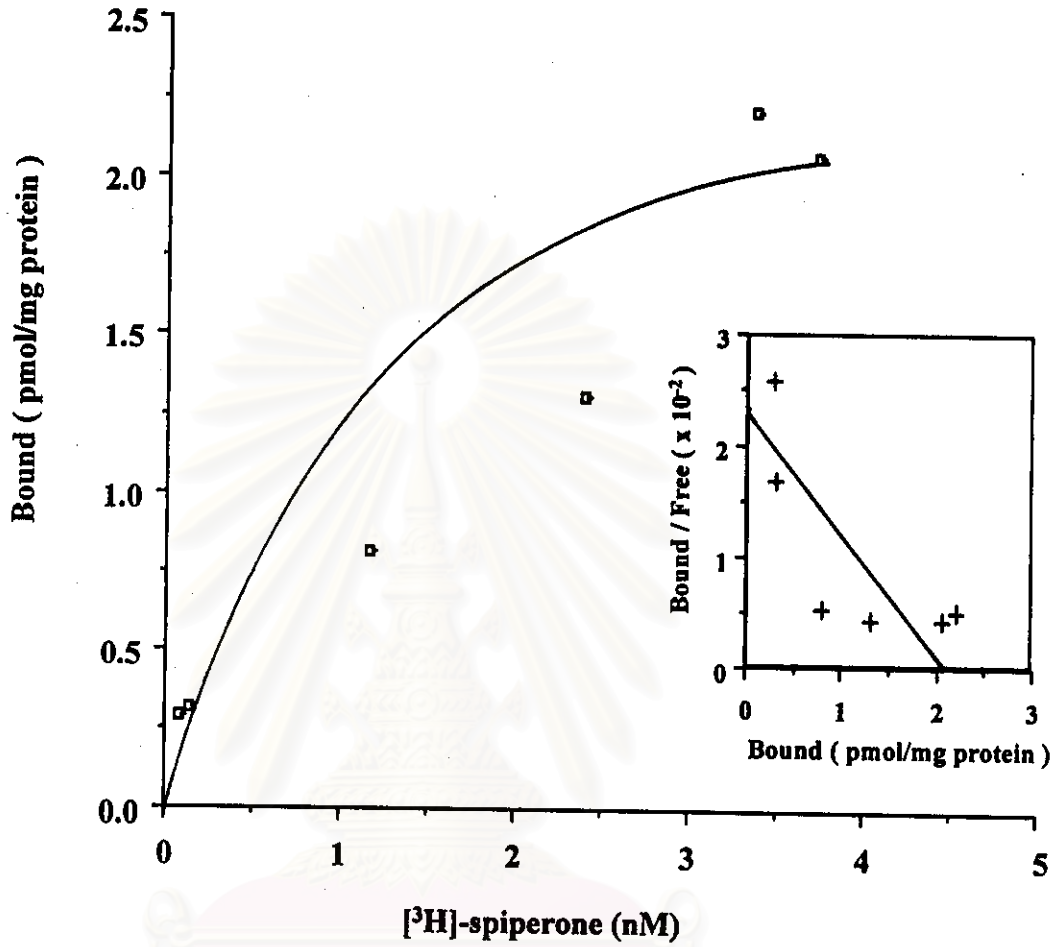

Figure 22. Saturation curve and Scatchard analysis (in the inset) of [^{3}H]-spiperone binding on brain stem membrane of rat number 19, treated with paracetamol 400 mg/kg/day i.p. for 15 days. The binding was carried out in six concentrations of [^{3}H]-spiperone, ranging from 0.05 - 5 nM. The plots were obtained from duplicate determinations and represented the specific binding of [^{3}H]-spiperone. The line of best fit was analysed by the LIGAND computer program. The result of this experiment was shown and provided a K_d value of 1.4 nM and B_{max} value of 2.07 pmol/mg protein.

3. Chronic Effect of Paracetamol Treatment for 15 Days on 5-HT Uptake Sites in Rat Frontal Cortex

After 15 days of drug administration, the B_{max} values in the frontal cortex for control and treated groups with paracetamol 300 and 400 mg/kg/day were 1.84 ± 0.25, 3.74 ± 0.60 and 4.59 ± 0.52 pmol/mg protein, respectively. The K_d values for these three groups were 1.69 ± 0.36, 1.70 ± 0.75 and 2.11 ± 0.35 nM, respectively. The difference of the B_{max} values between control and treated groups with the two doses of paracetamol in this area was statistically significant ($p < 0.001$). However, there was no significant difference in K_d values among the three groups (Table 7 and 8, Fig.23 and 24).

Table 7. Comparison of the binding characteristics of [^{3}H]imipramine to membranes of frontal cortex between control and 15-day paracetamol-treated rats

Groups	K_d (nM)	B_{max} (pmol/mg protein)
Control	1.69 ± 0.36	1.84 ± 0.25
Para 300	1.70 ± 0.75	3.74 ± 0.60**
Para 400	2.11 ± 0.35	4.59 ± 0.52***

The rats were i.p. injected with vehicle (control) and paracetamol (Para) 300 and 400 mg/kg/day. Data were expressed as means ± S.E.M. of 4-5 rats per group.

** indicate significant difference from control group ($p < 0.01$).

*** indicate significant difference from control group ($p < 0.001$).

Statistical comparisons were made using the non-paired Student's *t*-test.

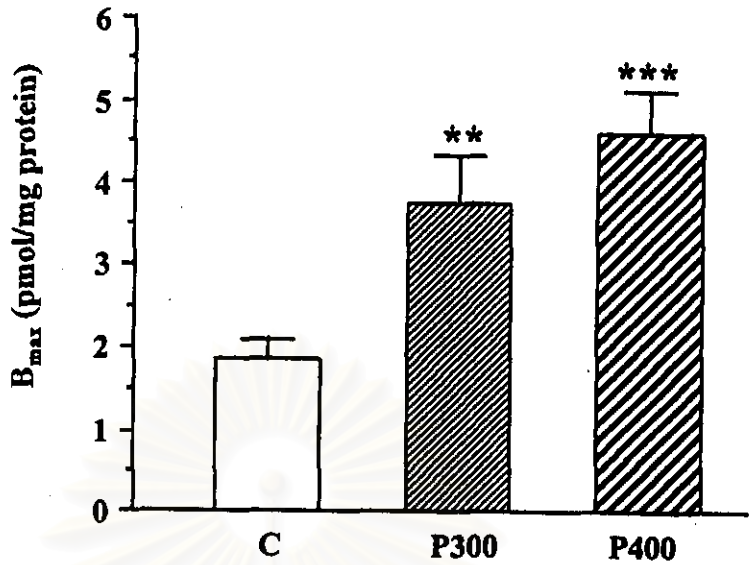

Figure 23. Comparison of the maximum number of binding sites (B_{max}) of [^{3}H]-imipramine in frontal cortex of control (C) and 15-day treated rats with paracetamol 300 (P300) and 400 (P400) mg/kg/day. B_{max} values were expressed as means ± S.E.M. of 4 rats per group.

** indicate significant difference from control group ($p < 0.01$).

*** indicate significant difference from control group ($p < 0.001$).

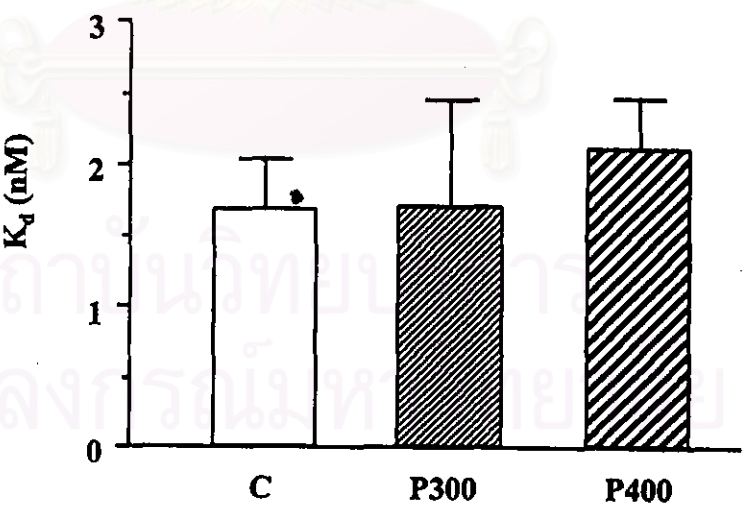

Figure 24. Comparison of the dissociation equilibrium constants (K_d) of [^{3}H]-imipramine binding sites in frontal cortex of control (C) and 15-day treated rats with paracetamol 300 (P300) and 400 (P400) mg/kg/day

K_d values were expressed as means ± S.E.M. of 5-6 rats per group.

Table 8. Binding characteristics of [^{3}H]imipramine to frontal cortex membranes in control and 15-day paracetamol-treated rats

Groups	Rat No.	K_d (nM)	B_{max} (pmol/mg protein)
Control	1	2.41	1.85
	2	1.86	2.28
	3	1.00	1.91
	4	1.29	1.63
	5	1.92	1.53
	means ± S.E.M.	**1.69 ± 0.36**	**1.84 ± 0.25**
Para 300	6	2.23	5.03
	7	1.24	3.11
	8	1.22	3.51
	9	2.11	3.30
	means ± S.E.M.	**1.70 ± 0.75**	**3.74 ± 0.60****
Para 400	10	2.37	4.75
	11	1.53	4.49
	12	2.52	5.02
	13	2.01	4.12
	means ± S.E.M.	**2.11 ± 0.35**	**4.59 ± 0.52*****

** indicate significant difference from control group ($p < 0.01$).

*** indicate significant difference from control group ($p < 0.001$).

Statistical comparisons were made by using the non-paired Student's *t*-test.

The saturation curve and Scatchard analysis of these data were shown in Fig. 25-27 and Fig. 95-97.

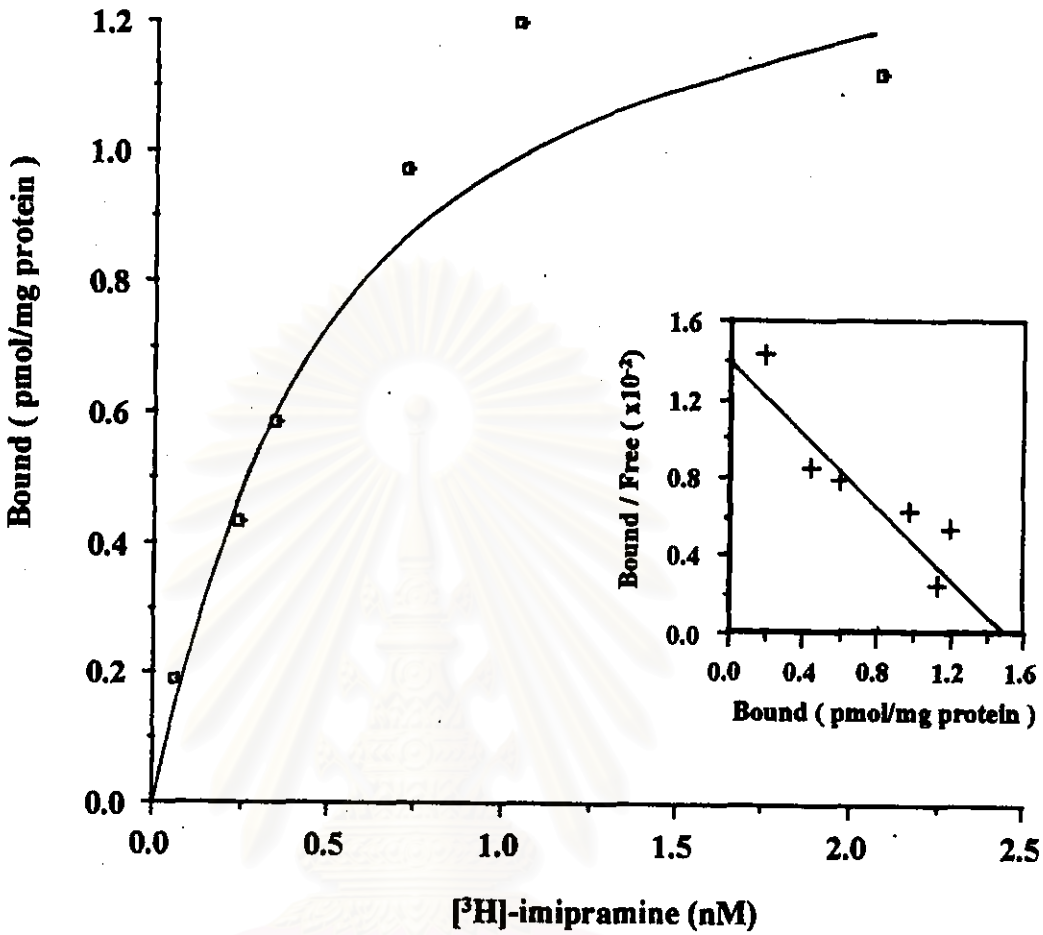

Figure 25. Saturation curve and Scatchard analysis (in the inset) of [^{3}H]-imipramine binding on frontal cortex membrane of control rat number 5, treated with vehicle once daily i.p. for 15 days. The binding was carried out in six concentrations of [^{3}H]-imipramine, ranging from 0.02 - 2.5 nM. The plots were obtained from duplicate determinations and represented the specific binding of [^{3}H]-imipramine. The line of best fit was analysed by the LIGAND computer program. The result of this experiment was shown and provided a K_d value of 1.92 nM and B_{max} value of 1.53 pmol/mg protein.

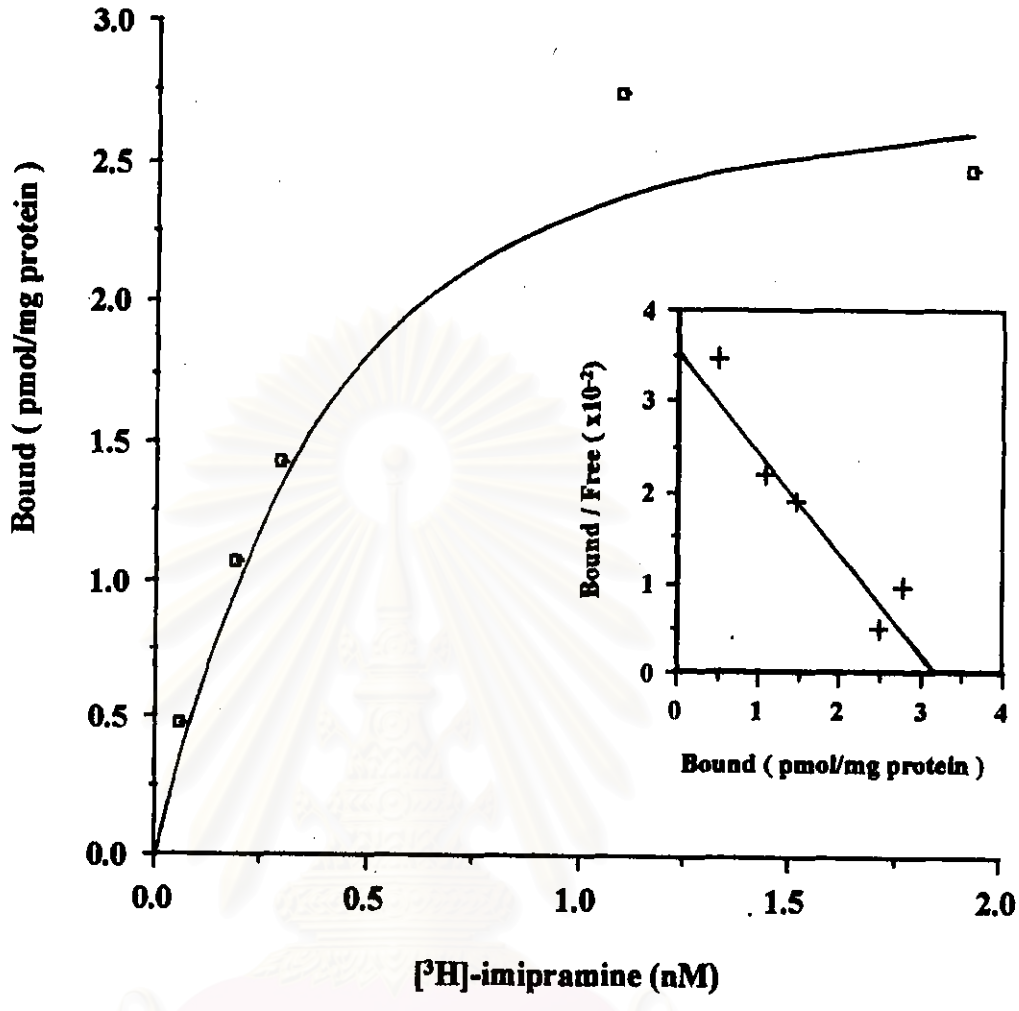

Figure 26. Saturation curve and Scatchard analysis (in the inset) of [^{3}H]-imipramine binding on frontal cortex membrane of rat number 7, treated with paracetamol 300 mg/kg/day i.p. for 15 days. The binding was carried out in five concentrations of [^{3}H]-imipramine, ranging from 0.02-2 nM. The plots were obtained from duplicate determinations and represented the specific binding of [^{3}H]-imipramine. The line of best fit was analysed by the LIGAND computer program. The result of this experiment was shown and provided a K_d value of 1.24 nM and B_{max} value of 3.11 pmol/mg protein.

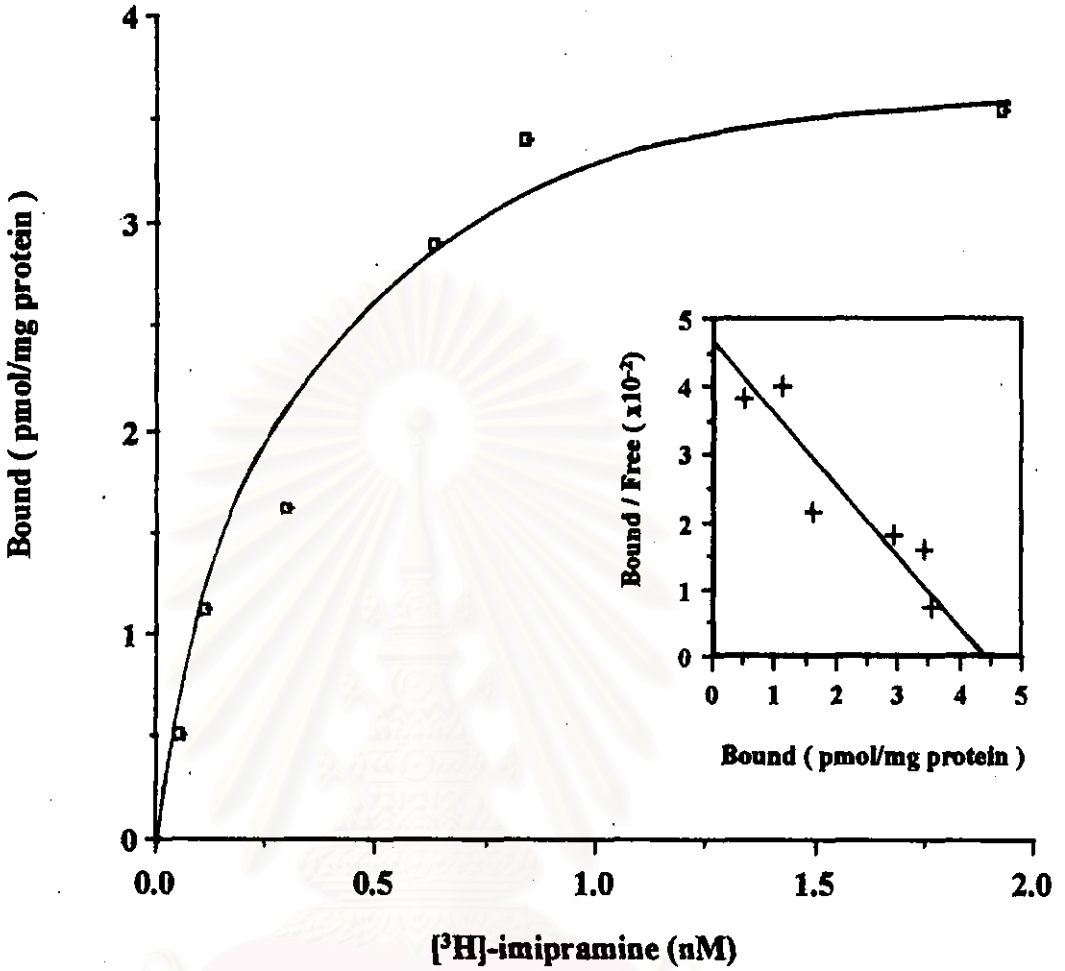

Figure 27. Saturation curve and Scatchard analysis (in the inset) of [^{3}H]-imipramine binding on frontal cortex membrane of rat number 11 , treated with paracetamol 400 mg/kg /day i.p. for 15 days. The binding was carried out in six concentrations of [^{3}H]-imipramine, ranging from 0.02 - 2 nM. The plots were obtained from duplicate determinations and represented the specific binding of [^{3}H]-imipramine. The line of best fit was analysed by the LIGAND computer program. The result of this experiment was shown and provided a K_d value of 1.53 nM and B_{max} value of 4.49 pmol/mg protein.

4. Chronic Effect of Paracetamol Treatment for 15 Days on 5-HT Uptake Sites in Rat Brain Stem

After 15 days of drug administration, the B_{max} values in the brain stem for control and treated groups with paracetamol 300 and 400 mg/kg/day were 1.06 ± 0.05, 1.16 ± 0.09 and 1.12 ± 0.09 pmol/mg protein, respectively. The K_d values for these three groups were 1.06 ± 0.14, 1.19 ± 0.13 and 1.31 ± 0.11 nM, respectively. The B_{max} and K_d values between control and treated groups with the two doses of paracetamol in this area were not different (Table 9 and 10, Fig. 28 and 29).

Table 9. Comparison of the binding characteristics of [^{3}H]imipramine to membranes of brain stem between control and 15-day paracetamol-treated rats

Groups	K_d (nM)	B_{max} (pmol/mg protein)
Control	1.06 ± 0.14	1.06 ± 0.05
Para 300	1.19 ± 0.13	1.16 ± 0.09
Para 400	1.31 ± 0.11	1.12 ± 0.09

The rats were i.p. injected with vehicle (control) and paracetamol (Para) 300 and 400 mg/kg/day. All data were expressed as means ± S.E.M. of 4 rats per group. Statistical comparisons were made using the non-paired Student's *t*-test.

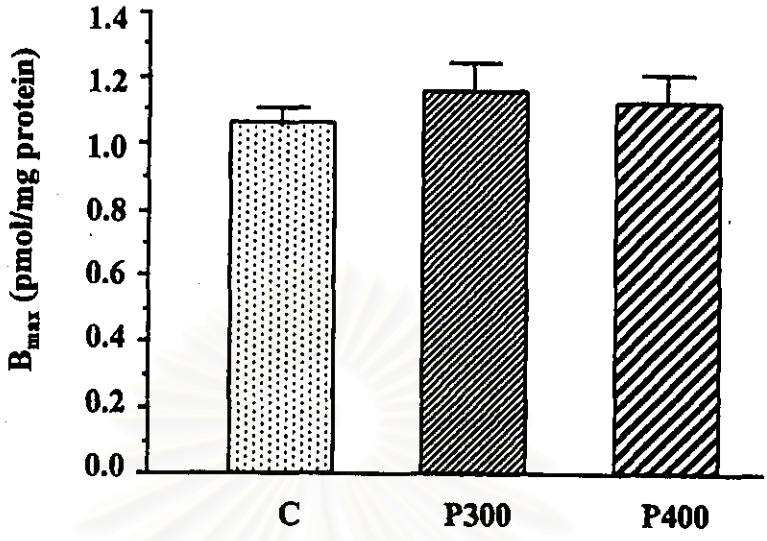

Figure 28. Comparison of the maximum number of binding sites (B_{max}) of [^{3}H]-imipramine in brain stem of control (C) and 15-day treated rats with paracetamol 300 (P300) and 400 (P400) mg/kg/day. B_{max} values were expressed as means ± S.E.M. of 4 rats per group.

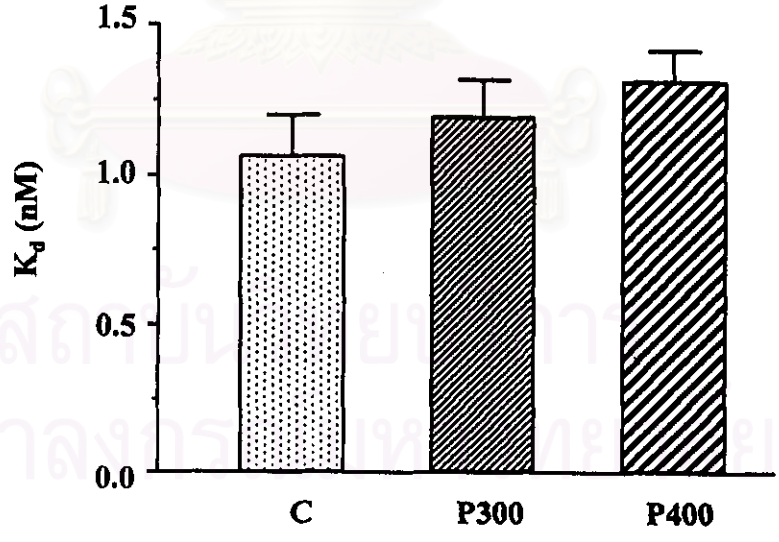

Figure 29. Comparison of the dissociation equilibrium constants (K_d) of [^{3}H]-imipramine binding sites in brain stem of control (C) and 15-day treated rats with paracetamol 300 (P300) and 400 (P400) mg/kg/day. K_d values were expressed as means ± S.E.M. of 4 rats per group.

Table 10. Binding characteristics of [^{3}H]imipramine to brain stem membranes in control and 15-day paracetamol-treated rats

Groups	Rat No.	K_d (nM)	B_{max} (pmol/mg protein)
Control	1	0.91	1.01
	2	0.89	0.95
	3	1.47	1.17
	4	0.95	1.10
	means ± S.E.M.	**1.06 ± 0.14**	**1.06 ± 0.05**
Para 300	5	1.29	1.34
	6	1.50	0.98
	7	1.04	0.98
	8	0.91	1.01
	means ± S.E.M.	**1.19 ± 0.13**	**1.16 ± 0.09**
Para 400	9	1.54	1.12
	10	1.32	1.37
	11	1.00	0.98
	12	1.36	1.00
	means ± S.E.M.	**1.31 ± 0.11**	**1.12 ± 0.09**

Statistical comparisons were made using the non-paired Student's *t*-test .

The saturation curve and Scatchard analysis of these data were shown in Fig. 30-32 and Fig. 98-100.

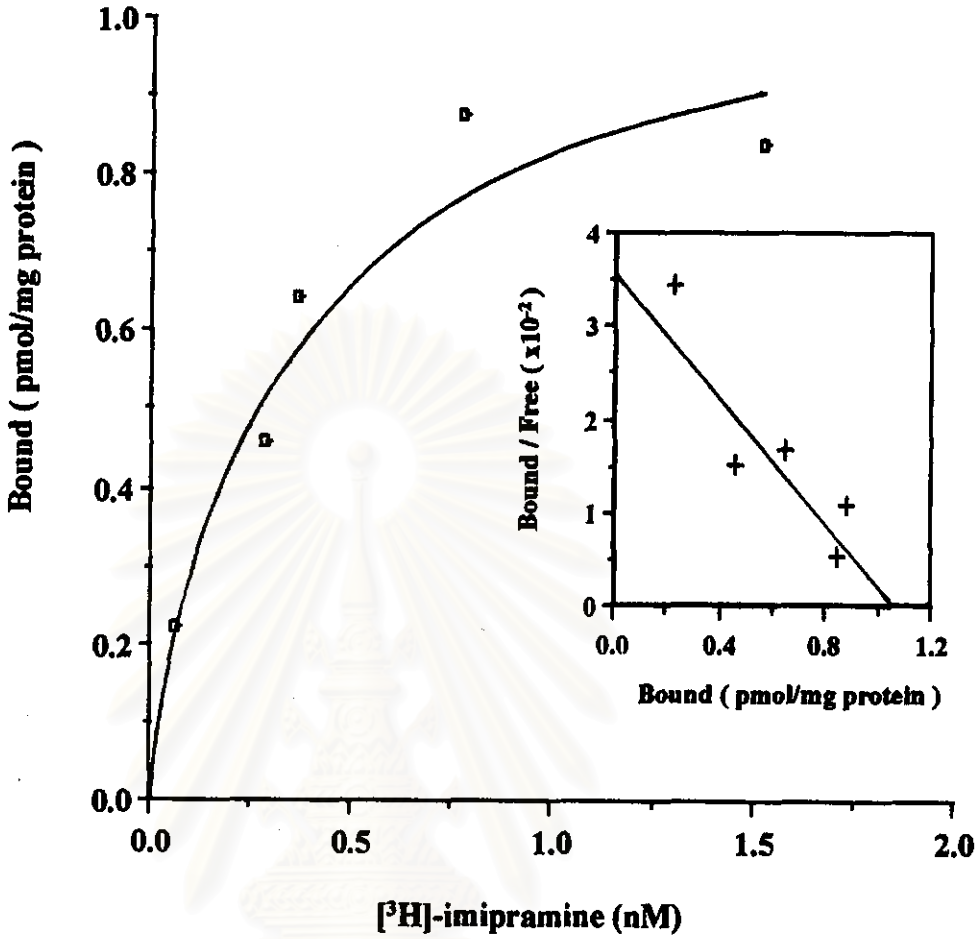

Figure 30. Saturation curve and Scatchard analysis (in the inset) of [^{3}H]-imipramine binding on brain stem membrane of control rat number 1, treated with vehicle i.p. once daily for 15 days. The binding was carried out in five concentrations of [^{3}H]-imipramine, ranging from 0.02 - 2 nM. The plots were obtained from duplicate determinations and represented the specific binding of [^{3}H]-imipramine. The line of best fit was analysed by the LIGAND computer program. The result of this experiment was shown and provided a K_d value of 0.91 nM and B_{max} value of 1.01 pmol/mg protein.

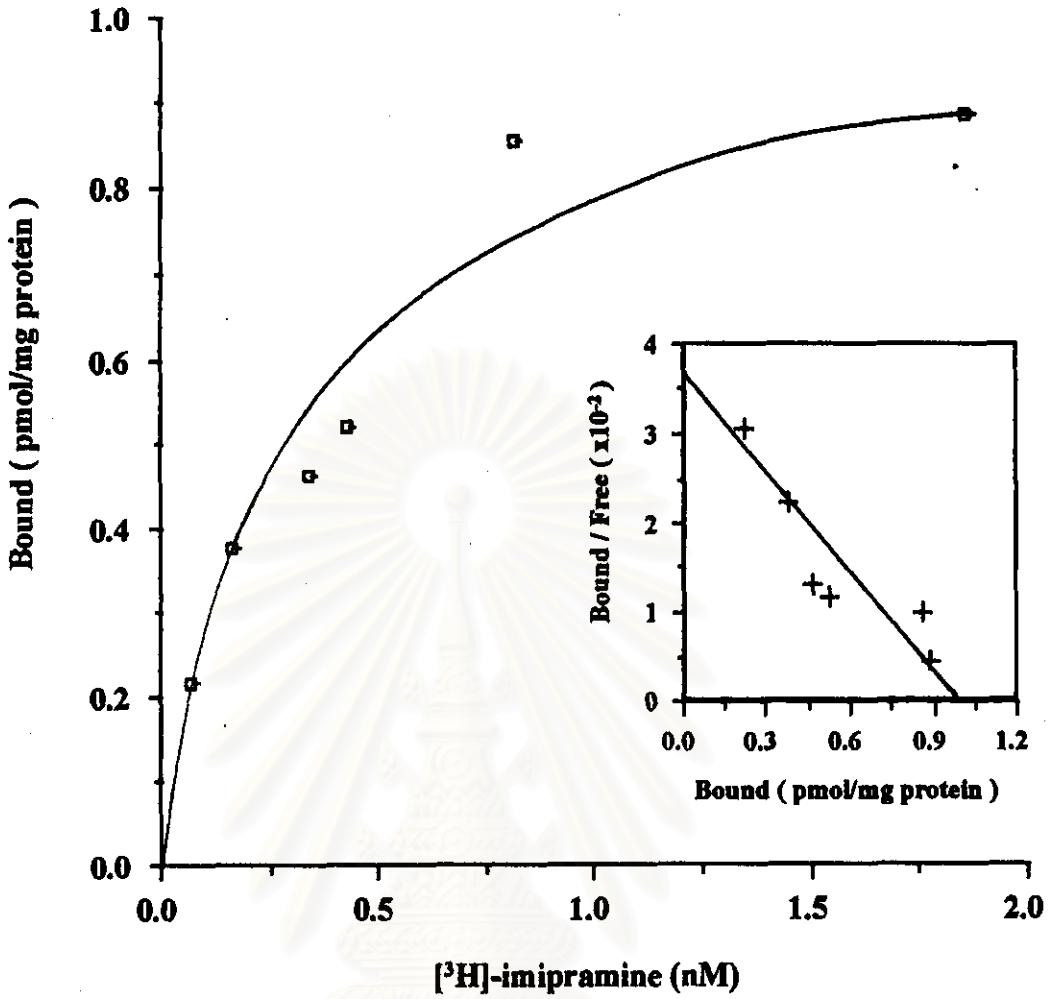

Figure 31. Saturation curve and Scatchard analysis (in the inset) of [^{3}H]-imipramine binding on brain stem membrane of rat number 7, treated with paracetamol 300 mg/kg/day i.p. for 15 days. The binding was carried out in six concentrations of [^{3}H]-imipramine, ranging from 0.02 - 2 nM. The plots were obtained from duplicate determinations and represented the specific binding of [^{3}H]-imipramine. The line of best fit was analysed by the LIGAND computer program. The result of this experiment was shown and provided a K_d value of 1.04 nM and B_{max} value of 0.98 pmol/mg protein.

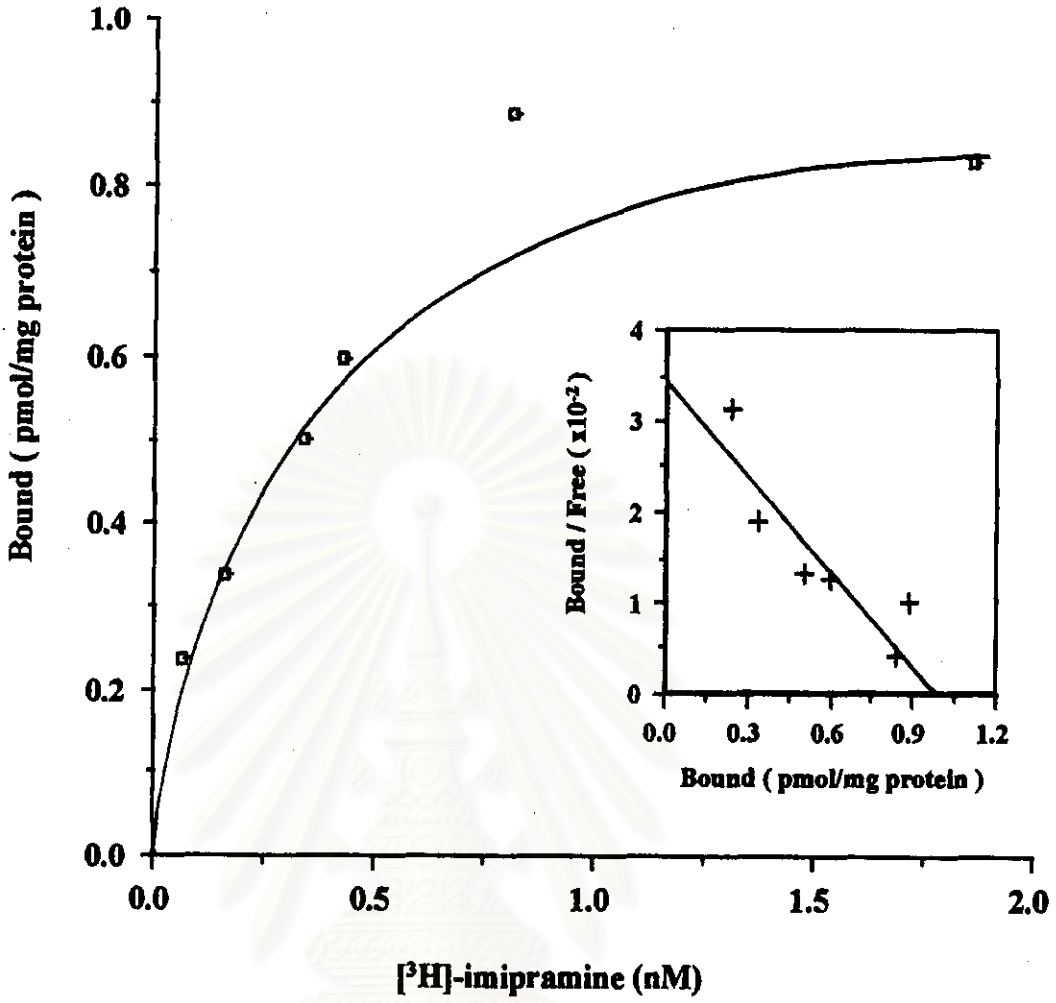

Figure 32. Saturation curve and Scatchard analysis (in the inset) of [^{3}H]-imipramine binding on brain stem membrane of rat number 11, treated with paracetamol 400 mg/kg/day i.p. for 15 days. The binding was carried out in six concentrations of [^{3}H]-imipramine, ranging from 0.02 - 2 nM. The plots were obtained from duplicate determinations and represented the specific binding of [^{3}H]-imipramine. The line of best fit was analysed by the LIGAND computer program. The result of this experiment was shown and provided a K_d value of 1.00 nM and B_{max} value of 0.98 pmol/mg protein.

5. Chronic Effect of Paracetamol Treatment for 15 Days on the Levels of Platelets 5-HT and Its Metabolite, 5-HIAA

After 15 days of drug administration, the mean values of 5-HT for control and treated rats with paracetamol 300 and 400 mg/kg/day were 3911.32 ± 438.07, 7024.67 ± 905.97 and 7342.78 ± 1041.35 ng/10^8 platelets, respectively. Whereas, the mean values of its metabolite, 5-HIAA, for these three groups were 7116.84 ± 1199.23, 5151.29 ± 792.60 and 5796.84 ± 465.69 ng/10^8 platelets, respectively. The levels of 5-HT in treated groups were significantly increased from control ($p < 0.01$), whereas those of 5-HIAA remained unchanged among these three groups (Table 11 and Fig. 33).

Table 11. Amounts of platelets 5-HT and its metabolite, 5-HIAA, in control and 15-day paracetamol-treated groups

Groups	5-HT (ng/10^8 platelets)	5-HIAA (ng/10^8platelets)
Control	3911.32 ± 438.07	7116.84 ± 1199.23
Para 300	7024.67 ± 905.97**	5151.29 ± 792.60
Para 400	7342.83 ± 1041.35**	5796.84 ± 465.69

** indicate significant difference from control group ($p < 0.01$). Statistical comparisons were made using the non-paired Student's *t*-test. Para 300 = paracetamol 300 mg/kg/day. and Para 400 = paracetamol 400 mg/kg/day.

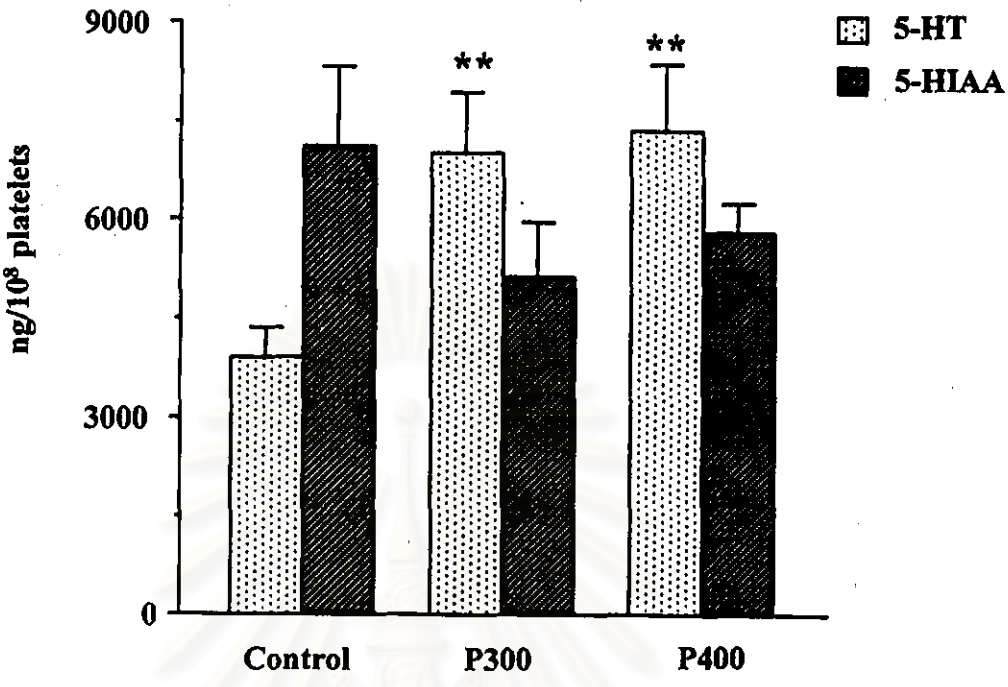

Figure 33. Comparison of the levels of 5-HT and 5-HIAA in platelets of control and 15-day treated rats with paracetamol 300 (P300), and 400 (P400) mg/kg/day. Values were expressed as means ± S.E.M. of 5-6 rats per group. ** indicate significant difference from control group ($p < 0.01$, non-paired Student's *t*-test).

6. Chronic Effect of Paracetamol Treatment for 30 Days on Central 5-HT_{2A} Serotonin Receptors in Rat Frontal Cortex

After 30 days of drug administration, the B_{max} values in the frontal cortex for control and treated groups with paracetamol 300 and 400 mg/kg/day were 2.05 ± 0.20, 1.38 ± 0.12 and 1.34 ± 0.13 pmol/mg protein, respectively. The K_d values for these three groups were 1.41 ± 0.07, 1.22 ± 0.11 and 1.12 ± 0.19 nM, respectively. The difference of the B_{max} values between control and treated groups with the two highest doses of paracetamol in this area was statistically significant ($p < 0.05$), whereas the K_d values remained unchanged (Table 12 and 13, Fig.34 and 35).

Table 12. Comparison of the binding characteristics of [^{3}H]spiperone to membranes of frontal cortex between control and 30-day paracetamol-treated rats

Groups	K_d (nM)	B_{max} (pmol/mg protein)
Control	1.41 ± 0.07	2.05 ± 0.20
Para 300	1.22 ± 0.11	1.38 ± 0.12*
Para 400	1.12 ± 0.19	1.34 ± 0.13*

The rats were i.p. injected with vehicle (control) and paracetamol (Para) 300 and 400 mg/kg/day. Data were expressed as means ± S.E.M. of 5-6 rats per group. Statistical comparisons were made using the non-paired Student's *t*-test.

* indicate significant difference from control ($p < 0.05$)

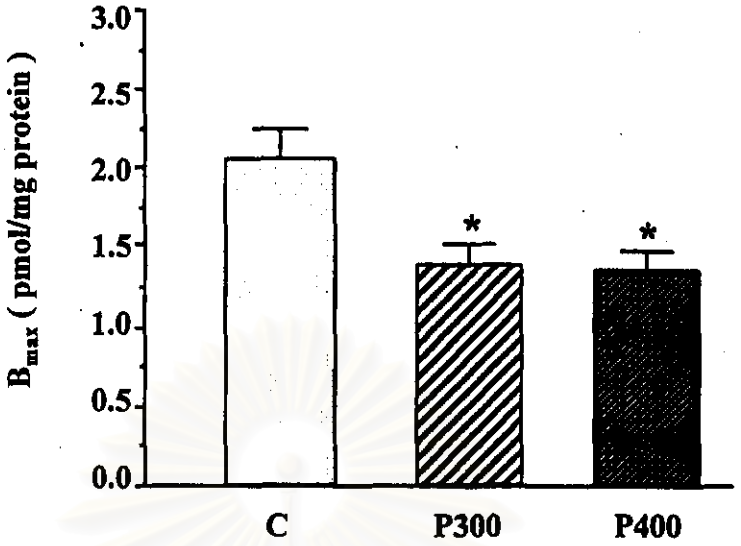

Figure 34. Comparison of the maximum number of binding sites (B_{max}) of [^{3}H]-spiperone in frontal cortex of control (C) and 30-day treated rats with paracetamol 300 (P300) and 400 (P400) mg/kg/day. B_{max} values were expressed as means ± S.E.M of 5-6 rats per group.

* indicate significant difference from control group ($p < 0.05$).

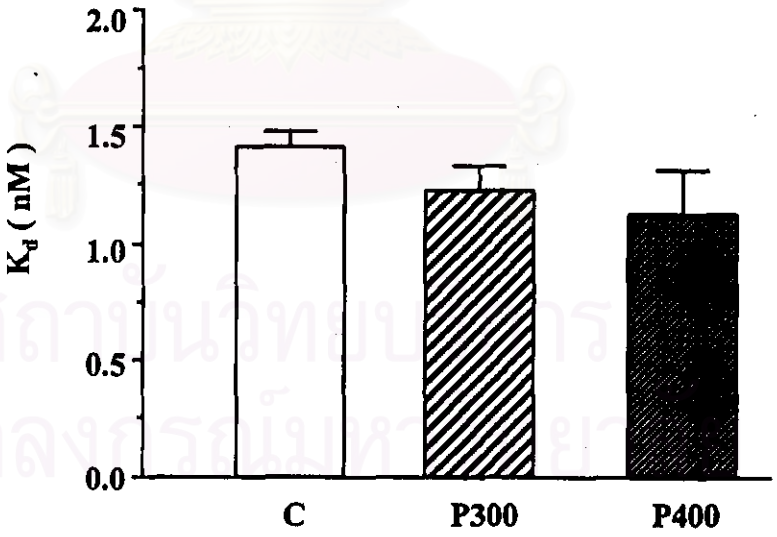

Figure 35. Comparison of the dissociation equilibrium constants (K_d) of [^{3}H]-spiperone binding sites in frontal cortex of control (C) and 30-day treated rats with paracetamol 300 (P300) and 400 (P400) mg/kg/day. K_d values were expressed as means ± S.E.M. of 5-6 rats per group.

Table 13. Binding characteristics of [^{3}H]spiperone to frontal cortex membranes in control and 30-day paracetamol-treated rats

Groups	Rat No.	K_d (nM)	B_{max} (pmol/mg protein)
Control	1	1.4	1.67
	2	1.2	1.55
	3	1.2	2.83
	4	1.5	2.14
	5	1.6	1.73
	6	1.6	2.38
	means ± S.E.M.	**1.41 ± 0.07**	**2.05 ± 0.20**
Para 300	7	1.0	1.51
	8	1.5	1.57
	9	0.9	1.14
	10	1.5	1.71
	11	1.2	1.01
	means ± S.E.M.	**1.22 ± 0.11**	**1.38 ± 0.12***
Para 400	12	1.5	1.65
	13	0.8	1.09
	14	0.9	1.52
	15	1.7	1.48
	16	0.7	0.97
	means ± S.E.M.	**1.12 ± 0.19**	**1.34 ± 0.13***

* indicate significant difference from control group ($p < 0.05$, the non-paired Student's *t*-test).

The saturation curve and Scatchard analysis of these data were shown in Fig. 36-39 and Fig. 101-103.

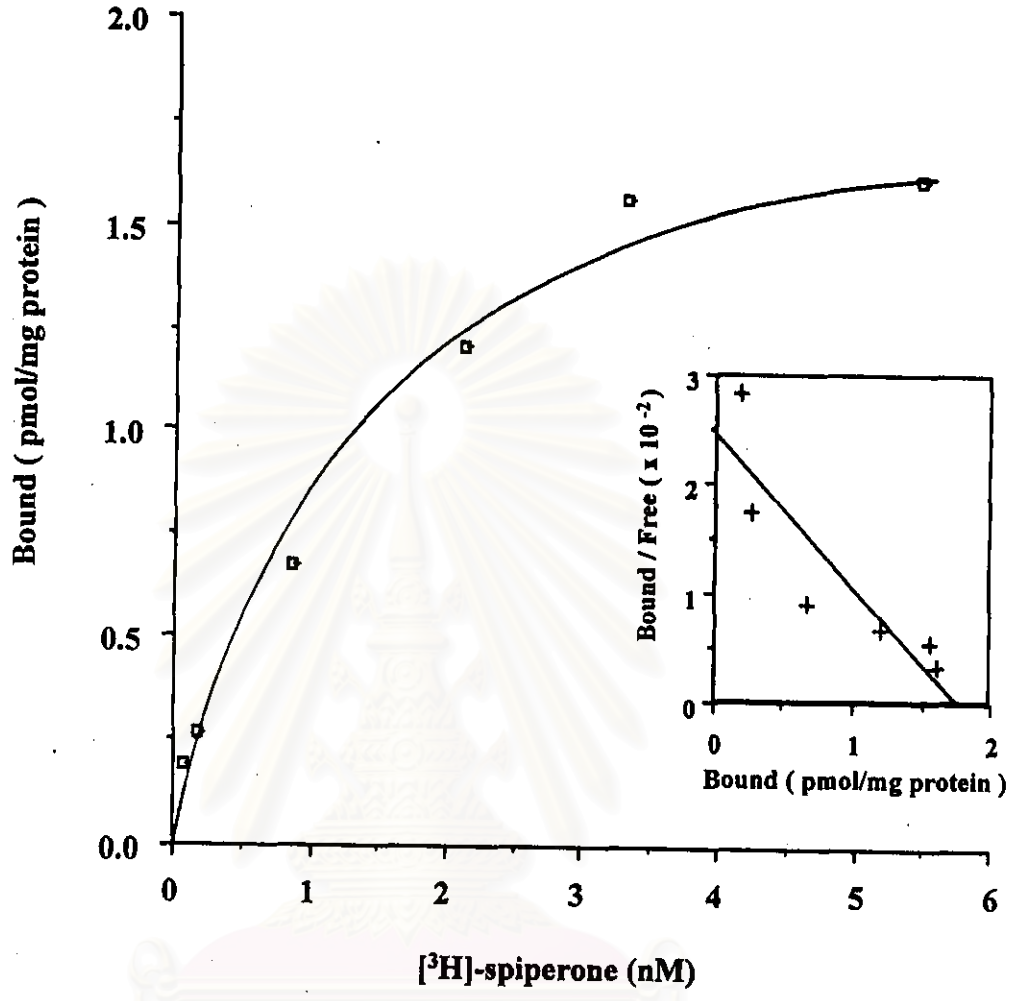

Figure 36. Saturation curve and Scatchard analysis (in the inset) of [^{3}H]-spiperone binding on frontal cortex membrane of control rat number 5, treated with vehicle i.p. once daily for 30 days. The binding was carried out in six concentrations of [^{3}H]-spiperone, ranging from 0.06 - 6 nM. The plots were obtained from duplicate determinations and represented the specific binding of [^{3}H]-spiperone. The line of best fit was analysed by the LIGAND computer program. The result of this experiment was shown and provided a K_d value of 1.6 nM and B_{max} value of 1.73 pmol/mg protein.

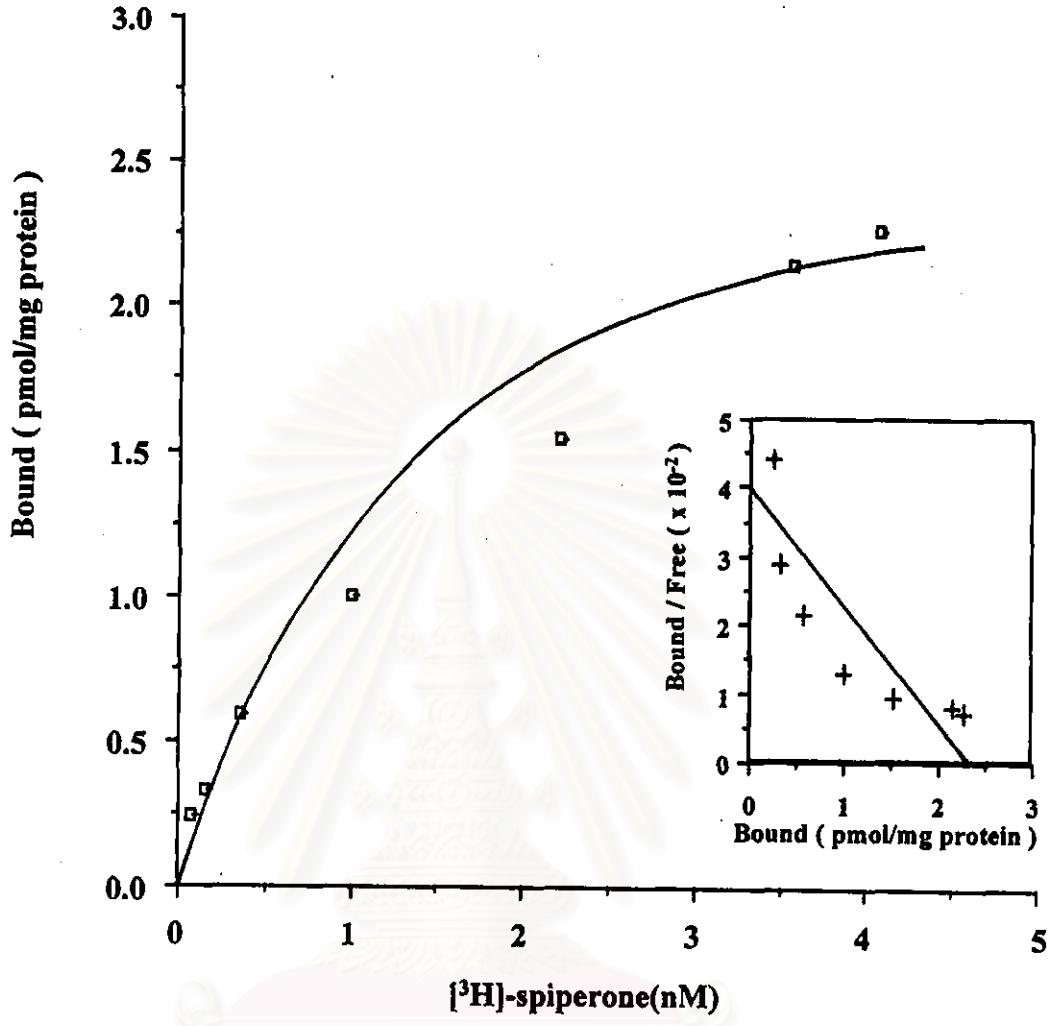

Figure 37. Saturation curve and Scatchard analysis (in the inset) of [^{3}H]-spiperone binding on frontal cortex membrane of control rat number 6, treated with vehicle i.p. once daily for 30 days. The binding was carried out in seven concentrations of [^{3}H]-spiperone, ranging from 0.05 - 5 nM. The plots were obtained from duplicate determinations and represented the specific binding of [^{3}H]-spiperone. The line of best fit was analysed by the LIGAND computer program. The result of this experiment was shown and provided a K_d value of 1.6 nM and B_{max} value of 2.38 pmol/mg protein.

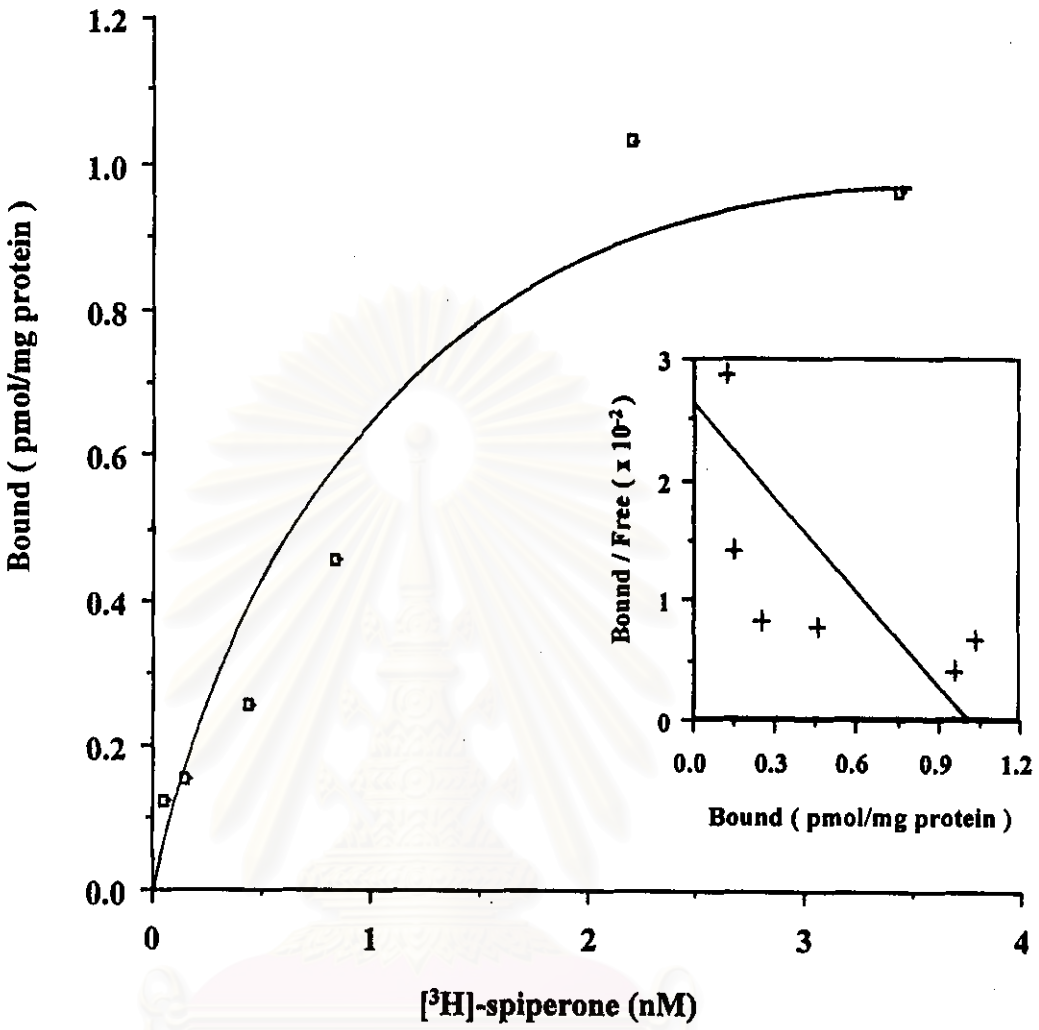

Figure 38. Saturation curve and Scatchard analysis (in the inset) of [^{3}H]-spiperone binding on frontal cortex membrane of rat number 11 , treated with paracetamol 300 mg/kg/day i.p. for 30 days. The binding was carried out in six concentrations of [^{3}H]-spiperone, ranging from 0.04 - 4 nM. The plots were obtained from duplicate determinations and represented the specific binding of [^{3}H]-spiperone. The line of best fit was analysed by the LIGAND computer program. The result of this experiment was shown and provided a K_d value of 1.2 nM and B_{max} value of 1.01 pmol/mg protein.

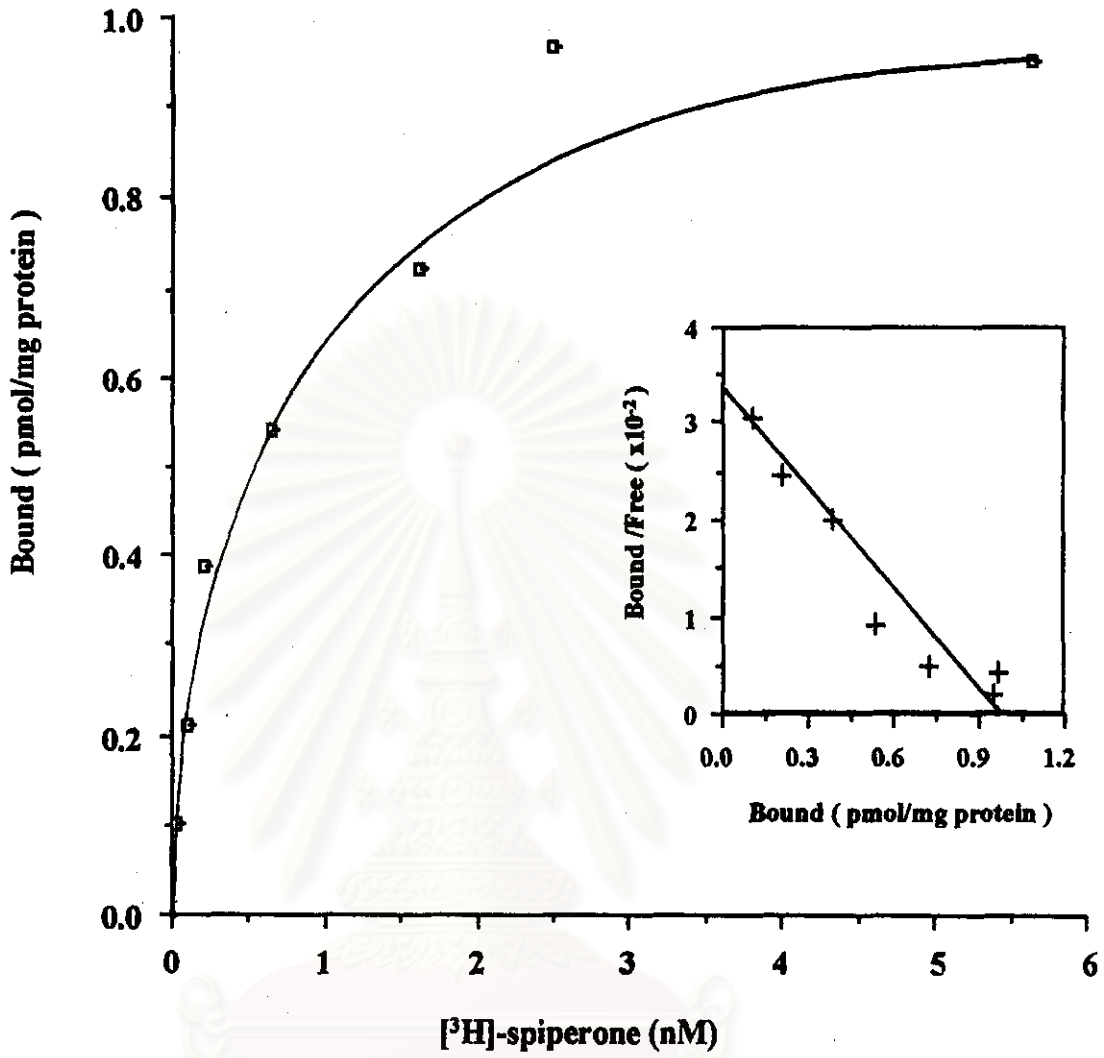

Figure 39. Saturation curve and Scatchard analysis (in the inset) of [^{3}H]-spiperone binding on frontal cortex membrane of rat number 16, treated with paracetamol 400 mg/kg/day i.p. for 30 days. The binding was carried out in seven concentrations of [^{3}H]-spiperone, ranging from 0.06-6 nM. The plots were obtained from duplicate determinations and represented the specific binding of [^{3}H]-spiperone. The line of best fit was analysed by the LIGAND computer program. The result of this experiment was shown and provided a K_d value of 0.7 nM and B_{max} value of 0.97 pmol/mg protein.

7. Chronic Effect of Paracetamol Treatment for 30 Days on Central 5-HT_{2A} Serotonin Receptors in Rat Brain Stem

After 30 days of drug administration, the B_{max} values in the brain stem for control and treated groups with paracetamol 300 and 400 mg/kg/day were 0.87 ± 0.06, 0.92 ± 0.12 and 0.82 ± 0.12 pmol/mg protein, respectively. The K_d values for these three groups were 0.80 ± 0.16, 0.70 ± 0.11 and 0.82 ± 0.08 nM, respectively. The B_{max} and K_d values between control and treated groups in this area were not different. (Table 14 and 15, Fig. 40 and 41).

Table 14. Comparison of the binding characteristics of [^{3}H]spiperone to membranes of brain stem between control and 30-day paracetamol-treated rats

Groups	K_d (nM)	B_{max} (pmol/mg protein)
Control	0.80 ± 0.16	0.87 ± 0.06
Para 300	0.70 ± 0.11	0.92 ± 0.12
Para 400	0.82 ± 0.08	0.82 ± 0.12

The rats were i.p. injected with vehicle (control) and paracetamol (Para) 300 and 400 mg/kg/day. Data were expressed as means ± S.E.M. of 5-6 rats per group. Statistical comparisons were made using the non-paired Student's *t*-test.

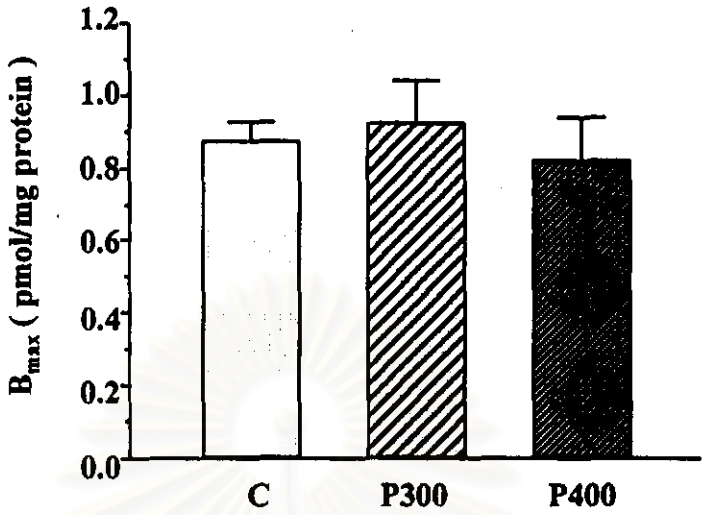

Figure 40. Comparison of the maximum number of binding sites (B_{max}) of [^{3}H]-spiperone in brain stem of control (C) and 30-day treated rats with paracetamol 300 (P300) and 400 (P400) mg/kg/day. B_{max} values were expressed as means ± S.E.M. of 5-6 rats per group.

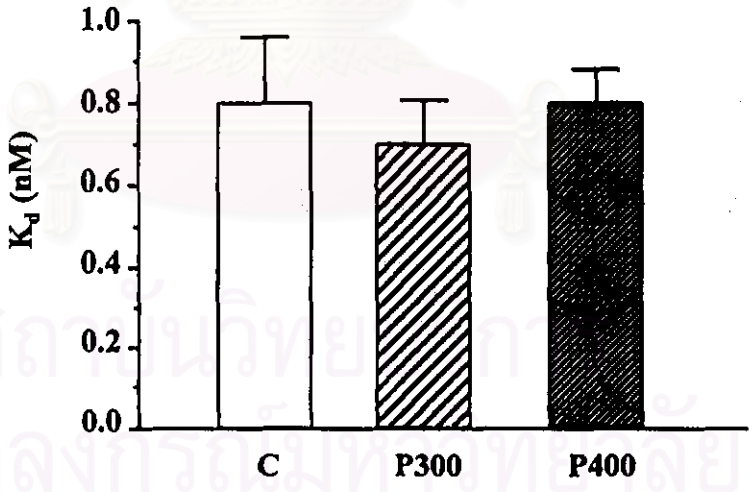

Figure 41. Comparison of the dissociation equilibrium constants (K_d) of [^{3}H]-spiperone binding sites in brain stem of control (C) and 30-day treated rats with paracetamol 300 (P300) and 400 (P400) mg/kg/day. K_d values were expressed as means ± S.E.M. of 5-6 rats per group.

Table 15. Binding characteristics of [^{3}H]spiperone to brain stem membranes in control and 30-day paracetamol-treated rats

Groups	Rat No.	K_d (nM)	B_{max} (pmol/mg protein)
Control	1	0.7	0.97
	2	0.5	0.66
	3	1.6	0.76
	4	0.6	0.82
	5	0.6	0.93
	6	0.8	1.08
	means ± S.E.M.	**0.80 ± 0.16**	**0.87 ± 0.06**
Para 300	7	1.0	1.28
	8	0.6	0.75
	9	0.7	0.87
	10	0.5	0.76
	means ± S.E.M.	**0.70 ± 0.11**	**0.92 ± 0.12**
Para 400	11	0.6	0.58
	12	1.0	0.98
	13	0.9	0.86
	14	0.6	0.52
	15	1.0	1.15
	means ± S.E.M.	**0.82 ± 0.08**	**0.82 ± 0.12**

Statistical comparisons were made using the non-paired Student's *t*-test .

The saturation curve and Scatchard analysis of these data were shown in Fig. 42-44 and Fig. 104-105.

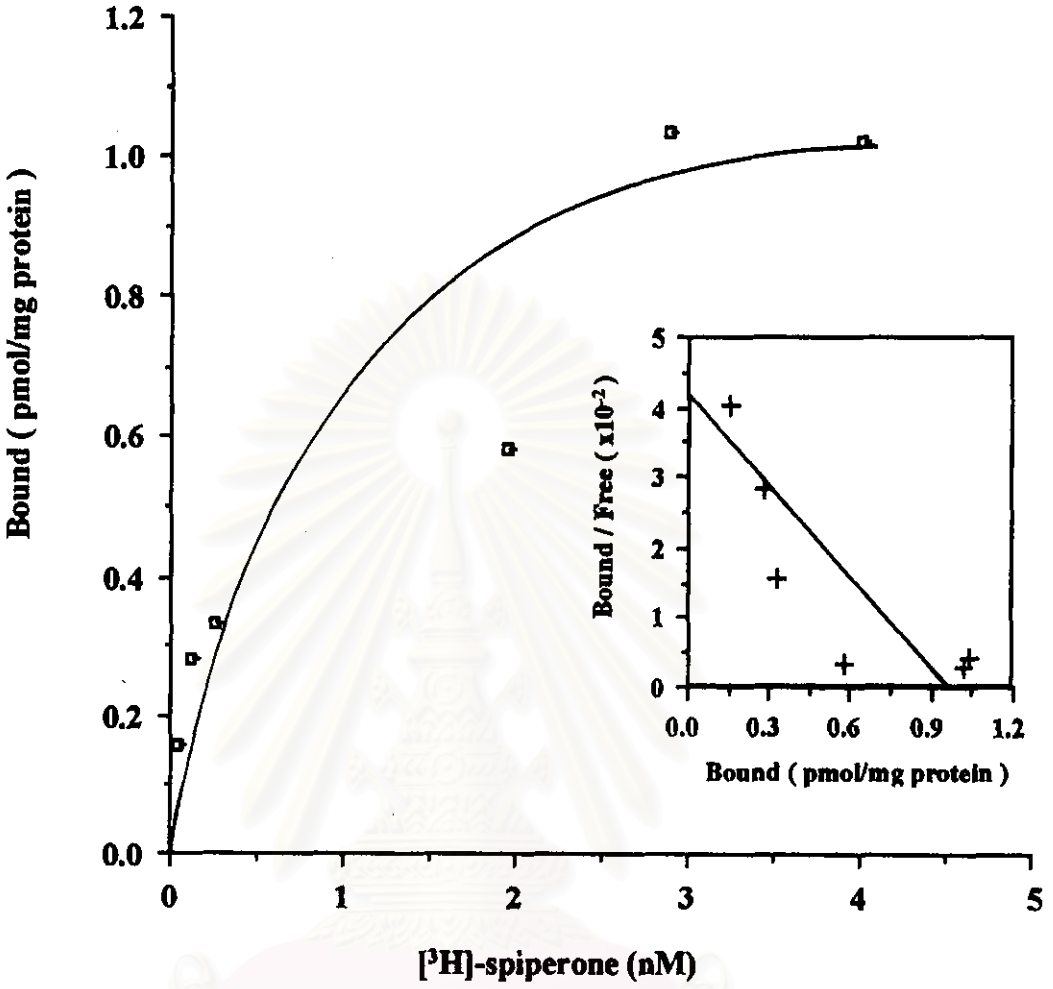

Figure 42. Saturation curve and Scatchard analysis (in the inset) of [³H]-spiperone binding on brain stem membrane of control rat number 5, treated with vehicle i.p. for 30 days. The binding was carried out in six concentrations of [³H]-spiperone, ranging from 0.05 - 5 nM. The plots were obtained from duplicate determinations and represented the specific binding of [³H]-spiperone. The line of best fit was analysed by the LIGAND computer program. The result of this experiment was shown and provided a K_d value of 0.6 nM and B_{max} value of 0.93 pmol/mg protein.

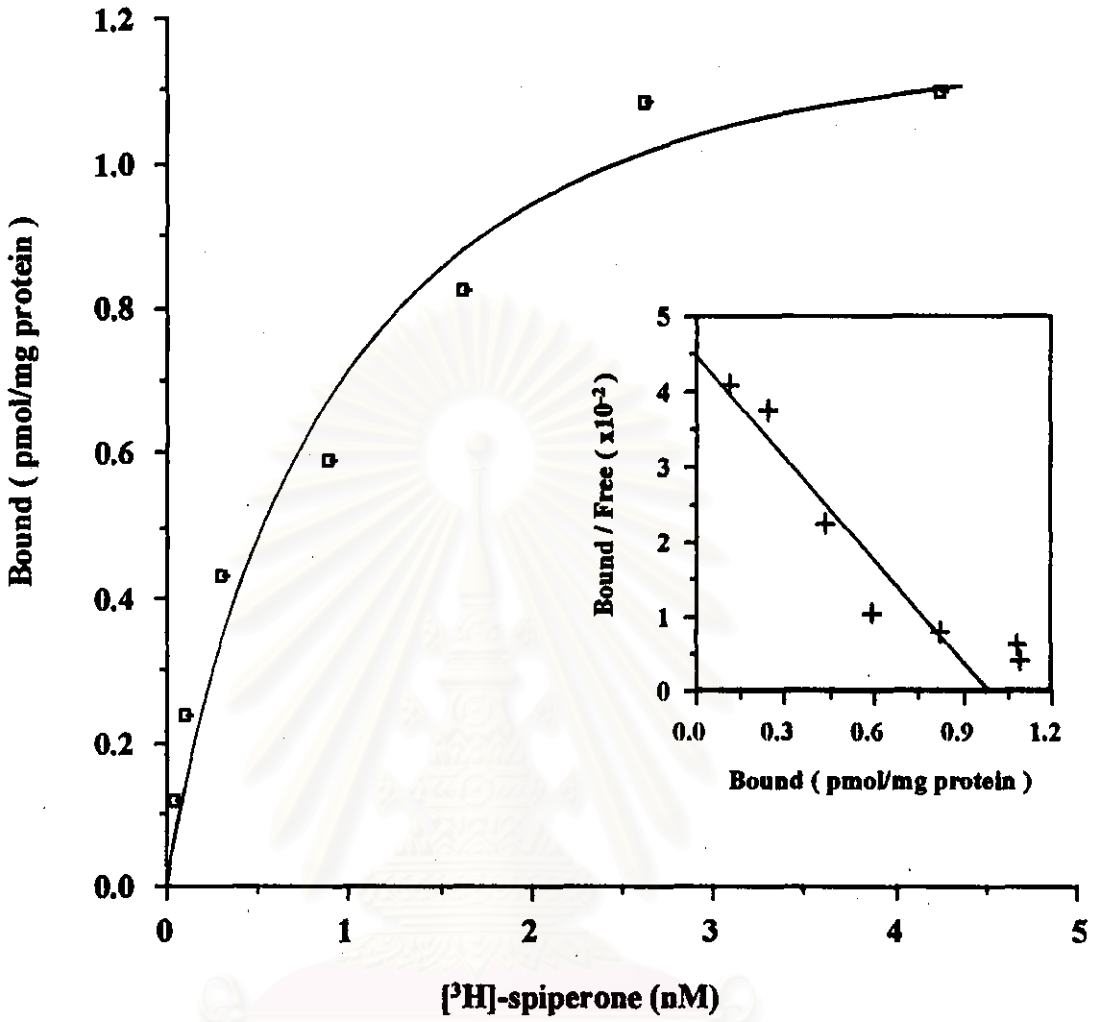

Figure 43. Saturation curve and Scatchard analysis (in the inset) of [³H]-spiperone binding on brain stem membrane of control rat number 6, treated with vehicle i.p. for 30 days. The binding was carried out in seven concentrations of [³H]-spiperone, ranging from 0.05 - 5 nM. The plots were obtained from duplicate determinations and represented the specific binding of [³H]-spiperone. The line of best fit was analysed by the LIGAND computer program. The result of this experiment was shown and provided a K_d value of 0.8 nM and B_{max} value of 1.08 pmol/mg protein.

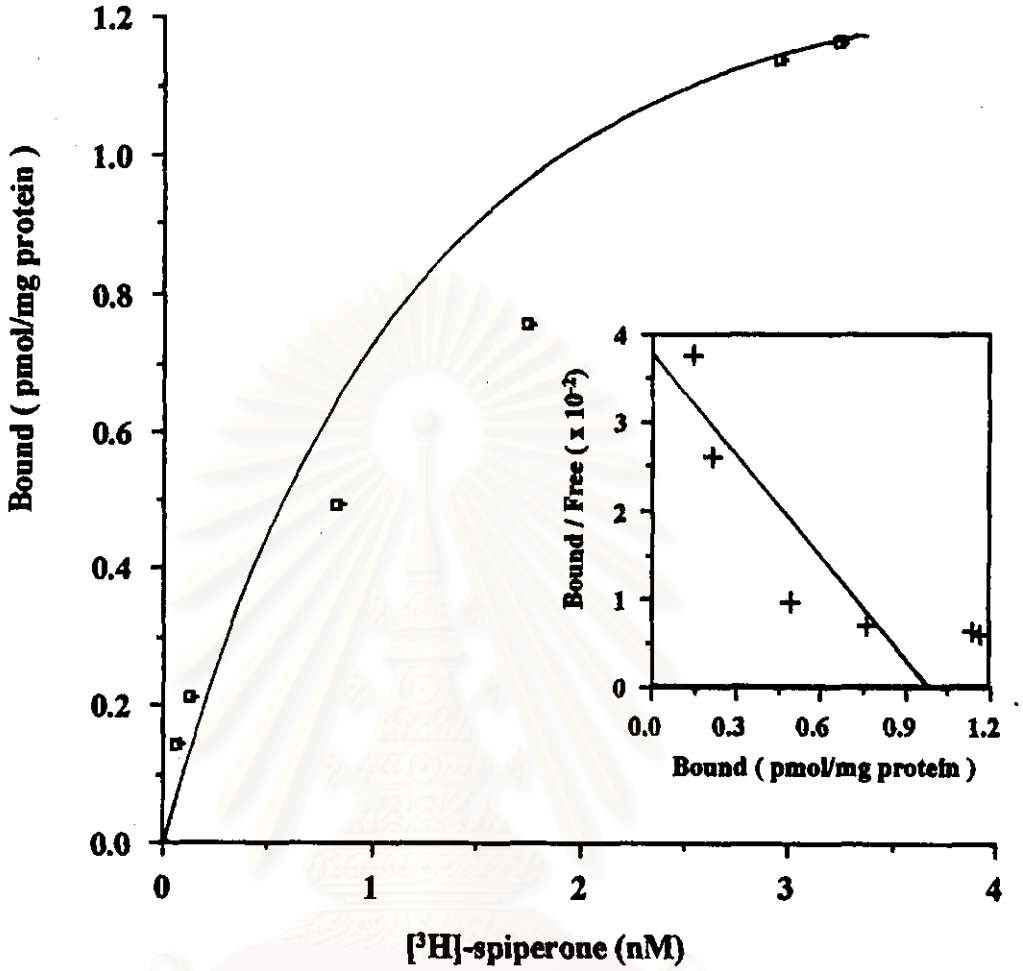

Figure 44. Saturation curve and Scatchard analysis (in the inset) of [^{3}H]-spiperone binding on brain stem membrane of rat number 15, treated with paracetamol 400 mg/kg/day i.p. for 30 days. The binding was carried out in six concentrations of [^{3}H]-spiperone, ranging from 0.2 - 8 nM. The plots were obtained from duplicate determinations and represented the specific binding of [^{3}H]-spiperone. The line of best fit was analysed by the LIGAND computer program. The result of this experiment was shown and provided a K_d value of 1.0 nM and B_{max} value of 1.15 pmol/mg protein.

8. Chronic Effect of Paracetamol Treatment for 30 Days on 5-HT Uptake Sites in Rat Frontal Cortex

After 30 days of drug administration, the B_{max} values in the frontal cortex for control and treated groups with paracetamol 300 and 400 mg/kg/day were 1.68 ± 0.13, 2.38 ± 0.14 and 2.71 ± 0.18 pmol/mg protein, respectively. The K_d values for these three groups were 1.28 ± 0.25, 1.62 ± 0.29 and 1.08 ± 0.18 nM, respectively. The difference of the B_{max} values between control and treated groups with the two doses of paracetamol in this area was statistically significant ($p < 0.01$). However, there was no significant difference in K_d values among the three groups (Table 16 and 17, Fig.45 and 46).

Table 16. Comparison of the binding characteristics of [^{3}H]imipramine to membranes of frontal cortex between control and 30-day paracetamol-treated rats

Groups	K_d (nM)	B_{max} (pmol/mg protein)
Control	1.28 ± 0.25	1.68 ± 0.13
Para 300	1.62 ± 0.29	2.38 ± 0.14*
Para 400	1.08 ± 0.18	2.71 ± 0.18*

The rats were i.p. injected with vehicle (control) and paracetamol (Para) 300 and 400 mg/kg/day. Data were expressed as means ± S.E.M. of 4-5 rats per group. Statistical comparisons were made using the non-paired Student's *t*-test.

* indicate significant difference from control ($p < 0.05$).

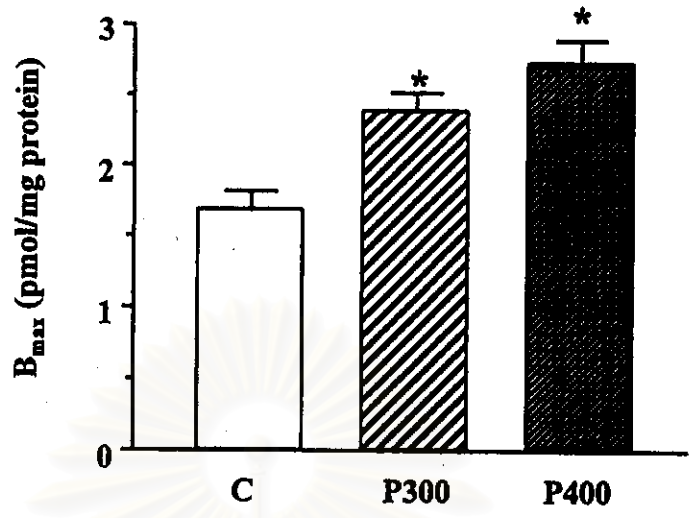

Figure 45. Comparison of the maximum number of binding sites (B_{max}) of [^{3}H]-imipramine in frontal cortex of control (C) and 30-day treated rats with paracetamol 300 (P300) and 400 (P400) mg/kg/day. B_{max} values were expressed as means ± S.E.M. of 4-5 rats per group.
* indicate significant difference from control group ($p < 0.05$).

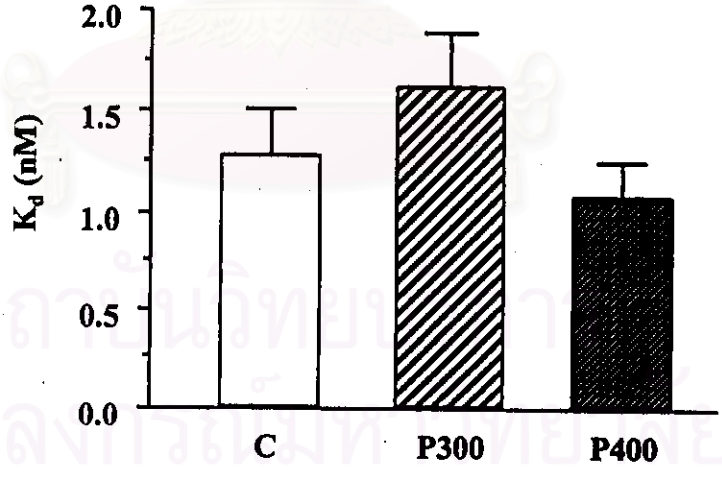

Figure 46. Comparison of the dissociation equilibrium constants (K_d) of [^{3}H]-imipramine binding sites in frontal cortex of control (C) and 30-day treated rats with paracetamol 300 (P300) and 400 (P400) mg/kg/day. K_d values were expressed as means ± S.E.M. of 4-5 rats per group.

Table 17. Binding characteristics of [^{3}H]imipramine to frontal cortex membranes in control and 30-day paracetamol-treated rats

Groups	Rat No.	K_d (nM)	B_{max} (pmol/mg protein)
Control	1	1.08	1.53
	2	1.58	1.88
	3	0.68	1.40
	4	1.76	1.91
	5	1.06	1.95
	means ± S.E.M.	**1.28 ± 0.25**	**1.68 ± 0.13**
Para 300	6	1.99	2.67
	7	1.19	2.30
	8	1.07	2.03
	9	2.23	2.51
	means ± S.E.M.	**1.62 ± 0.29**	**2.38 ± 0.14***
Para 400	10	0.76	2.37
	11	0.85	2.52
	12	1.58	3.19
	13	1.12	2.76
	means ± S.E.M.	**1.08 ± 0.18**	**2.71 ± 0.18***

* indicate significant difference from control group ($p < 0.05$, non-paired Student's *t*-test).

The saturation curve and Scatchard analysis of these data were shown in Fig. 47-49 and Fig. 107-109.

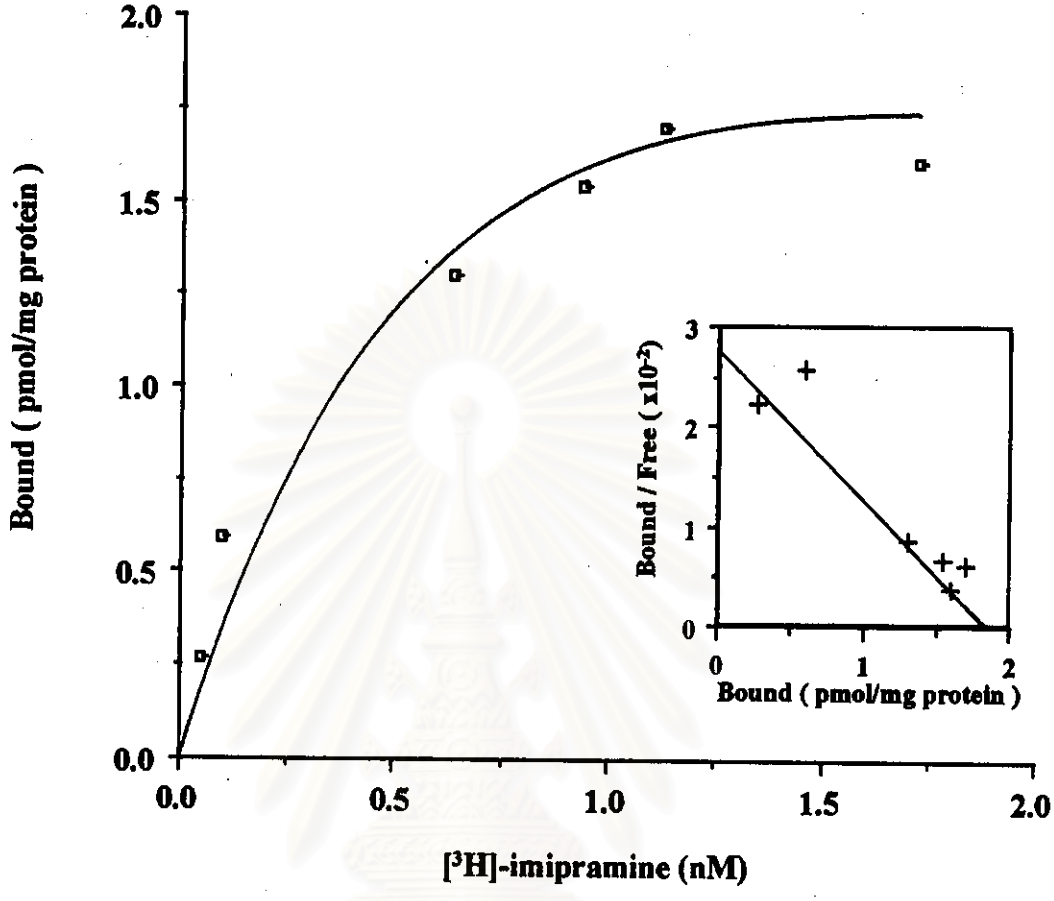

Figure 47. Saturation curve and Scatchard analysis (in the inset) of [^{3}H]-imipramine binding on frontal cortex membrane of control rat number 5, treated with vehicle i.p. once daily for 30 days. The binding was carried out in six concentrations of [^{3}H]-imipramine, ranging from 0.02 - 2 nM. The plots were obtained from duplicate determinations and represented the specific binding of [^{3}H]-imipramine. The line of best fit was analysed by the LIGAND computer program. The result of this experiment was shown and provided a K_d value of 1.06 nM and B_{max} value of 1.95 pmol/mg protein.

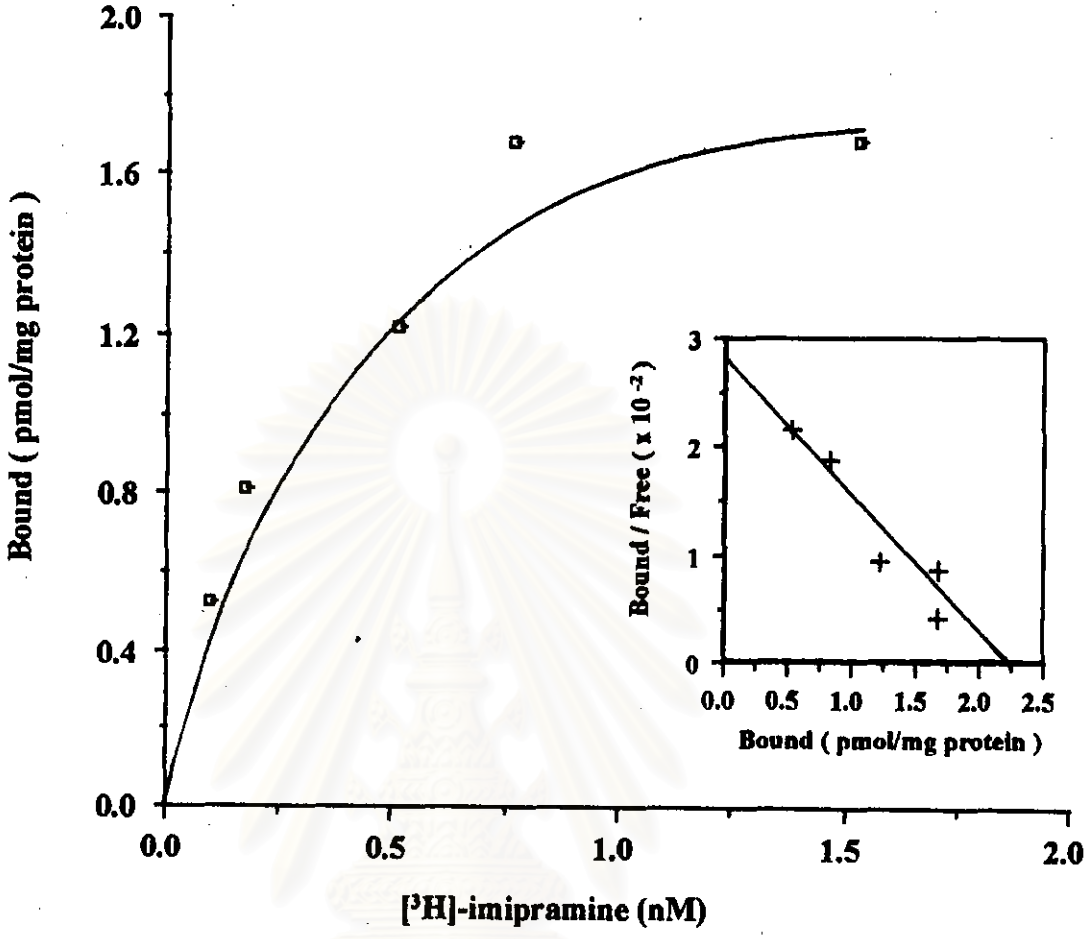

Figure 48. Saturation curve and Scatchard analysis (in the inset) of [³H]-imipramine binding on frontal cortex membrane of rat number 8 , treated with paracetamol 300 mg/kg/day i.p. for 30 days. The binding was carried out in five concentrations of [³H]-imipramine, ranging from 0.02 - 2 nM. The plots were obtained from duplicate determinations and represented the specific binding of [³H]-imipramine. The line of best fit was analysed by the LIGAND computer program. The result of this experiment was shown and provided a K_d value of 1.07 nM and B_{max} value of 2.30 pmol/mg protein.

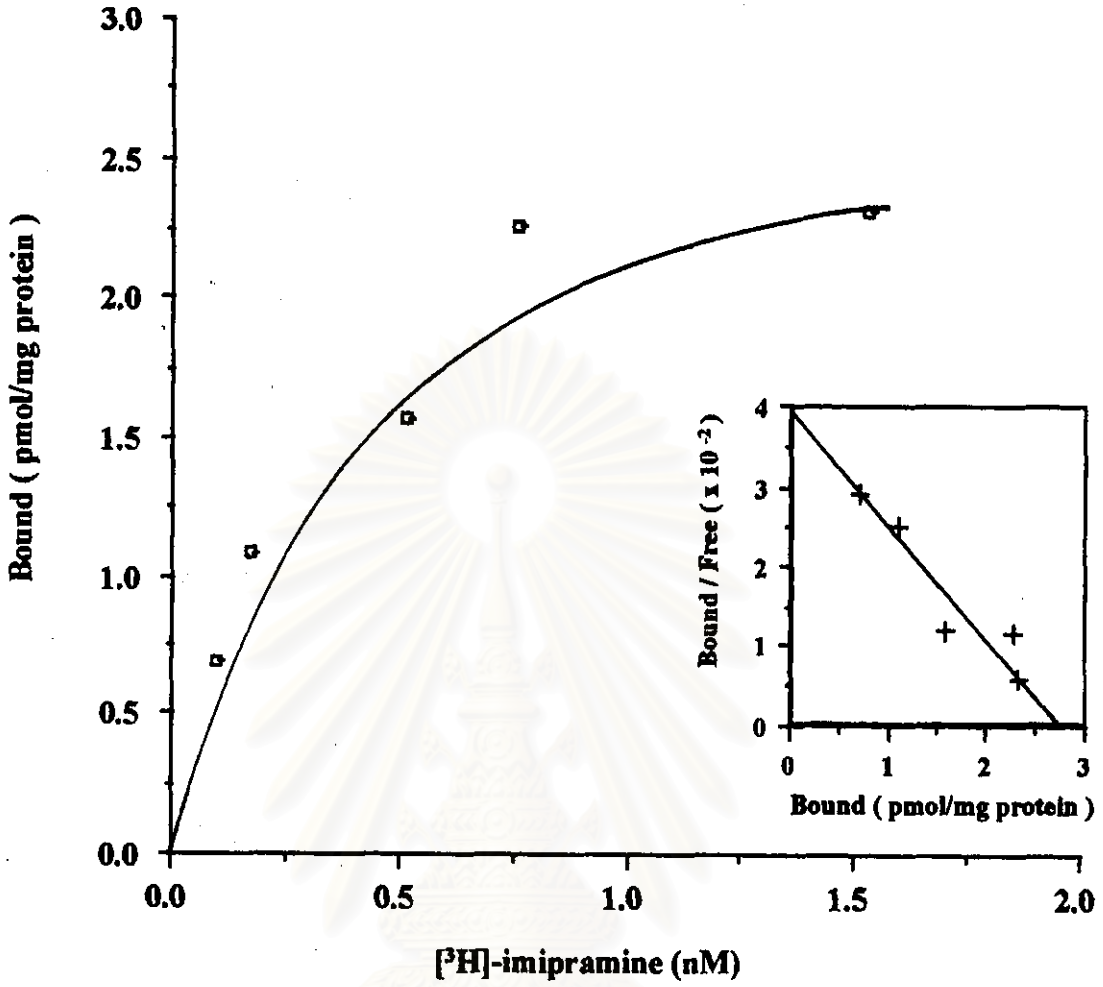

Figure 49. Saturation curve and Scatchard analysis (in the inset) of [^{3}H]-imipramine binding on frontal cortex membrane of rat number 13 , treated with paracetamol 400 mg/kg/day i.p. for 30 days. The binding was carried out in five concentrations of [^{3}H]-imipramine, ranging from 0.02 - 2 nM. The plots were obtained from duplicate determinations and represented the specific binding of [^{3}H]-imipramine. The line of best fit was analysed by the LIGAND computer program. The result of this experiment was shown and provided a K_d value of 1.12 nM and B_{max} value of 2.76 pmol/mg protein.

9. Chronic Effect of Paracetamol Treatment for 30 Days on 5-HT Uptake Sites in Rat Brain Stem

After 30 days of drug administration, the B_{max} values in the brain stem for control and treated groups with paracetamol 300 and 400 mg/kg/day were 1.01 ± 0.07, 1.04 ± 0.06 and 1.12 ± 0.09 pmol/mg protein, respectively. The K_d values for these three groups were 1.25 ± 0.15, 1.14 ± 0.18 and 1.04 ± 0.09 nM, respectively. The B_{max} and K_d values between control and treated groups with the two doses of paracetamol in this area were not different (Table 18 and 19, Fig.50 and 51).

Table 18. Comparison of the binding characteristics of [^{3}H]imipramine to membranes of brain stem between control and 30-day paracetamol-treated rats

Groups	K_d (nM)	B_{max} (pmol/mg protein)
Control	1.25 ± 0.15	1.01 ± 0.07
Para 300	1.14 ± 0.18	1.04 ± 0.06
Para 400	1.04 ± 0.09	1.12 ± 0.07

The rats were i.p. injected with vehicle (control) and paracetamol (Para) 300 and 400 mg/kg/day. All data were expressed as means ± S.E.M. of 4 rats per group. Statistical comparisons were made using the non-paired Student's *t*-test.

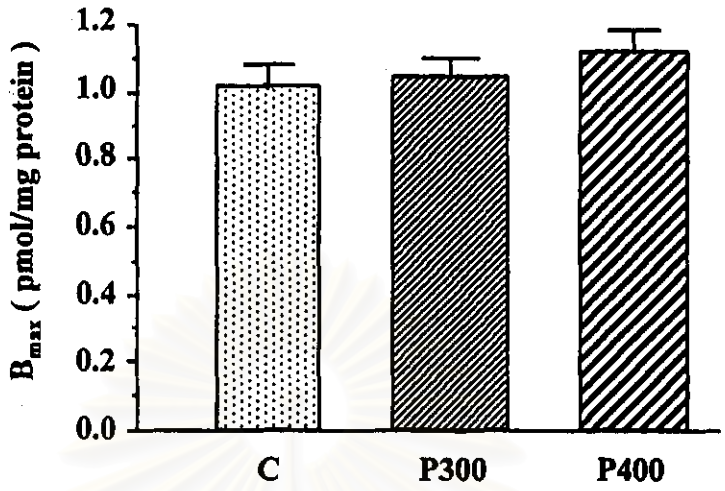

Figure 50. Comparison of the maximum number of binding sites (B_{max}) of [^{3}H]-imipramine in brain stem of control (C) and 30-day treated rats with paracetamol 300 (P300) and 400 (P400) mg/kg/day. B_{max} values were expressed as means ± S.E.M. of 5-6 rats per group.

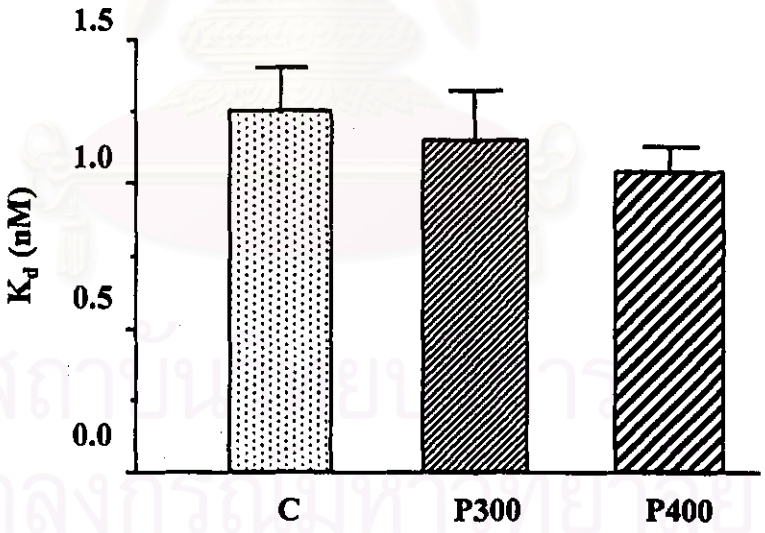

Figure 51. Comparison of the dissociation equilibrium constants (K_d) of [^{3}H]-imipramine binding sites in brain stem of control (C) and 30-day treated rats with paracetamol 300 (P300) and 400 (P400) mg/kg/day. K_d values were expressed as means ± S.E.M. of 5-6 rats per group.

Table 19. Binding characteristics of [^{3}H]imipramine to brain stem membranes in control and 30-day paracetamol-treated rats

Groups	Rat No.	K_d (nM)	B_{max} (pmol/mg protein)
Control	1	1.30	1.05
	2	1.60	1.16
	3	0.88	0.84
	4	1.23	0.98
	means ± S.E.M.	**1.25 ± 0.15**	**1.01 ± 0.07**
Para 300	5	0.73	0.98
	6	1.25	0.96
	7	1.59	1.21
	8	0.98	1.01
	means ± S.E.M.	**1.14 ± 0.18**	**1.04 ± 0.06**
Para 400	9	0.93	1.15
	10	0.89	1.29
	11	1.06	0.98
	12	1.28	1.04
	means ± S.E.M.	**1.04 ± 0.09**	**1.12 ± 0.07**

Statistical comparisons were made using the non-paired Student's *t*-test.

The saturation curve and Scatchard analysis of these data were shown in Fig. 52-54 and Fig. 110-112.

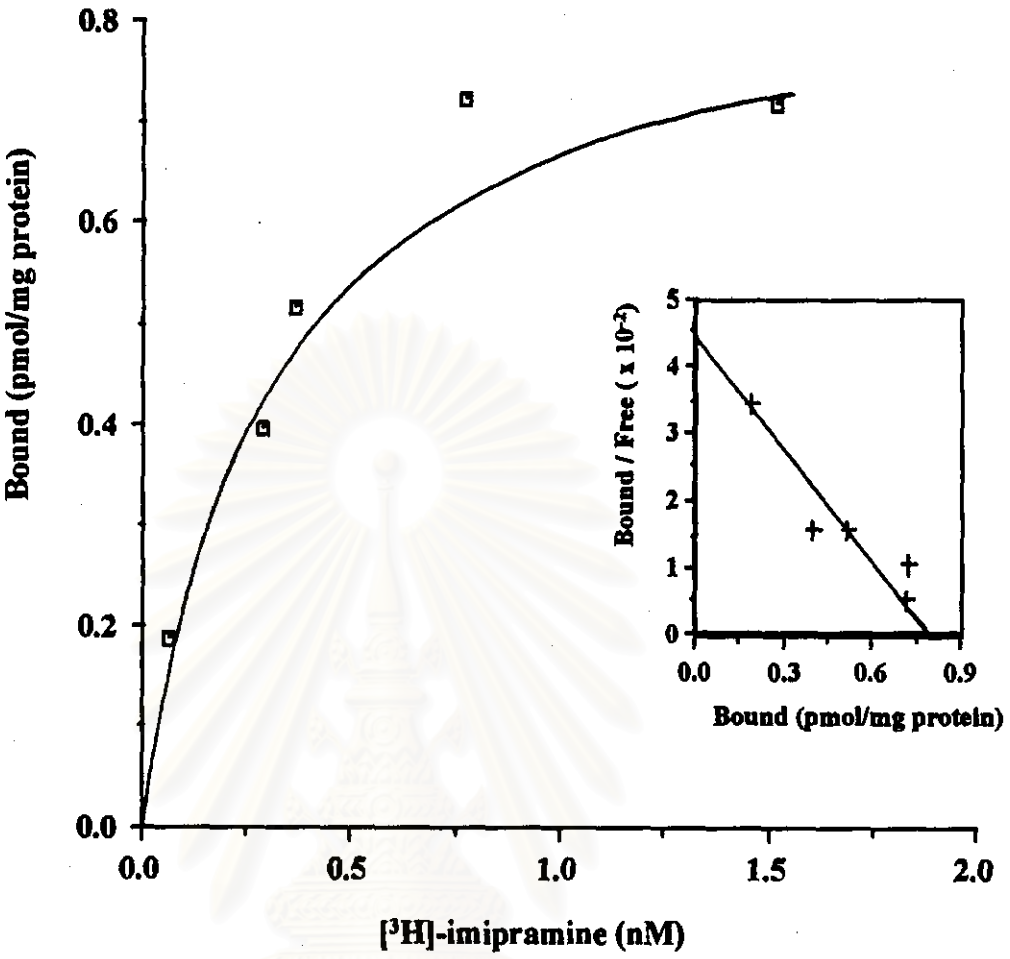

Figure 52. Saturation curve and Scatchard analysis (in the inset)of [^{3}H]-imipramine binding on brain stem membrane of control rat number 3 , treated with vehicle i.p. once daily for 30 days. The binding was carried out in five concentrations of [^{3}H]-imipramine ranging from 0.02 - 2 nM. The plots were obtained from duplicate determinations and represented the specific binding of [^{3}H]-imipramine. The line of best fit was analysed by the LIGAND computer program. The result of this experiment was shown and provided a K_d value of 0.88 nM and B_{max} value of 0.84 pmol/mg protein.

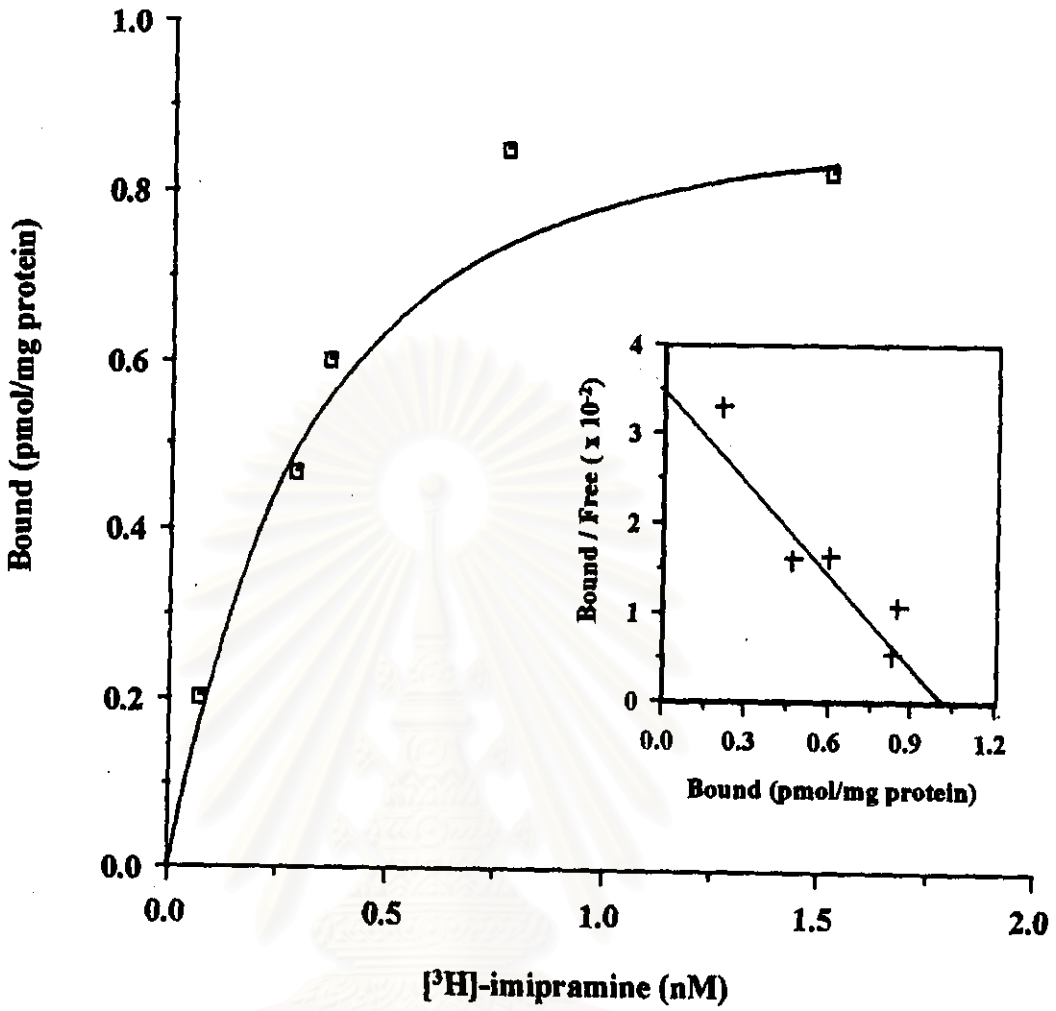

Figure 53. Saturation curve and Scatchard analysis (in the inset) of [^{3}H]-imipramine binding on brain stem membrane of rat number 8, treated with paracetamol 300 mg/kg/day i.p. for 30 days. The binding was carried out in five concentrations of [^{3}H]-imipramine, ranging from 0.02 - 2 nM. The plots were obtained from duplicate determinations and represented the specific binding of [^{3}H]-imipramine. The line of best fit was analysed by the LIGAND computer program. The result of this experiment was shown and provided a K_d value of 0.98 nM and B_{max} value of 1.01 pmol/mg protein.

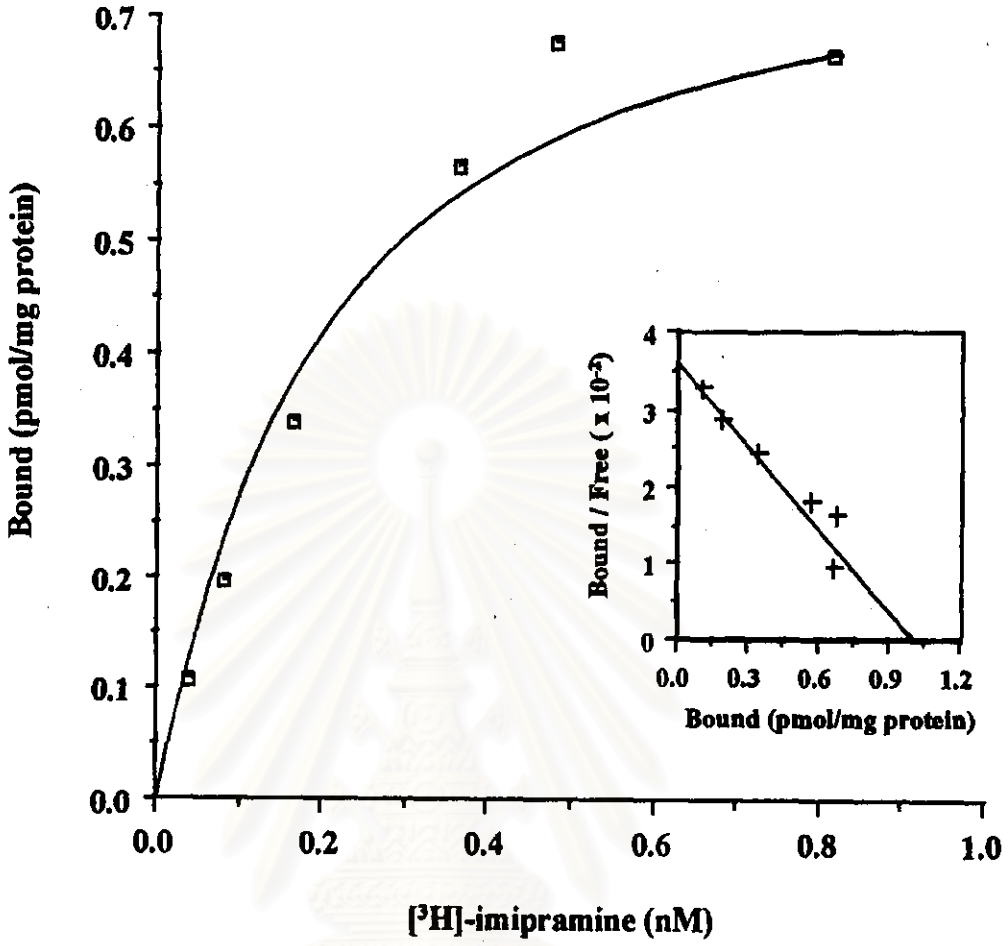

Figure 54. Saturation curve and Scatchard analysis (in the inset) of [^{3}H]-imipramine binding on brain stem membrane of rat number 12, treated with paracetamol 400 mg/kg/day i.p. for 30 days. The binding was carried out in six concentrations of [^{3}H]-imipramine, ranging from 0.01 - 1 nM. The plots were obtained from duplicate determinations and represented the specific binding of [^{3}H]-imipramine. Theline of best fit was analysed by the LIGAND computer program. The result of this experiment was shown and provided a K_d value of 1.28 nM and B_{max} value of 1.04 pmol/mg protein.

10. Chronic Effect of Paracetamol Treatment for 30 Days on the Levels of Platelets 5-HT and Its Metabolite, 5-HIAA

After 30 days of drug administration, the mean values of 5-HT for control and treated rats with paracetamol 300 and 400 mg/kg were 3911.32 ± 438.07, 3329.99 ± 895.51 and 3636.51 ± 714.21 ng/10^8 platelets, respectively. Whereas, the mean values of metabolite of 5-HT, 5-HIAA, for these three groups were 7116.84 ± 1199.23, 13788.65 ± 2373.95 and 13866.77 ± 2517.06 ng/10^8platelets, respectively. The levels of 5-HIAA were significantly increased in treated groups ($p < 0.05$), whereas those of 5-HT remained unchanged among these three groups (Table 20 and Fig.55).

Table 20. Amounts of 5-HT and its metabolite, 5-HIAA, in control and 30-day paracetamol-treated groups

Groups	5-HT (ng/10^8 platelets)	5-HIAA (ng/10^8 platelets)
Control	3911.32 ± 438.07	7116.84 ± 1137.69
Para 300	3329.99 ± 895.51	13788.65 ± 2373.95*
Para 400	3636.51 ± 714.21	13866.77 ± 2517.06*

Values were expressed as means ± S.E.M. of 8-9 rats per group. * indicate significantly difference from control group ($p < 0.05$). Statistical comparisons were made using the non-paired Student's *t*-test. Para 300 = paracetamol 300 mg/kg/day and Para 400 = paracetamol 400 mg/kg/day.

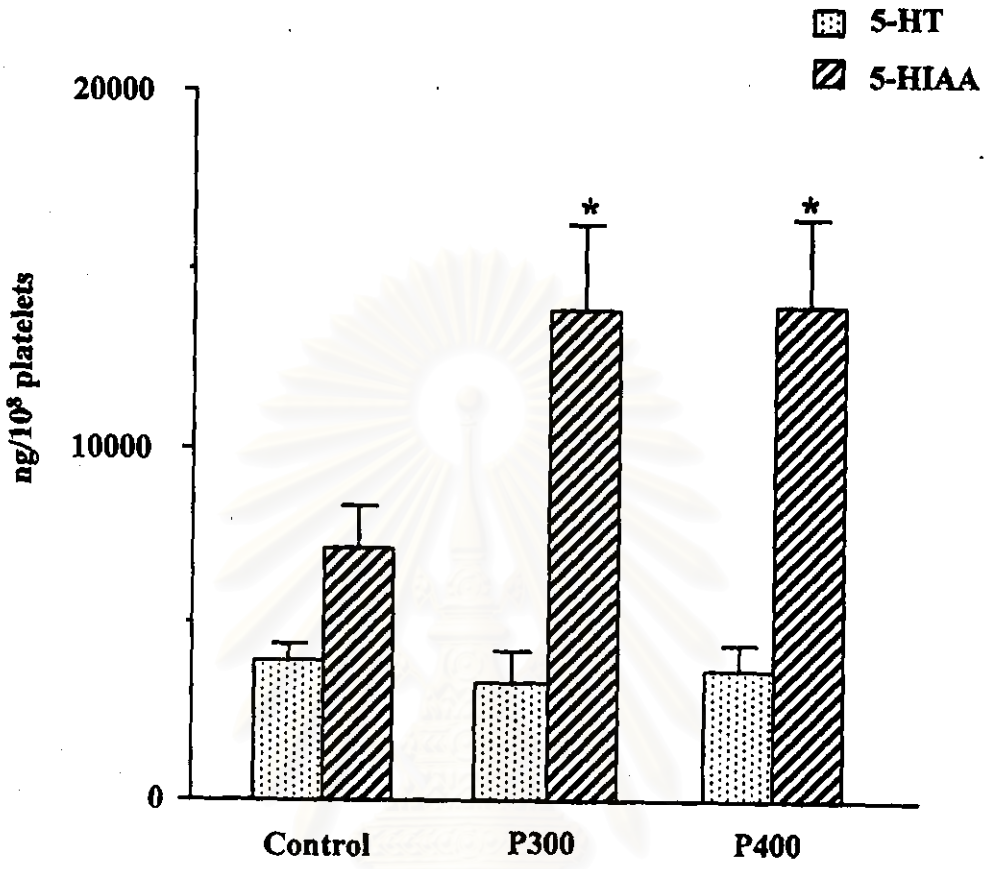

Figure 55. Comparison of the levels of 5-HT and 5-HIAA in platelets of control and 30-day treated rats with paracetamol 300 (P300), and 400 (P400) mg/kg/day. Values were expressed as means ± S.E.M. of 8-9 rats per group. * indicate significant difference from control group ($p < 0.05$, non-paired Student's *t*-test).

Comparison of [^{3}H]spiperone binding sites in frontal cortex between 15-day and 30-day paracetamol-treated groups

After 30 days of drug administration, B_{max} values of 5-HT_{2A} receptor sites in frontal cortex increased significantly compared to 15-day treated groups at a dose of 400 mg/kg/day ($p< 0.05$)(1.34 ± 0.13 and 0.94 ± 0.01 pmol/mg protein for 30-day and 15-day treated groups, respectively) (Fig 56).

Comparison of [^{3}H]imipramine binding sites in frontal cortex between 15-day and 30-day paracetamol-treated groups

After 30 days of drug administration, B_{max} values of 5-HT uptake sites in frontal cortex decreased significantly compared to 15-day treated groups at a dose of 300 ($p< 0.05$) and 400 mg/kg/day ($p< 0.001$)(2.38 ± 0.14 and 3.74 ± 0.60 pmol/mg protein for 30-day and 15-day treated groups in a dose of 300 mg/kg/day, respectively and 2.71 ± 0.18 and 4.59 ± 0.52 pmol/mg protein for 30-day and 15-day treated groups in a dose of 400 mg/kg/day, respectively) (Fig. 57).

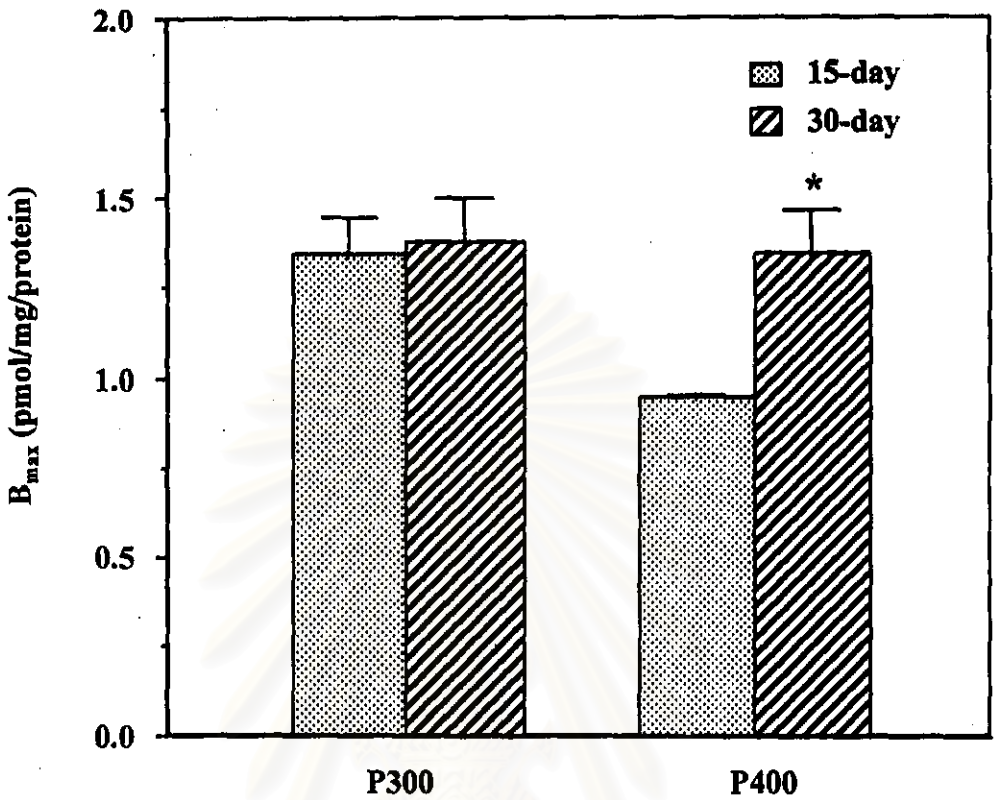

Figure 56. Comparison of B_{max} of [^{3}H]-spiperone binding sites in frontal cortex after 15 and 30 day paracetamol administration in a dose of 300 (P300) and 400 (P400) mg/kg/day. Data were shown as means ± S.E.M. of 5 rats per group. * indicate significant difference from 15-day treated groups in a dose of 400 mg/kg/day ($p < 0.05$, non-paired Student's *t*-test).

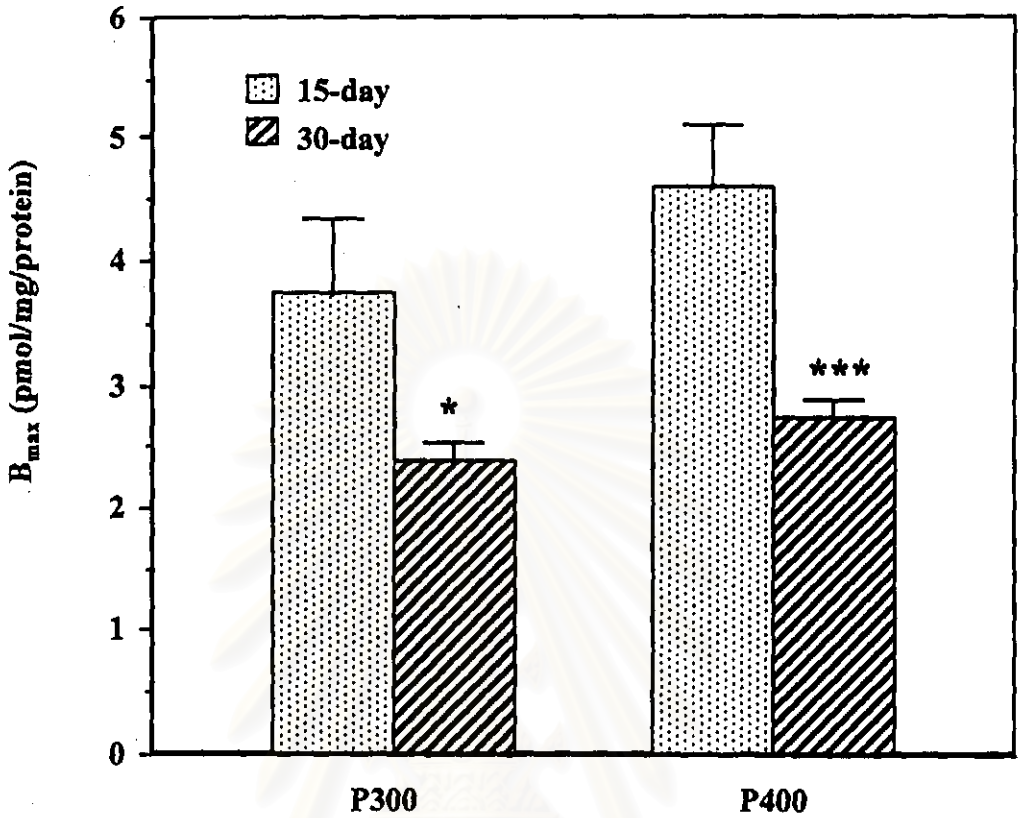

Figure 57. Comparison of B_{max} of [^{3}H]-imipramine binding sites in frontal cortex after 15 and 30 day paracetamol administration in a dose of 300 (P300) and 400 (P400) mg/kg/day. Data were shown as means ± S.E.M. of 4 rats per group. * indicate significant difference from 15-day treated groups in a dose of 300 mg/kg/day.($p< 0.05$). *** indicate significant difference from 15-day treated groups in a dose of 400 mg/kg/day. ($p< 0.001$, non-paired Student's *t*-test).

Comparison of the levels of 5-HT in platelets between 15-day and 30-day paracetamol-treated groups

After 30 days of drug administration, the levels of 5-HT in platelets decreased significantly compared to 15-day treated groups at a dose of 300 ($p<0.05$) and 400 ($p<0.01$) mg/kg/day:- 3329.99 ± 895.51 and 7024.67 ± 905.97 ng/10^8 platelets for 30-day and 15-day treated groups in a dose of 300 mg/kg/day, respectively and 3636.51 ± 714.21 and 7342.83 ± 1041.35 ng/10^8 platelets for 30-day and 15-day treated groups in a dose of 400 mg/kg/day, respectively (Fig. 58).

Comparison of the levels of 5-HIAA in platelets between 15-day and 30-day paracetamol-treated groups

After 30 days of drug administration, the levels of 5-HIAA in platelets increased significantly compared to 15-day treated groups at a dose of 300 and 400 mg/kg/day ($p<0.01$):- 13788.65 ± 2373.95 and 5151.29 ± 792.60 ng/10^8 platelets for 30-day and 15-day treated groups in a dose of 300 mg/kg/day, respectively and 13866.77 ± 2517.06 and 5796.84 ± 465.69 ng/10^8 platelets for 30-day and 15-day treated groups in a dose of 400 mg/kg/day, respectively (Fig.59).

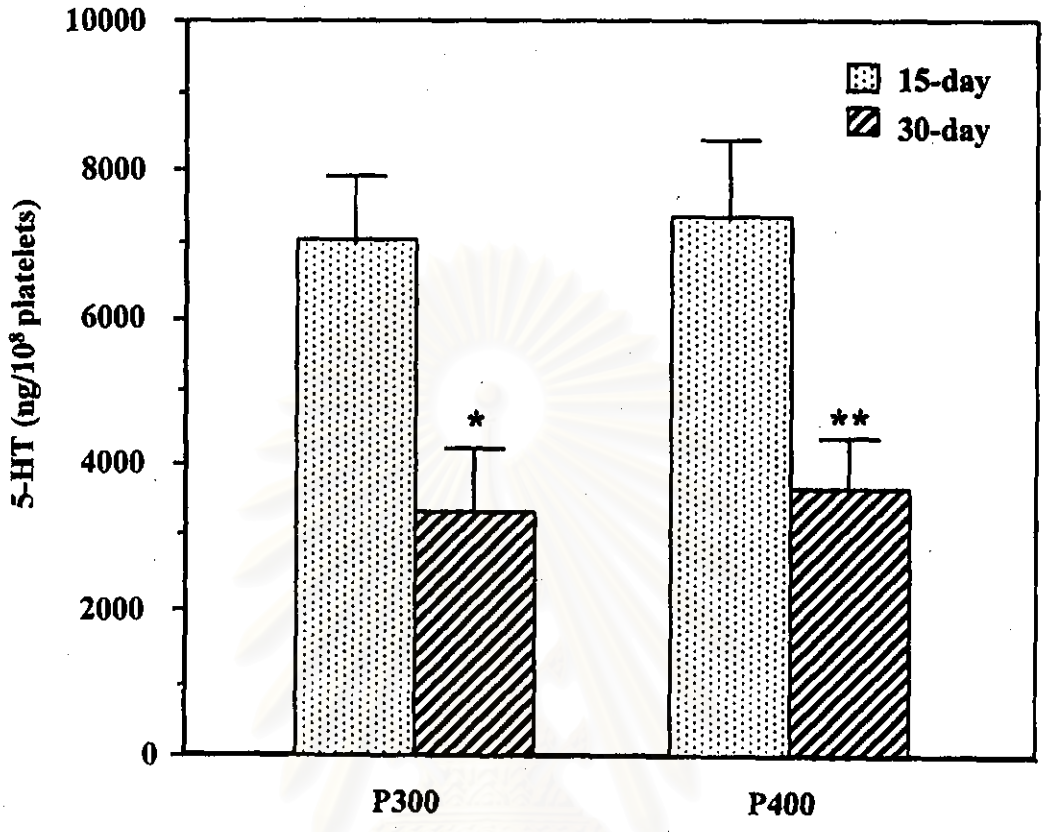

Figure 58. Comparison of the levels of 5-HT in platelets after 15 and 30 day paracetamol administration in a dose of 300 (P300) and 400 (P400) mg/kg/day. Data were shown as means ± S.E.M. of 8-9 rats per group.

* indicate significant difference from 15-day treated groups in a dose of 300 mg/kg/day ($p < 0.05$, non-paired Student's *t*-test).

** indicate significant difference from 15-day treated groups in a dose of 400 mg/kg/day ($p < 0.01$, non-paired Student's *t*-test).

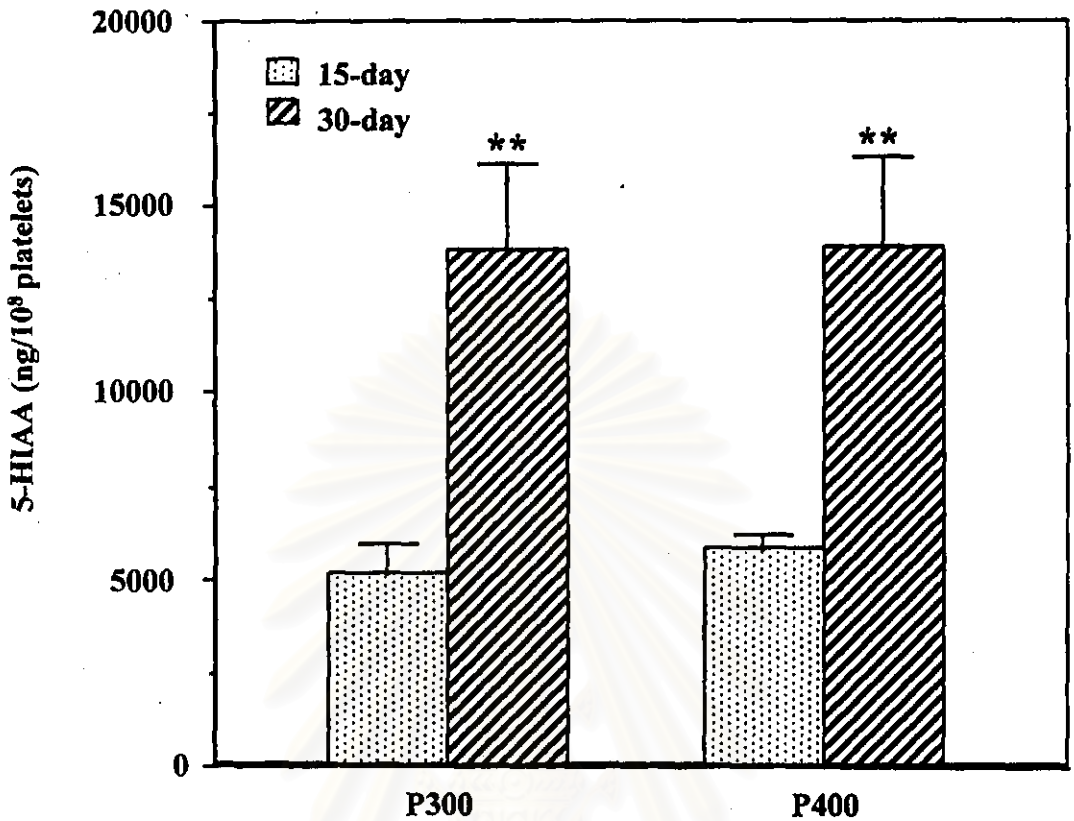

Figure 59. Comparison of the levels of 5-HIAA in platelets after 15 and 30 day paracetamol administration in a dose of 300 (P300) and 400 (P400) mg/kg/day. Data were shown as means ± S.E.M. of 8-9 rats per group. ** indicate significant difference from 15-day treated groups in a dose of 300 and 400 mg/kg/day ($p < 0.01$, non-paired Student's t-test).

11. Acute Effect of Paracetamol Treatment on Central 5-HT_{2A} Serotonin Receptors in Rat Frontal Cortex

After 90 min of drug administration, the B_{max} values in the frontal cortex for control and treated groups with paracetamol 300 and 400 mg/kg/day were 2.13 ± 0.09, 1.14 ± 0.08 and 1.20 ± 0.15 pmol/mg protein, respectively. The K_d values for these three groups were 1.36 ± 0.05, 1.20 ± 0.32 and 1.06 ± 0.23 nM, respectively. The difference of the B_{max} values between control and treated groups with the two doses of paracetamol in this area was statistically significant ($p < 0.001$). However, there was no significant difference in K_d values among the three groups (Table 21, Fig. 60 and 61).

Table 21. Comparison of the binding characteristics of [^{3}H]spiperone to membranes of frontal cortex between control and acute paracetamol-treated rats

Groups	K_d (nM)	B_{max} (pmol/mg protein)
Control	1.36 ± 0.05	2.13 ± 0.09
Para 300	1.20 ± 0.32	1.14 ± 0.08***
Para 400	1.06 ± 0.23	1.20 ± 0.15***

The rats were i.p. injected with vehicle (control) and paracetamol (Para) 300 and 400 mg/kg/day. Data were expressed as means ± S.E.M. of 5-6 rats per group. Statistical comparisons were made using the non-paired Student's *t*-test.

*** indicate significant difference from control group ($p < 0.001$).

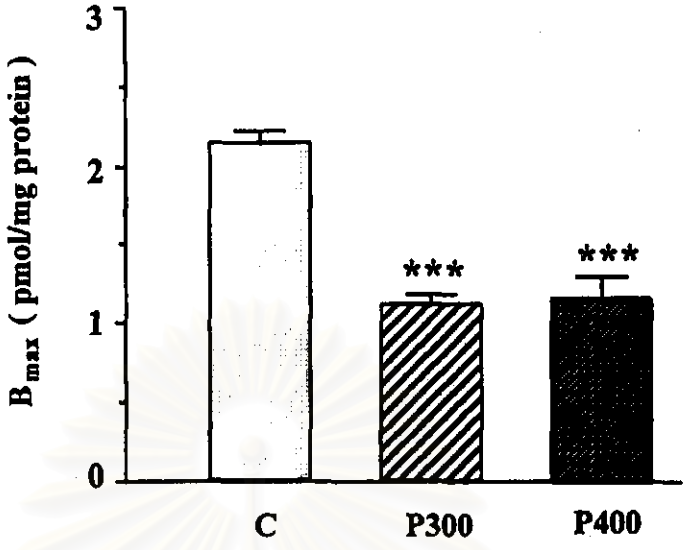

Figure 60. Comparison of the maximum number of binding sites (B_{max}) of [^{3}H]-spiperone in frontal cortex of control (C) and acute treated rats with paracetamol 300 (P300) and 400 (P400) mg/kg/day. B_{max} values were expressed as means ± S.E.M of 5-6 rats per group.
*** indicate significant difference from control group ($p < 0.001$).

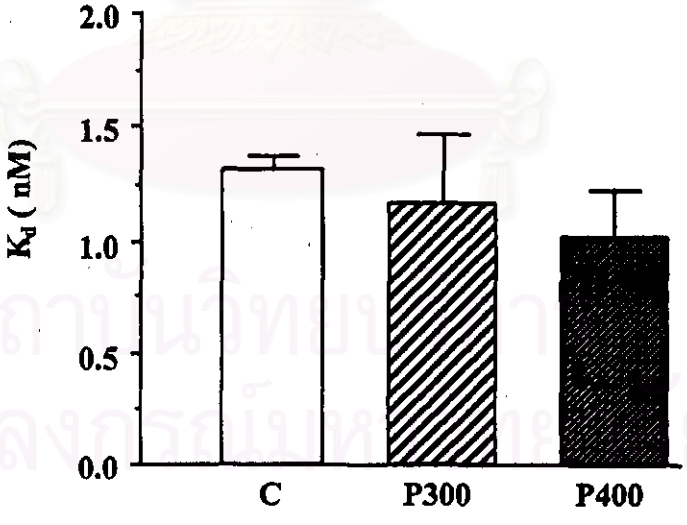

Figure 61. Comparison of the dissociation equilibrium constants (K_d) of [^{3}H]-spiperone binding sites in frontal cortex of control (C) and acute treated rats with paracetamol 300 (P300) and 400 (P400) mg/kg/day. K_d values were expressed as means ± S.E.M. of 5-6 rats per group.

Table 22. Binding characteristics of [^{3}H]spiperone to frontal cortex membranes in control and acute paracetamol-treated rats

Groups	Rat No.	K_d (nM)	B_{max} (pmol/mg protein)
Control	1	1.5	2.44
	2	1.4	1.92
	3	1.3	1.93
	4	1.5	2.26
	5	1.2	2.32
	6	1.3	1.92
	means ± S.E.M.	**1.36 ± 0.05**	**2.13 ± 0.09**
Para 300	7	0.5	0.89
	8	2.3	1.43
	9	0.7	1.09
	10	1.5	1.12
	11	1.0	1.19
	means ± S.E.M.	**1.20 ± 0.32**	**1.14 ± 0.08*****
Para 400	12	1.9	0.94
	13	1.1	1.73
	14	1.1	1.15
	15	0.6	0.85
	16	0.6	1.33
	means ± S.E.M.	**1.06 ± 0.23**	**1.20 ± 0.15*****

*** indicate significant difference from control group ($p < 0.001$, non-paired Student's *t*-test).

The saturation curve and Scatchard analysis of these data were shown in Fig. 62-65 and Fig. 113-115.

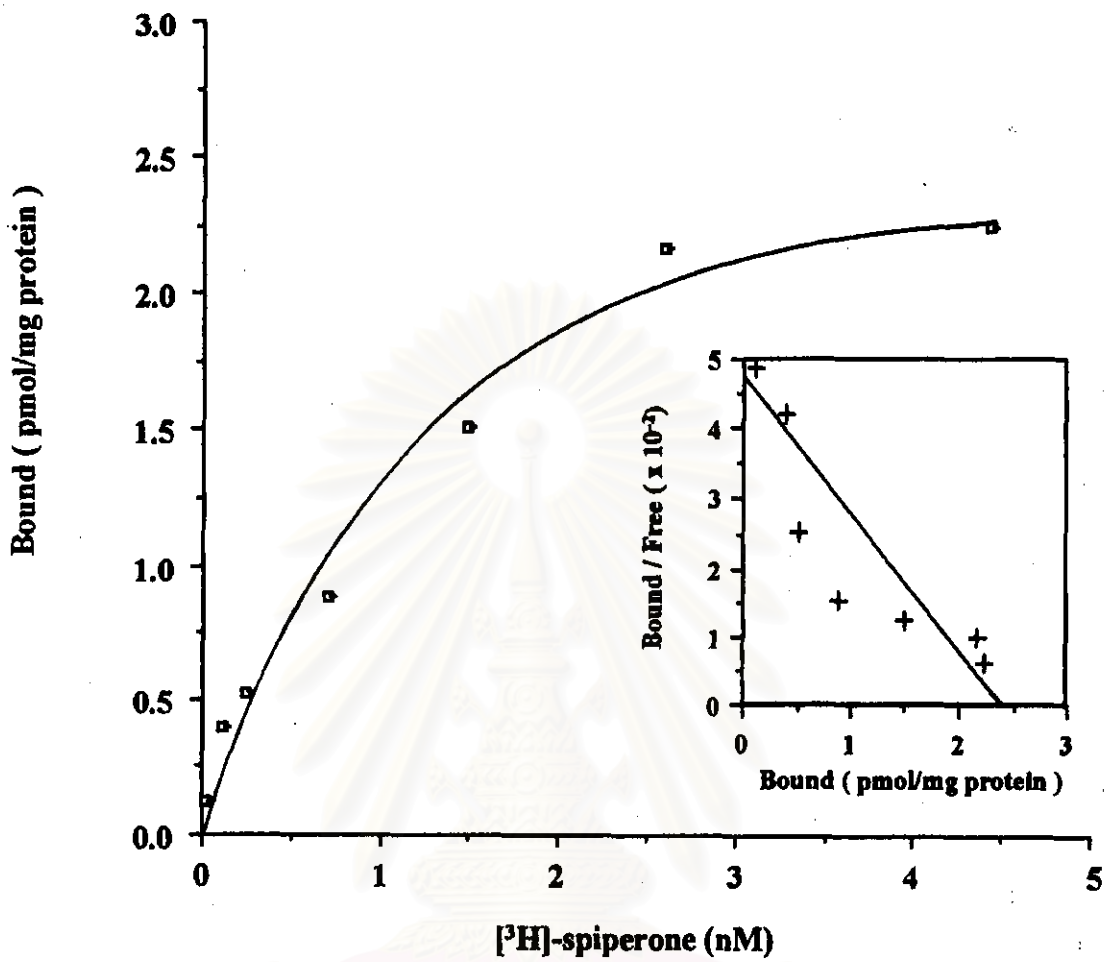

Figure 62. Saturation curve and Scatchard analysis (in the inset) of [^{3}H]-spiperone binding on frontal cortex membrane of control rat number 5, treated with vehicle i.p. for 90 min.The binding was carried out in seven concentrations of [^{3}H]-spiperone, ranging from 0.05 - 5 nM. The plots were obtained from duplicate determinations and represented the specific binding of [^{3}H]-spiperone. The line of best fit was analysed by the LIGAND computer program. The result of this experiment was shown and provided a K_d value of 1.2 nM and B_{max} value of 2.32 pmol/mg protein.

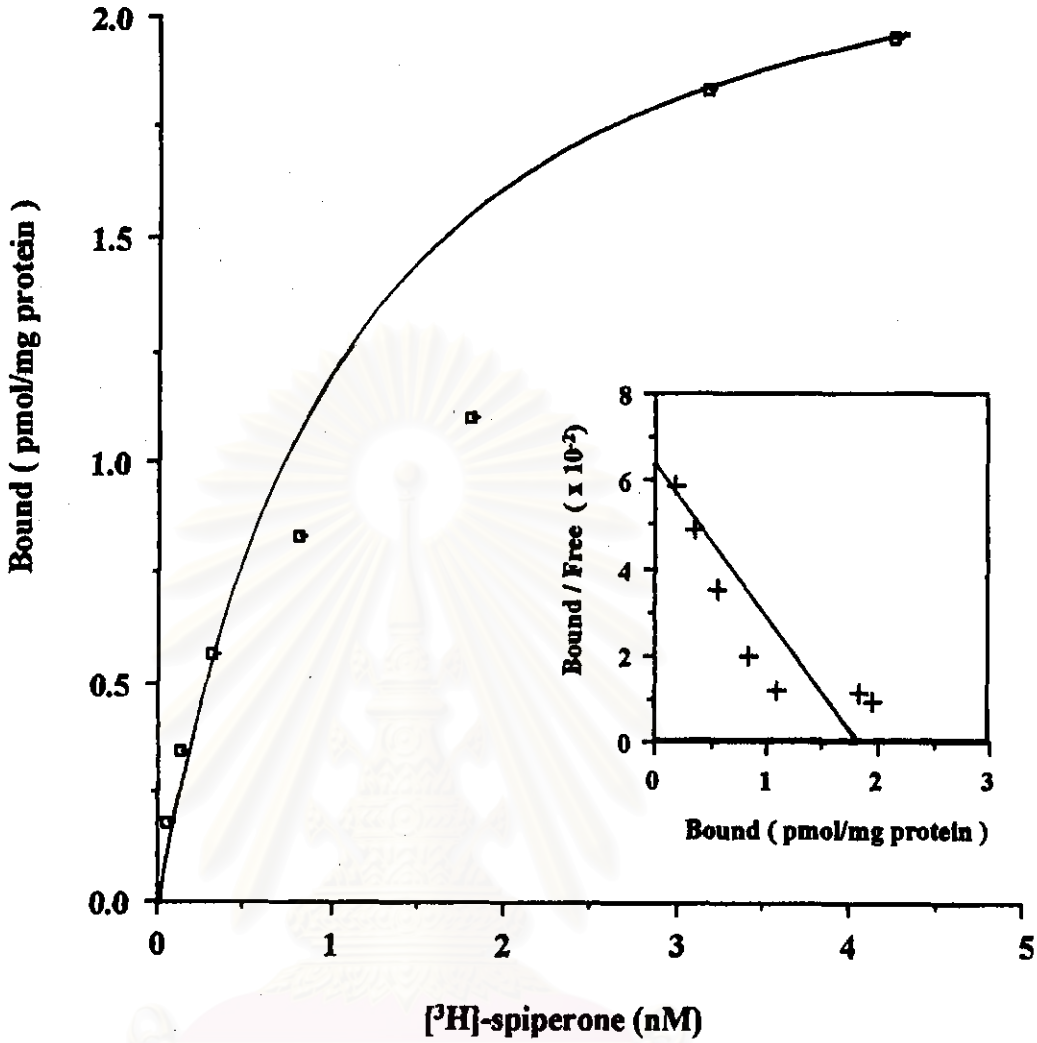

Figure 63. Saturation curve and Scatchard analysis (in the inset) of [³H]-spiperone binding on frontal cortex membrane of control rat number 6, treated with vehicle i.p. for 90 min.The binding was carried out in seven concentrations of [³H]-spiperone, ranging from 0.05 - 5 nM. The plots were obtained from duplicate determinations and represented the specific binding of [³H]-spiperone. The line of best fit was analysed by the LIGAND computer program. The result of this experiment was shown and provided a K_d value of 1.3 nM and B_{max} value of 1.92 pmol/mg protein.

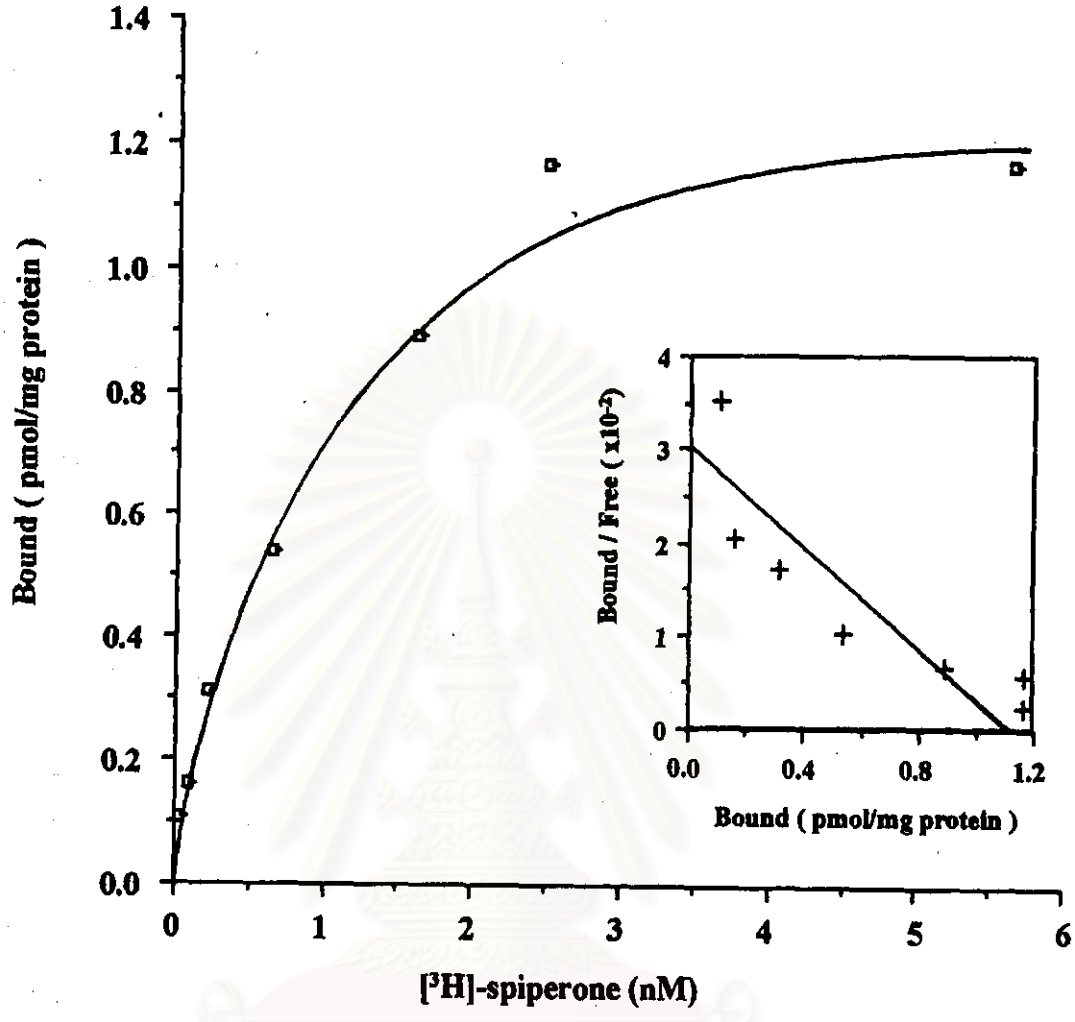

Figure 64. Saturation curve and Scatchard analysis (in the inset) of [³H]-spiperone binding on frontal cortex membrane of rat number 11, treated with paracetamol 300 mg/kg i.p. for 90 min. The binding was carried out in six concentrations of [³H]-spiperone, ranging from 0.2-10 nM. The plots were obtained from duplicate determinations and represented the specific binding of [³H]-spiperone. The line of best fit was analysed by the LIGAND computer program. The result of this experiment was shown and provided a K_d value of 1.0 nM and B_{max} value of 1.19 pmol/mg protein.

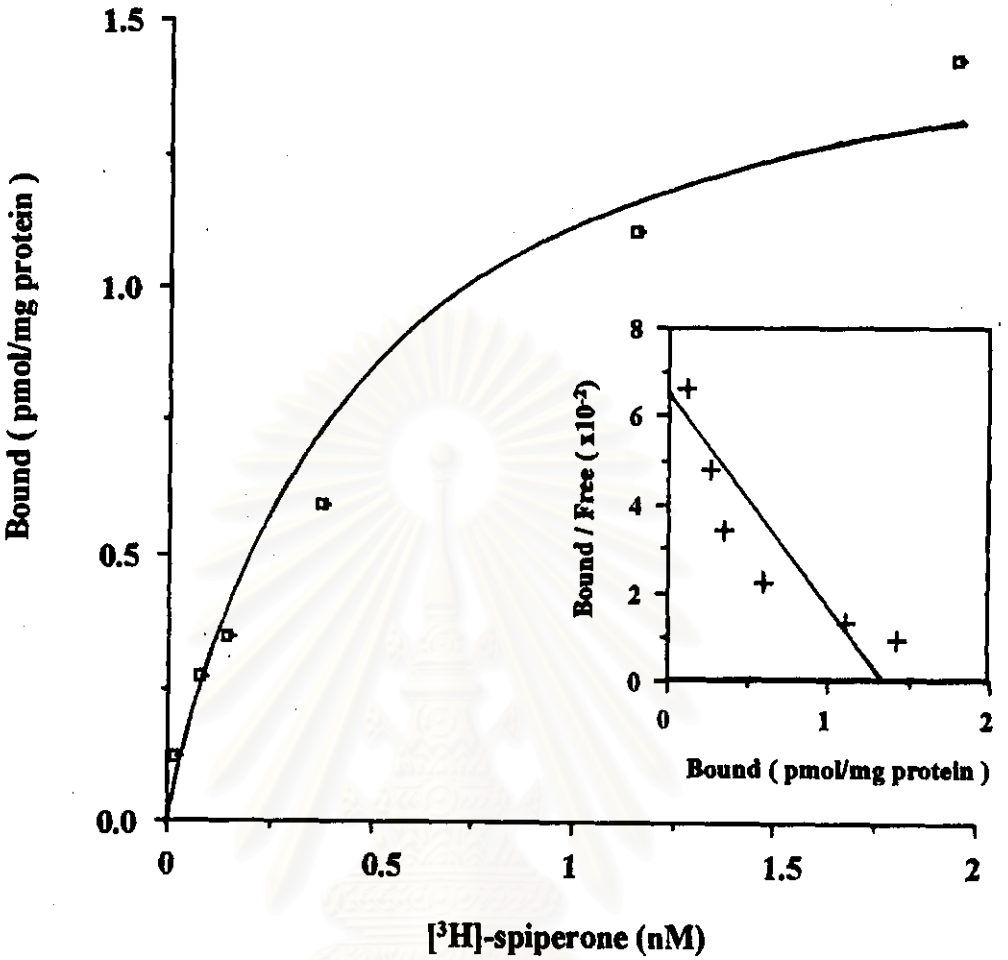

Figure 65. Saturation curve and Scatchard analysis (in the inset) of [^{3}H]-spiperone binding on frontal cortex membrane of rat number 16, treated with paracetamol 400 mg/kg i.p. for 90 min. The binding was carried out in six concentrations of [^{3}H]-spiperone, ranging from 0.02 -2 nM. The plots were obtained from duplicate determinations and represented the specific binding of [^{3}H]-spiperone. The line of best fit was analysed by the LIGAND computer program. The result of this experiment was shown and provided a K_d value of 0.6 nM and B_{max} value of 1.33 pmol/mg protein.

12. Acute Effect of Paracetamol Treatment on Central 5-HT_{2A} Serotonin Receptors in Rat Brain Stem

After 90 min of drug administration, the B_{max} values in the brain stem for control and treated groups with paracetamol 300 and 400 mg/kg/day were 1.10 ± 0.16, 1.01 ± 0.08 and 1.07 ± 0.05 pmol/mg protein, respectively. The K_d values for these three groups were 0.93 ± 0.09, 0.92 ± 0.12 and 0.88 ± 0.07 nM, respectively. The B_{max} and K_d values between control and treated groups with the two doses of paracetamol in this area were not different (Table 23 and 24, Fig. 66 and 67).

Table 23. Comparison of the binding characteristics of [^{3}H]spiperone to membranes of brain stem between control and acute paracetamol-treated rats

Groups	K_d (nM)	B_{max} (pmol/mg protein)
Control	0.93 ± 0.09	1.10 ± 0.16
Para 300	0.92 ± 0.12	1.01 ± 0.08
Para 400	0.88 ± 0.07	1.07 ± 0.05

The rats were i.p. injected with vehicle (control) and paracetamol (Para) 300 and 400 mg/kg/day. Data were expressed as means ± S.E.M. of 5-6 rats per group. Statistical comparisons were made using the non-paired Student's *t*-test.

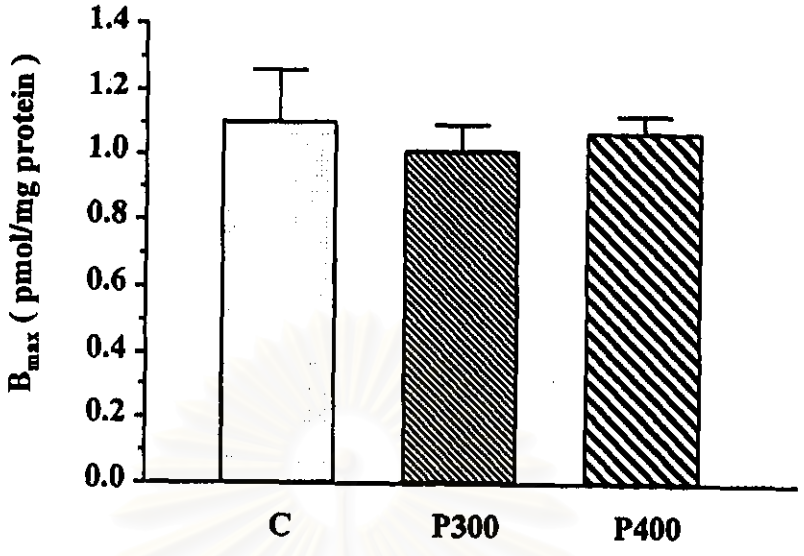

Figure 66. Comparison of the maximum number of binding sites (B_{max}) of [^{3}H]-spiperone in brain stem of control (C) and acute treated rats with paracetamol 300 (P300) and 400 (P400) mg/kg/day. B_{max} values were expressed as means ± S.E.M. of 5-6 rats per group.

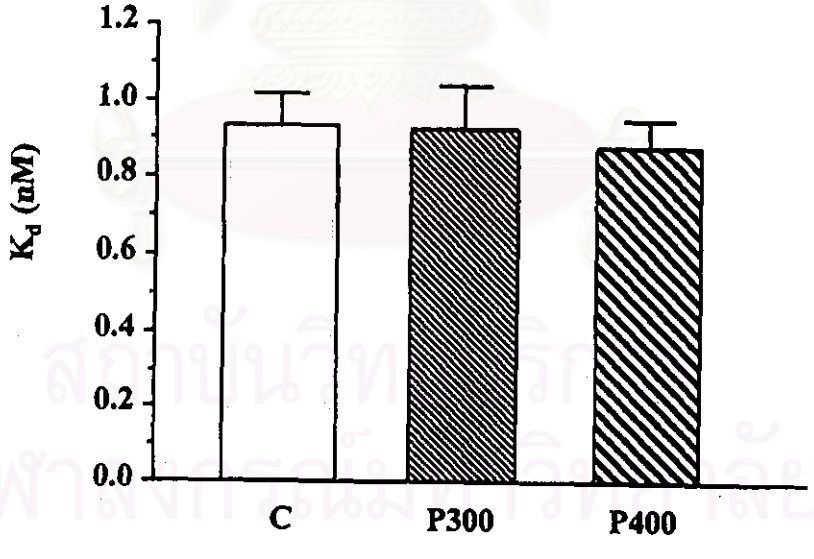

Figure 67. Comparison of the dissociation equilibrium constants (K_d) of [^{3}H]-spiperone binding sites in brain stem of control (C) and acute treated rats with paracetamol 300 (P300) and 400 (P400) mg/kg/day. K_d values were expressed as means ± S.E.M. of 5-6 rats per group.

Table 24. Binding characteristics of [^{3}H]spiperone to brain stem membranes in control and acute paracetamol-treated rats

Groups	Rat No.	K_d (nM)	B_{max} (pmol/mg protein)
Control	1	1.0	1.19
	2	1.3	0.82
	3	1.0	0.98
	4	0.8	0.84
	5	0.9	0.94
	6	0.6	1.84
	means ± S.E.M.	**0.93 ± 0.09**	**1.10 ± 0.16**
Para 300	7	1.0	1.13
	8	1.1	0.83
	9	0.7	0.95
	10	0.7	1.25
	11	1.1	0.91
	means ± S.E.M.	**0.92 ± 0.12**	**1.01 ± 0.08**
Para 400	12	1.2	1.12
	13	0.7	1.08
	14	0.9	1.29
	15	1.0	1.18
	16	0.6	0.68
	means ± S.E.M.	**0.88 ± 0.07**	**1.07 ± 0.05**

Statistical comparisons were made using the non-paired Student's *t*-test.

The saturation curve and Scatchard analysis of these data were shown in Fig. 68-71. and Fig. 116-118.

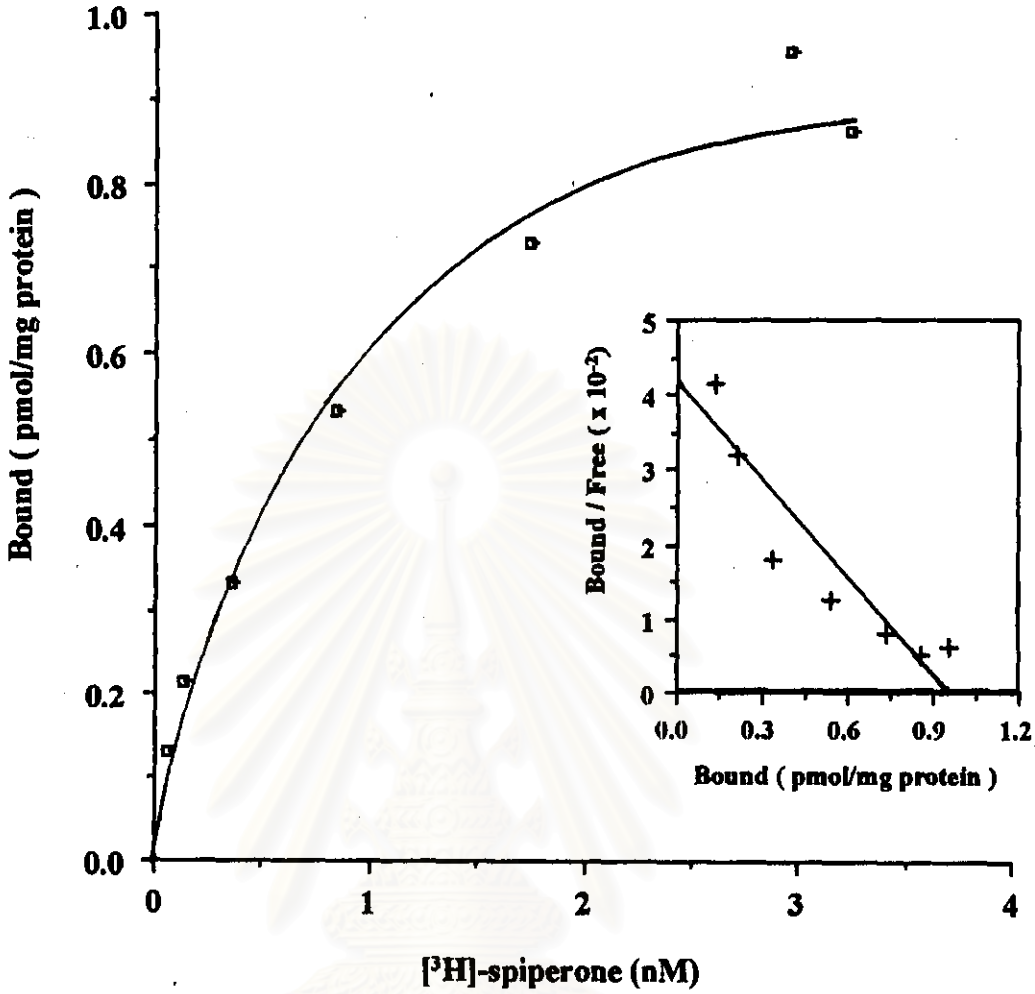

Figure 68. Saturation curve and Scatchard analysis (in the inset) of [³H]-spiperone binding on brain stem membrane of control rat number 5 , treated with vehicle i.p. for 90 min. The binding was carried out in seven concentrations of [³H]-spiperone, ranging from 0.04 - 4 nM. The plots were obtained from duplicate determinations and represented the specific binding of [³H]-spiperone. The line of best fit was analysed by the LIGAND computer program. The result of this experiment was shown and provided a K_d value of 0.9 nM and B_{max} value of 0.94 pmol/mg protein.

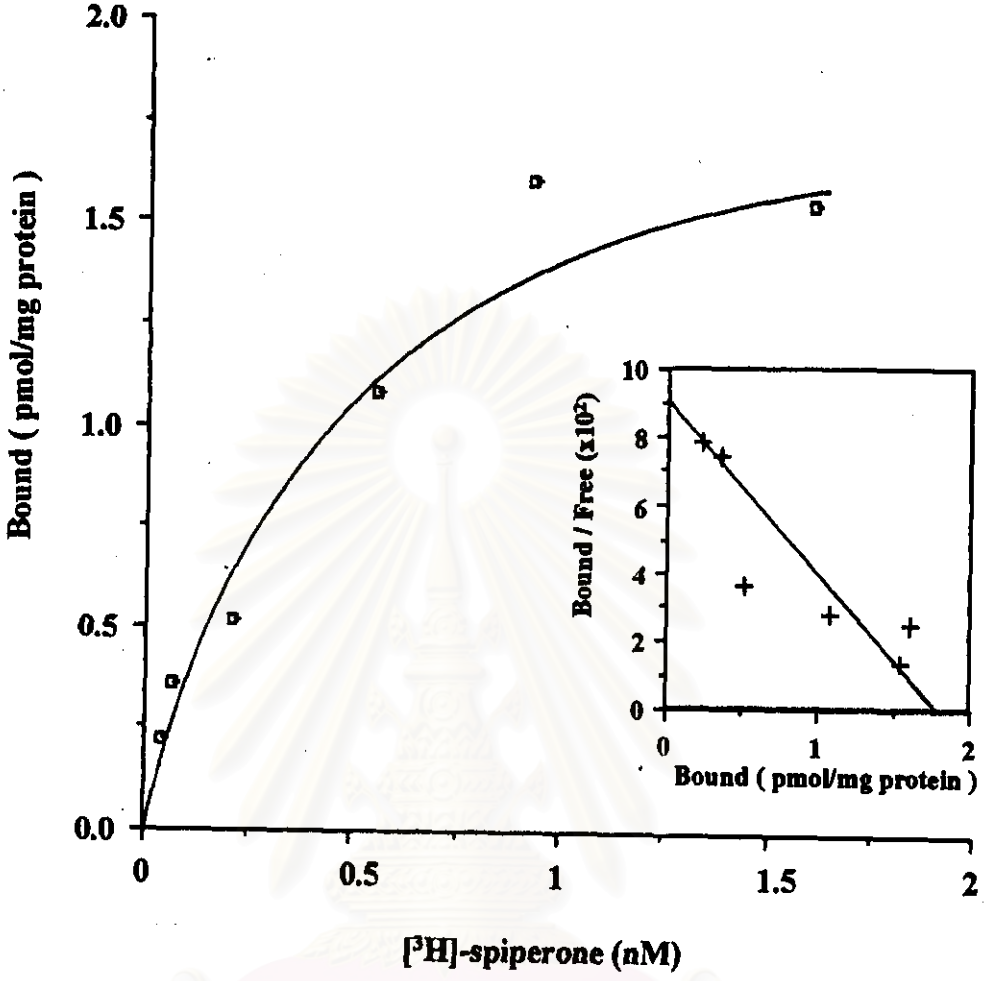

Figure 69. Saturation curve and Scatchard analysis (in the inset) of [^{3}H]-spiperone binding on brain stem membrane of control rat number 6, treated with vehicle i.p. for 90 min. The binding was carried out in six concentrations of [^{3}H]-spiperone, ranging from 0.02 - 2 nM. The plots were obtained from duplicate determinations and represented the specific binding of [^{3}H]-spiperone. The line of best fit was analysed by the LIGAND computer program. The result of this experiment was shown and provided a K_d value of 0.6 nM and B_{max} value of 1.84 pmol/mg protein.

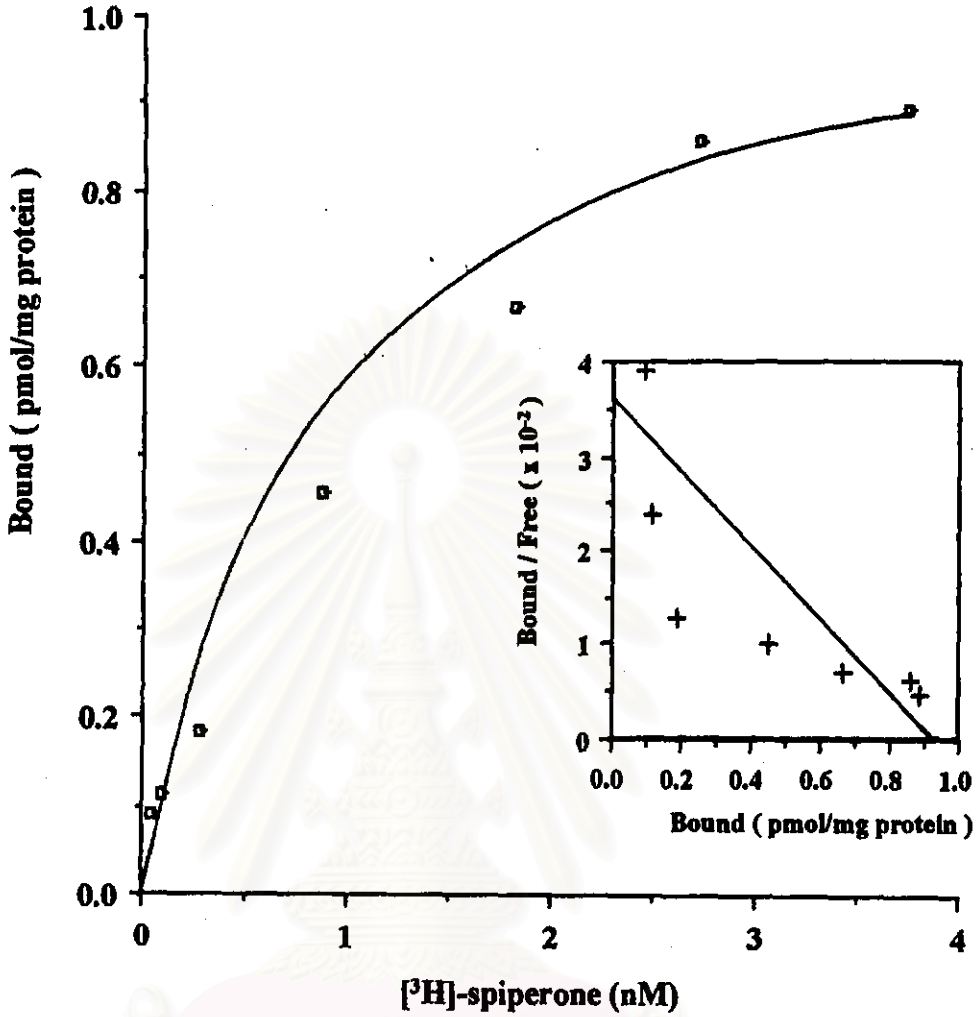

Figure 70. Saturation curve and Scatchard analysis (in the inset) of [^{3}H]-spiperone binding on brain stem membrane of rat number 11, treated with paracetamol 300 mg/kg/day i.p. for 90 min. The binding was carried out in seven concentrations of [^{3}H]-spiperone, ranging from 0.04 - 4 nM. The plots were obtained from duplicate determinations and represented the specific binding of [^{3}H]-spiperone. The line of best fit was analysed by the LIGAND computer program. The result of this experiment was shown and provided a K_d value of 1.1 nM and B_{max} value of 0.91 pmol/mg protein.

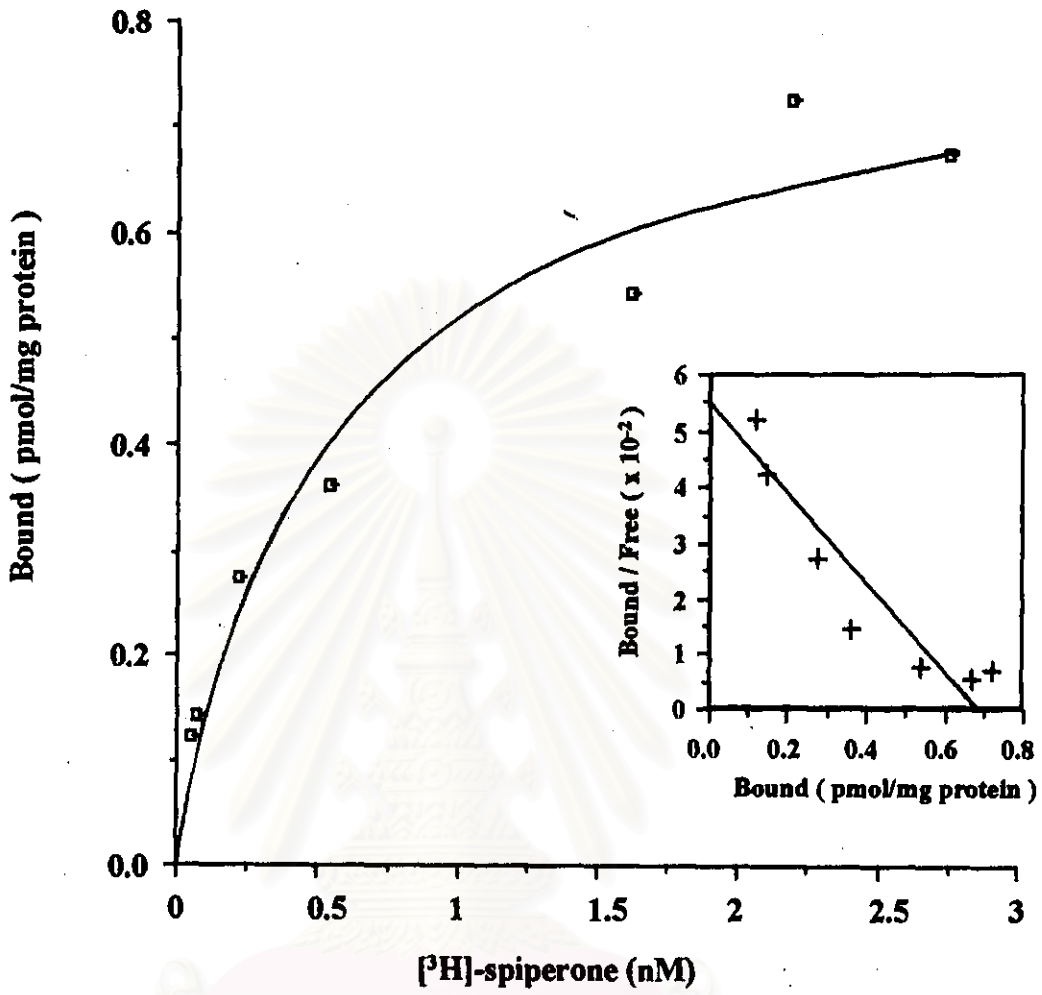

Figure 71. Saturation curve and Scatchard analysis (in the inset) of [³H]-spiperone binding on brain stem membrane of rat number 16, treated with paracetamol 400 mg/kg/day i.p. for 90 min. The binding was carried out in seven concentrations of [³H]-spiperone, ranging from 0.03 - 3 nM. The plots were obtained from duplicate determinations and represented the specific binding of [³H]-spiperone. The line of best fit was analysed by the LIGAND computer program. The result of this experiment was shown and provided a K_d value of 0.6 nM and B_{max} value of 0.68 pmol/mg protein.

13. Acute Effect of Paracetamol Treatment on 5-HT Uptake Sites in Rat Frontal Cortex

After 90 min of drug administration, the B_{max} values in the frontal cortex for control and treated groups with paracetamol 300 and 400 mg/kg/day were 1.78 ± 0.09, 3.14 ± 0.09 and 3.41 ± 0.13 pmol/mg protein, respectively. The K_d values for these three groups were 1.47 ± 0.24, 1.83 ± 0.16 and 1.82 ± 0.32 nM, respectively. The difference of the B_{max} values between control and treated groups with the two doses of paracetamol in this area was statistically significant ($p < 0.001$). However, there was no significant difference in K_d values among the three groups. (Table 25 and 26, Fig.72 and 73).

Table 25. Comparison of the binding characteristics of [^{3}H]imipramine to membranes of frontal cortex between control and acute paracetamol-treated rats

Groups	K_d (nM)	B_{max} (pmol/mg protein)
Control	1.47 ± 0.24	1.78 ± 0.09
Para 300	1.83 ± 0.16	3.14 ± 0.09***
Para 400	1.82 ± 0.32	3.41 ± 0.13***

The rats were i.p. injected with vehicle (control) and paracetamol (Para) 300 and 400 mg/kg/day. Data were expressed as means ± S.E.M. of 4-5 rats per group. Statistical comparisons were made using the non-paired Student's *t*-test.

*** indicate significant difference from control ($p < 0.001$)

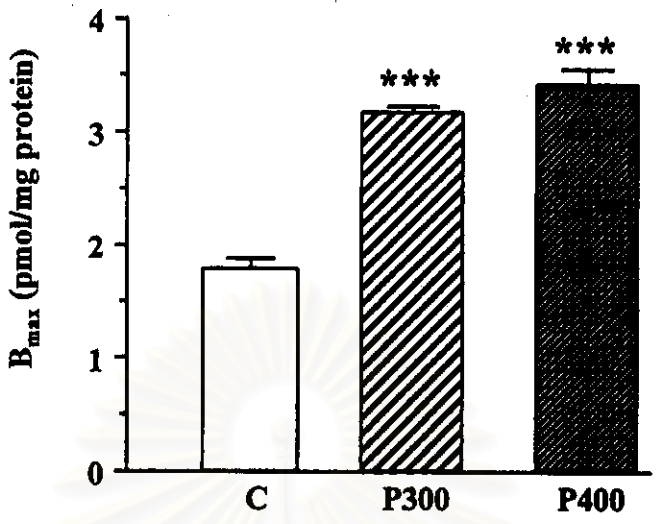

Figure 72. Comparison of the maximum number of binding sites (B_{max}) of [^{3}H]-imipramine in frontal cortex of control (C) and acute treated rats with paracetamol 300 (P300) and 400 (P400) mg/kg/day. B_{max} values were expressed as means ± S.E.M. of 4-5 rats per group. *** indicate significant difference from control group ($p < 0.001$)

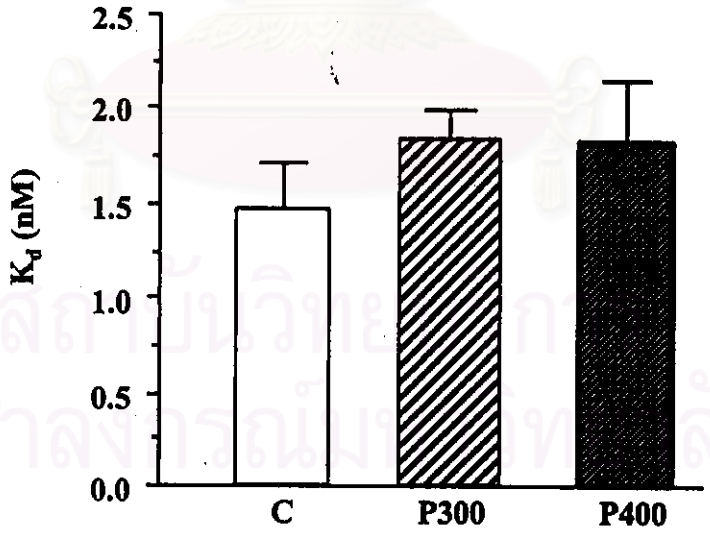

Figure 73. Comparison of the dissociation equilibrium constants (K_d) of [^{3}H]-imipramine binding sites in frontal cortex of control (C) and acute treated rats with paracetamol 300 (P300) and 400 (P400)mg/kg/day. K_d values were expressed as means ± S.E.M. of 4-5 rats per group.

Table 26. Binding characteristics of [^{3}H]imipramine to frontal cortex membranes in control and acute paracetamol-treated rats

Groups	Rat No.	K_d (nM)	B_{max} (pmol/mg protein)
Control	1	1.77	1.96
	2	0.86	1.52
	3	1.90	1.66
	4	1.76	1.79
	5	1.06	1.95
	means ± S.E.M.	**1.47 ± 0.24**	**1.78 ± 0.09**
Para 300	6	2.05	3.21
	7	1.81	2.99
	8	2.46	3.09
	9	0.98	3.27
	means ± S.E.M.	**1.83 ± 0.16**	**3.14 ± 0.09*** **
Para 400	10	1.79	3.45
	11	1.51	3.07
	12	2.51	3.68
	13	1.48	3.45
	means ± S.E.M.	**1.82 ± 0.32**	**3.41 ± 0.13*** **

*** indicate significant difference from control group ($p < 0.001$, non-paired Student's *t*-test)

The saturation curve and Scatchard analysis of these data were shown in Fig. 74-76 and Fig. 119-121.

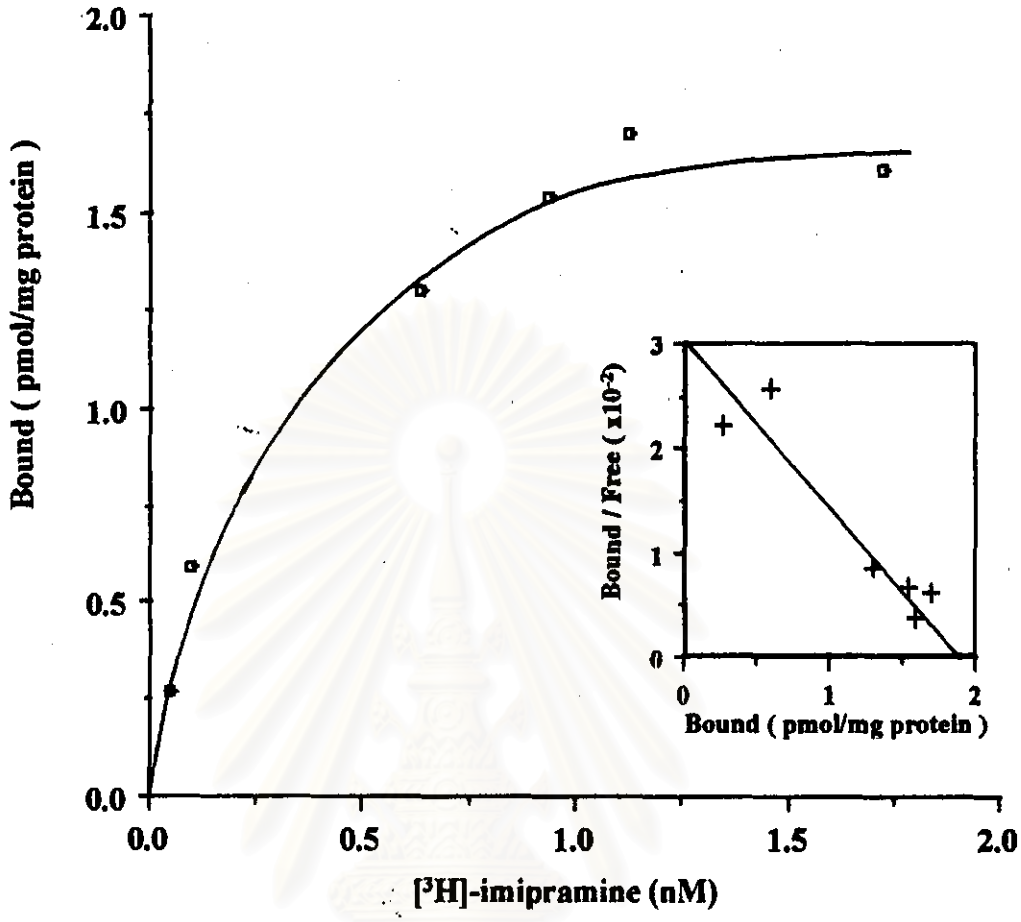

Figure 74. Saturation curve and Scatchard analysis (in the inset) of [^{3}H]-imipramine binding on frontal cortex membrane of control rat number 5, treated with vehicle i.p. for 90 min. The binding was carried out in six concentrations of [^{3}H]-imipramine, ranging from 0.02 - 2 nM. The plots were obtained from duplicate determinations and represented the specific binding of [^{3}H]-imipramine. The line of best fit was analysed by the LIGAND computer program. The result of this experiment was shown and provided a K_d value of 1.06 nM and B_{max} value of 1.95 pmol/mg protein.

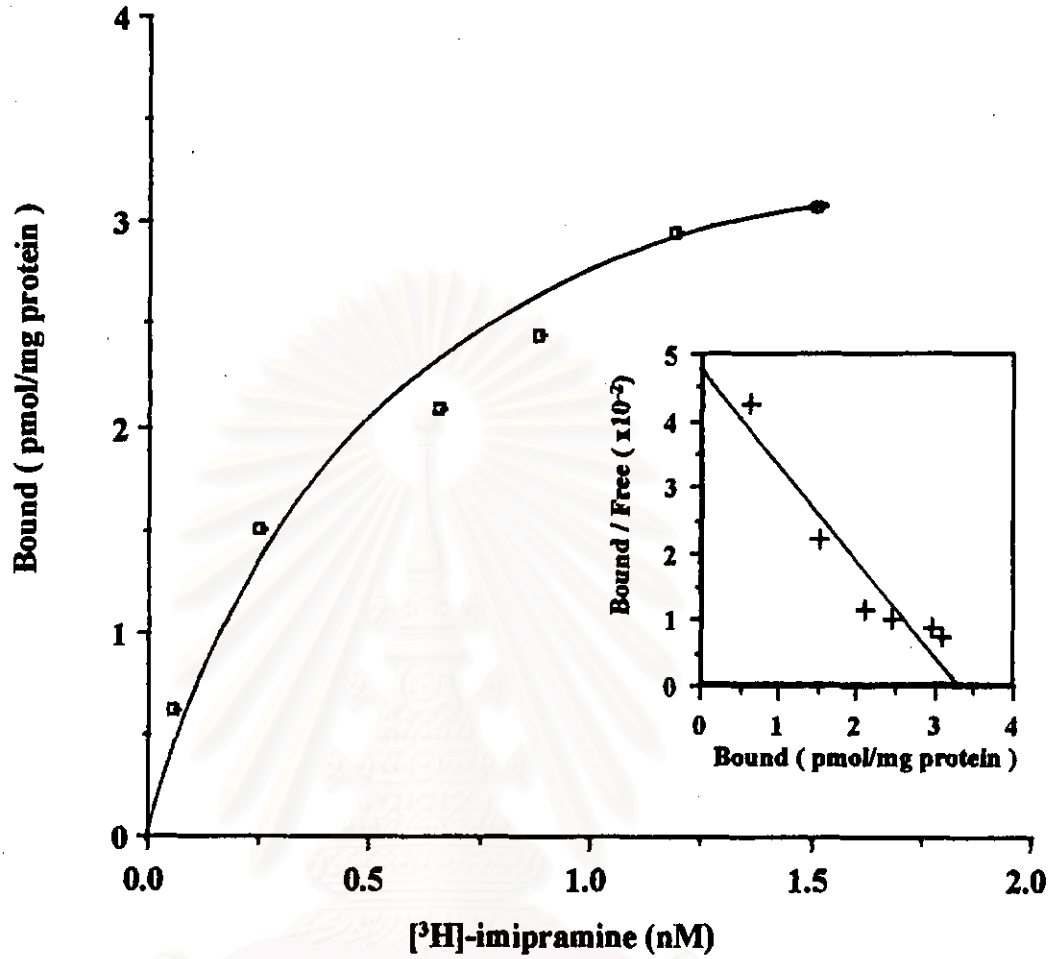

Figure 75. Saturation curve and Scatchard analysis (in the inset) of [³H]-imipramine binding on frontal cortex membrane of rat number 9, treated with paracetamol 300 mg/kg i.p. for 90 min. The binding was carried out in six concentrations of [³H]-imipramine, ranging from 0.02-2 nM. The plots were obtained from duplicate determinations and represented the specific binding of [³H]-imipramine.The line of best fit was analysed by the LIGAND computer program. The result of this experiment was shown and provided a K_d value of 0.98 nM and B_{max} value of 3.27 pmol/mg protein.

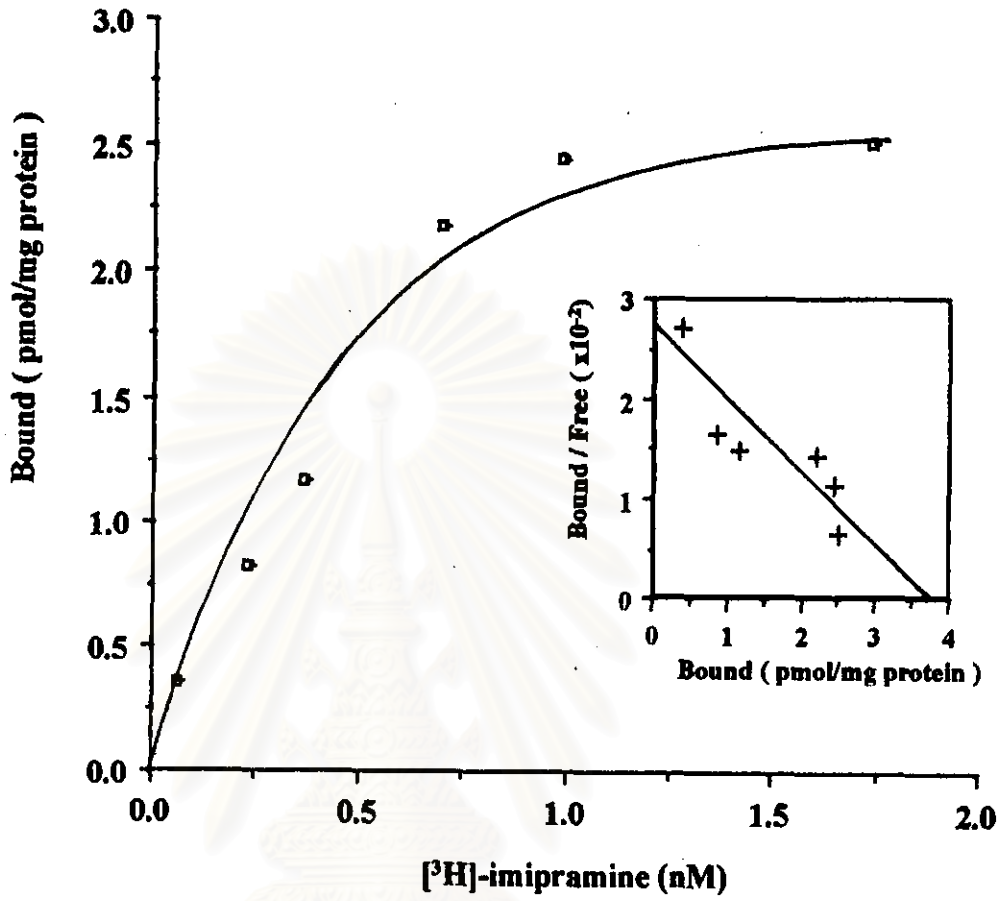

Figure 76. Saturation curve and Scatchard analysis (in the inset) of [^{3}H]-imipramine binding on frontal cortex membrane of rat number 12, treated with paracetamol 400 mg/kg i.p. for 90 min. The binding was carried out in six concentrations of [^{3}H]-imipramine, ranging from 0.02-2 nM. The plots were obtained from duplicate determinations and represented the specific binding of [^{3}H]-imipramine.The line of best fit was analysed by the LIGAND computer program. The result of this experiment was shown and provided a K_d value of 2.51 nM and B_{max} value of 3.68 pmol/mg protein.

14. Acute Effect of Paracetamol Treatment on 5-HT Uptake Sites in Rat Brain Stem

After 90 min of drug administration, the B_{max} values in the brain stem for control and treated groups with paracetamol 300 and 400 mg/kg/day were 1.21 ± 0.20, 1.13 ± 0.08 and 1.09 ± 0.06 pmol/mg protein, respectively. The K_d values for these three groups were 1.28 ± 0.29, 1.22 ± 0.11 and 1.19 ± 0.24 nM, respectively. The B_{max} and K_d values between control and treated groups with the two doses of paracetamol in this area were not different (Table 27 and 28, Fig.77 and 78).

Table 27. Comparison of the binding characteristics of [^{3}H]imipramine to membranes of brain stem between control and acute paracetamol-treated rats

Groups	K_d (nM)	B_{max} (pmol/mg protein)
Control	1.28 ± 0.29	1.21 ± 0.20
Para 300	1.22 ± 0.11	1.13 ± 0.08
Para 400	1.19 ± 0.24	1.09 ± 0.06

The rats were i.p. injected with vehicle (control) and paracetamol (Para) 300 and 400 mg/kg/day. All data were expressed as means ± S.E.M. of 4-5 rats per group. Statistical comparisons were made using the non-paired Student's *t*-test.

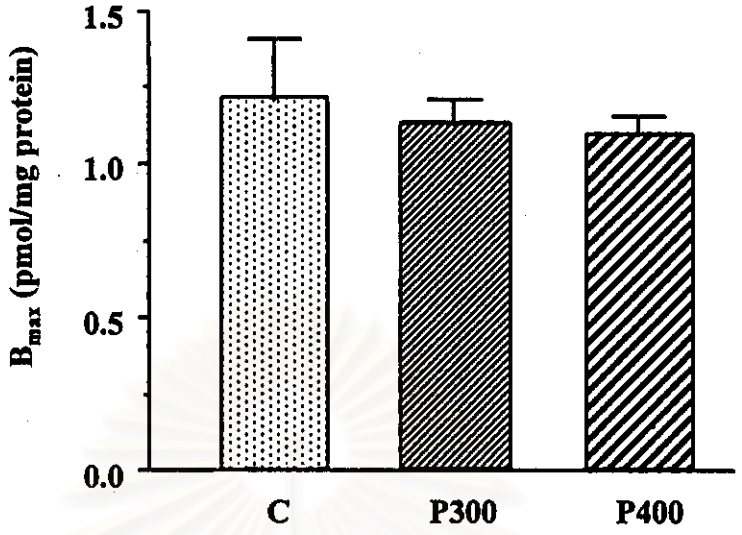

Figure 77. Comparison of the maximum number of binding sites (B_{max}) of [^{3}H]-imipramine in brain stem of control (C) and acute treated rats with paracetamol 300 (P300) and 400 (P400) mg/kg/day. Bmax values were expressed as means ± S.E.M. of 4-5 rats per group.

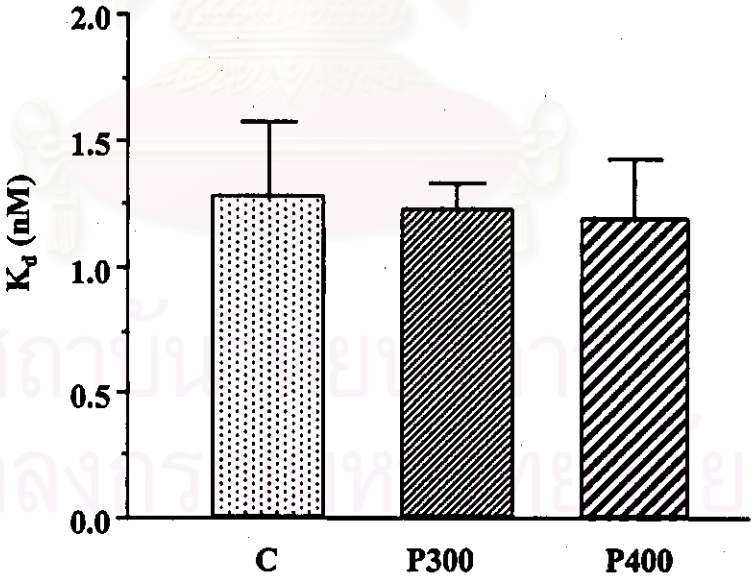

Figure 78. Comparison of the dissociation affinity constants (K_d) of [^{3}H]-imipramine binding sites in brain stem of control (C) and acute treated rats with paracetamol 300 (P300) and 400 (P400) mg/kg/day. K_d values were expressed as means ± S.E.M. of 4-5 rats per group

Table 28. Binding characteristics of [^{3}H]imipramine to brain stem membranes in control and acute paracetamol-treated rats

Groups	Rat No.	K_d (nM)	B_{max} (pmol/mg protein)
Control	1	2.3	1.98
	2	0.7	1.03
	3	1.6	1.16
	4	0.95	1.10
	5	0.85	0.76
	means ± S.E.M.	**1.28 ± 0.29**	**1.21 ± 0.20**
Para 300	6	0.98	0.96
	7	1.37	1.34
	8	1.10	1.09
	9	1.03	1.11
	means ± S.E.M.	**1.22 ± 0.11**	**1.13 ± 0.08**
Para 400	10	0.93	1.11
	11	0.98	1.13
	12	0.94	0.92
	13	1.92	1.21
	means ± S.E.M.	**1.19 ± 0.24**	**1.09 ± 0.06**

Statistical comparisons were made using the non-paired Student's *t*-test.

The saturation curve and Scatchard analysis of these data were shown in Fig. 79-81 and Fig. 122-124.

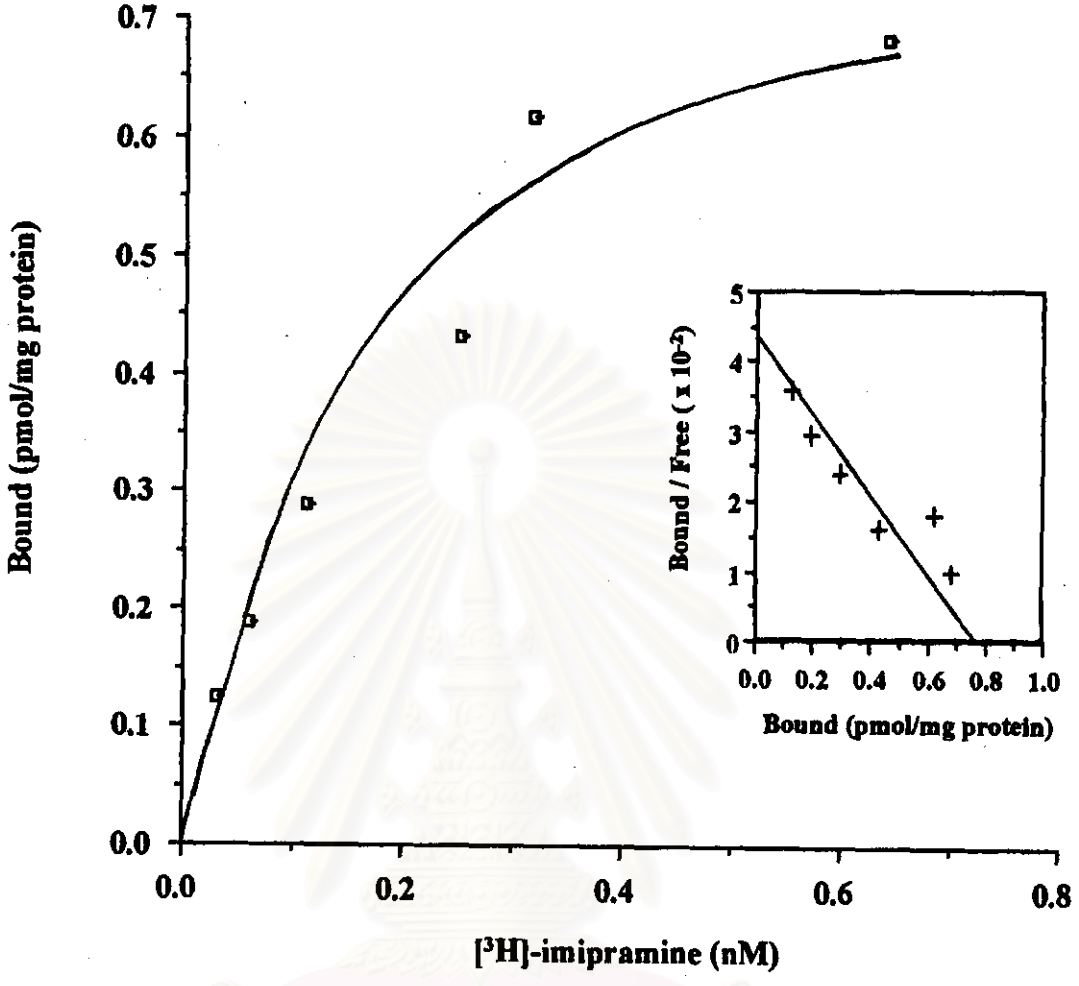

Figure 79. Saturation curve and Scatchard analysis (in the inset) of [^{3}H]-imipramine binding on brain stem membrane of control rat number 5, treated with vehicle i.p. for 90 min. The binding was carried out in six concentrations of [^{3}H]-imipramine ranging from 0.02 - 0.8 nM. The plots were obtained from duplicate determinations and represented the specific binding of [^{3}H]-imipramine. The line of best fit was analysed by the LIGAND computer program. The result of this experiment was shown and provided a K_d value of 0.85 nM and B_{max} value of 0.76 pmol/mg protein.

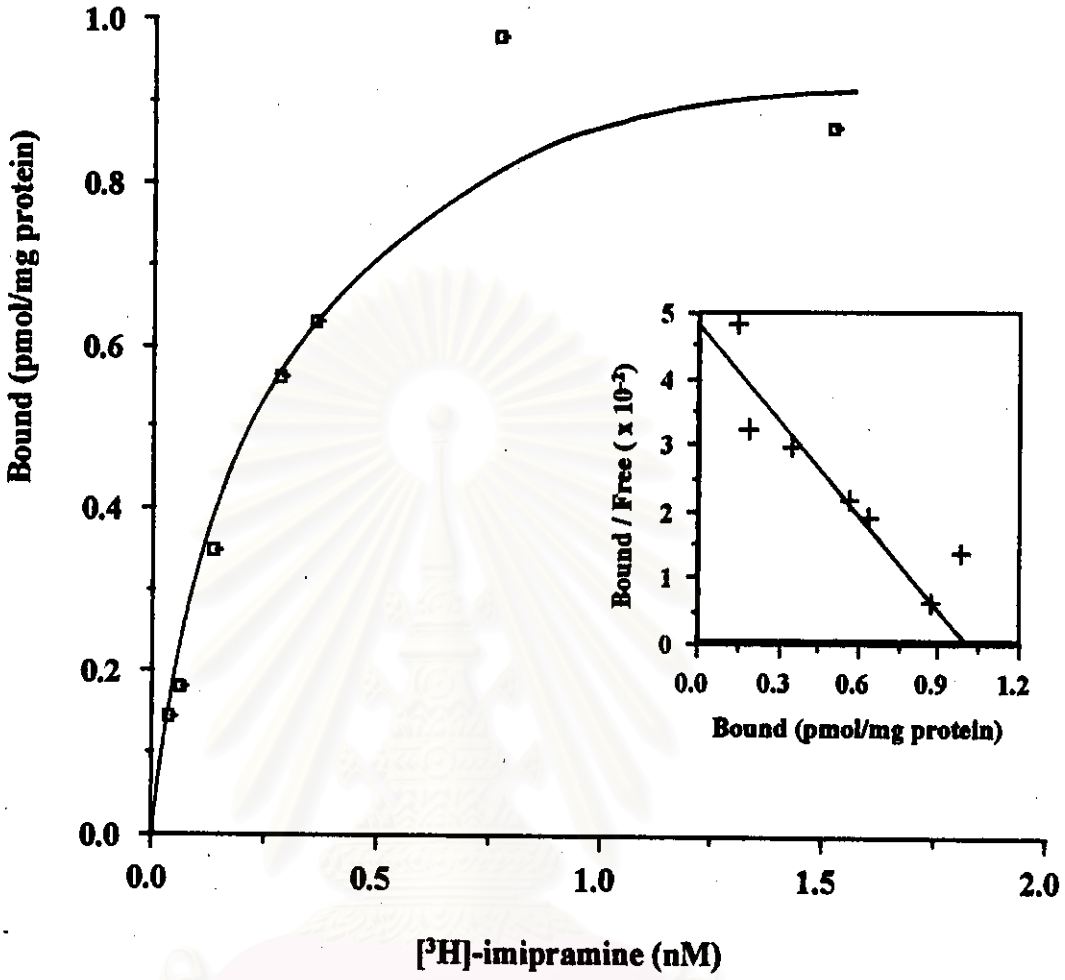

Figure 80. Saturation curve and Scatchard analysis (in the inset) of [^{3}H]-imipramine binding on brain stem membrane of rat number 9, treated with paracetamol 300 mg/kg i.p. for 90 min. The binding was carried out in seven concentrations of [^{3}H]-imipramine, ranging from 0.01 - 2 nM.The plots were obtained from duplicate determinations and represented the specific binding of [^{3}H]-imipramine. The line of best fit was analysed by the LIGAND computer program. The result of this experiment was shown and provided a K_d value of 1.03 nM and B_{max} value of 1.11 pmol/mg protein.

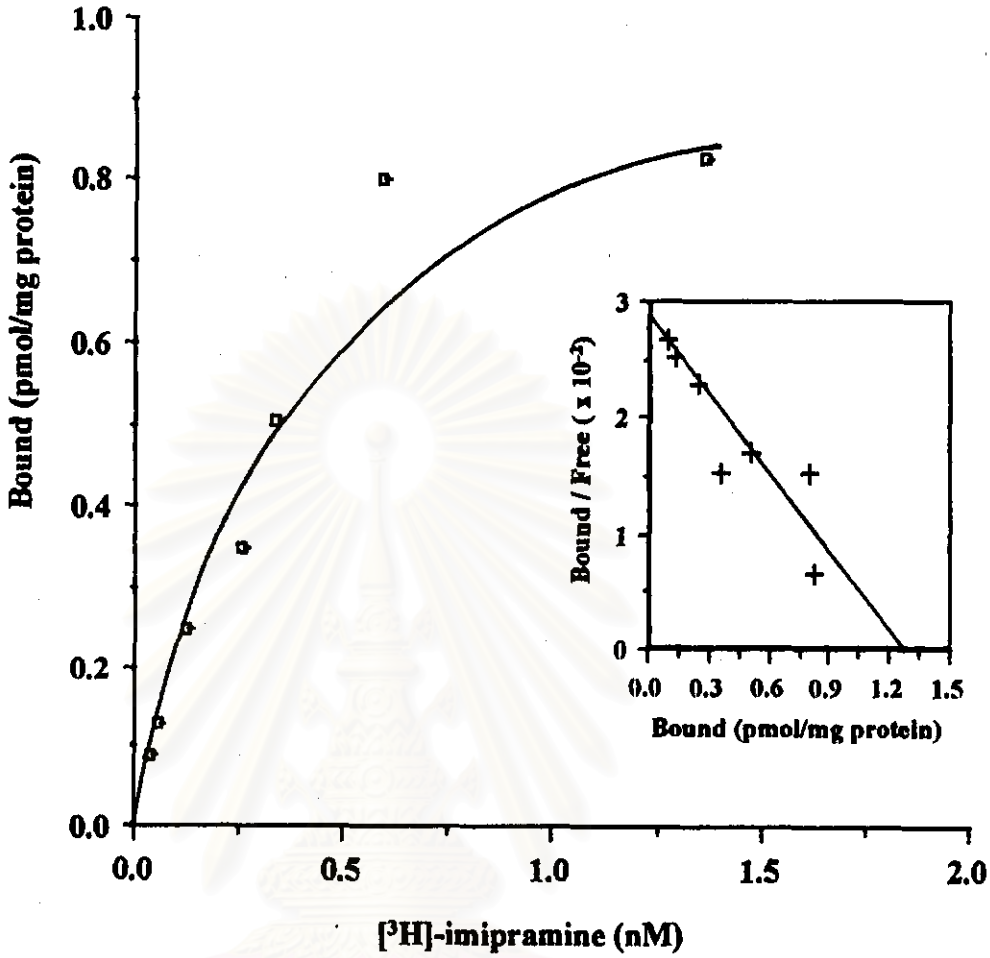

Figure 81. Saturation curve and Scatchard analysis (in the inset) of [^{3}H]-imipramine binding on brain stem membrane of rat number 13, treated with paracetamol 400 mg/kg i.p. for 90 min. The binding was carried out in seven concentrations of [^{3}H]-imipramine, ranging from 0.02 - 2 nM. The plots were obtained from duplicate determinations and represented the specific binding of [^{3}H]-imipramine. The line of best fit was analysed by the LIGAND computer program. The result of this experiment was shown and provided a K_d value of 1.91 nM and B_{max} value of 1.21 pmol/mg protein.

15. Acute and Chronic Effect of Paracetamol Treatment on antinociceptive activity

The tail flick latency for control and treated groups with paracetamol 400 mg/kg/day after 90 min, 15 and 30 days was 8.52 ± 1.18, 16.52 ± 4.48, 25.79 ± 4.78 and 6.46 ± 0.36 sec, respectively. A significant increase of tail flick latency was observed in the treated rats with paracetamol after 90 min and 15 days , but not 30-day treated rats, as compared to that in control rats. (Table 29 and Fig. 82).

Table 29. Comparison of tail flick latency in control and paracetamol-treated rats 400 mg/kg/day after 90 min, 15 and 30 days

Groups of treatment	Tail flick latency (sec)
vehicle treated rats	8.52 ± 1.18
90 min-paracetamol-treated rats	16.52 ± 4.48*
15 day-paracetamol-treated rats	25.79 ± 4.78**
30 day-paracetamol-treated rats	6.46 ± 0.36

Data were expressed as means ± S.E.M. of 6 rats per group.

* indicate significant difference from control ($p < 0.05$).

** indicate significant difference from control ($p < 0.01$).

Statistical comparisons were made using the non-paired Student's *t*-test.

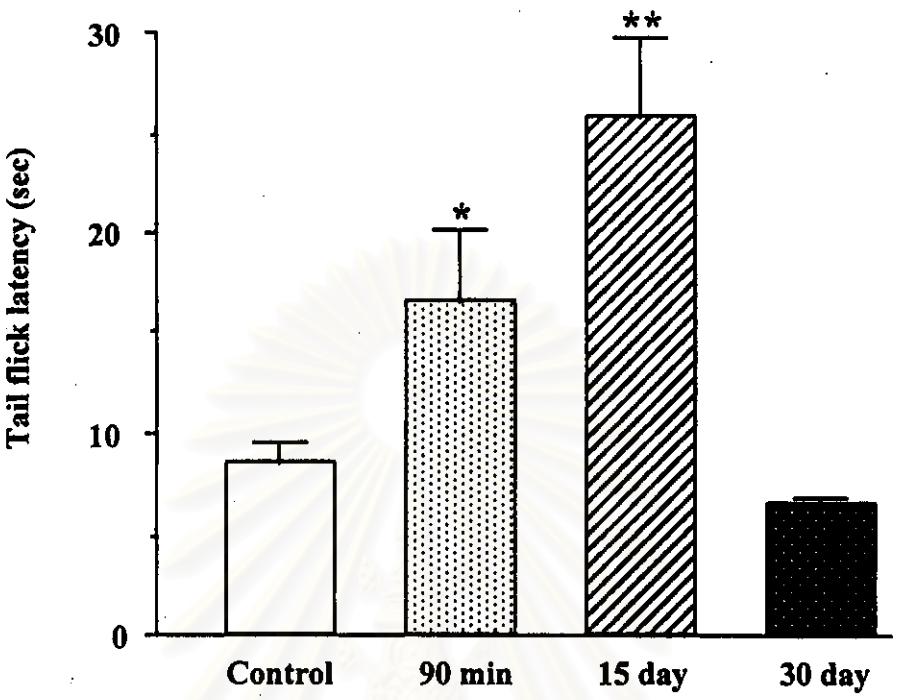

Figure 82. Comparison of tail flick latency in control and treated rats with paracetamol 400 mg/kg/day after 90 min, 15 and 30 days .Data were expressed as means ± S.E.M. of 6 rats per group.

* indicate significant difference from control group ($p < 0.05$).

** indicate significant difference from control group ($p < 0.01$).

Statistical comparisons were made using the non-paired Student's *t*-test.

16. Chronic Effect of Paracetamol Treatment for 15 days on Rat Body Weight

In chronic 15 day-treatment, the mean body weight of rats was 288.13 ± 2.66, 284.38 ± 1.99, 253.13 ± 3.52 and 245 ± 3.40 g for control and treated groups with paracetamol 200, 300 and 400 mg/kg/day respectively. The rats treated with paracetamol 300 and 400 mg/kg, but not 200 mg/kg, showed a significant decrease of body weight ($p < 0.001$). The overall loss of weight was 35 g (12% decrease) and 43 g (15% decrease) for the group of treatment with paracetamol 300 and 400 mg/kg, respectively as compared to the control group (Table 30 and Fig. 83).

Table 30. Comparison of the rat body weight (g) between control and 15-day treated-rats with paracetamol 200, 300 and 400 mg/kg/day

Group of treatment	rat body weight (g)
Control	288.13 ± 2.66
Para 200	284.38 ± 1.99
Para 300	253.13 ± 3.52***
Para 400	245.00 ± 3.40***

Data were shown as means ± S.E.M.of 8 rats per group.

*** indicate significant difference from control group ($p < 0.001$, the non-paired Student's *t*-test).

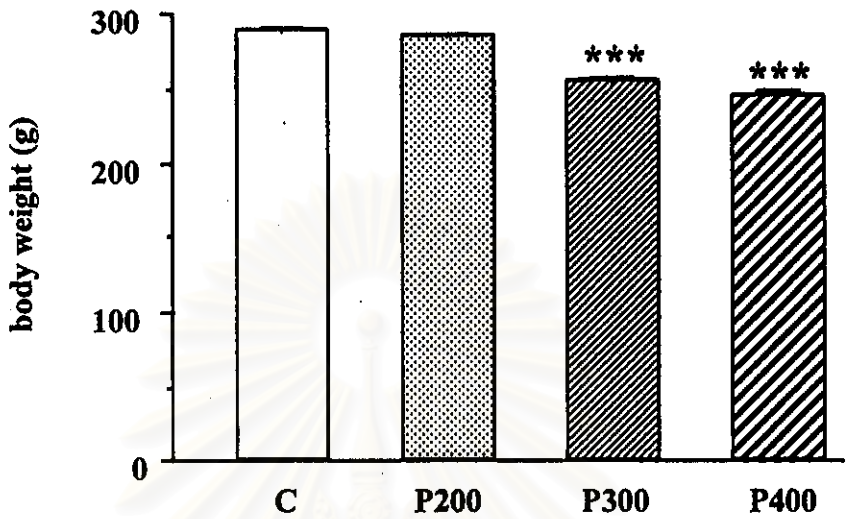

Figure 83. Comparison of body weight of control (C) and 15-day treated rats with paracetamol 200 (P200), 300 (P300) and 400(P400) mg/kg/day. Data were shown as means ± S.E.M.of 8 rats per group.
*** indicate significant difference from control group ($p < 0.001$, the non-paired Student's *t*-test) .

17. Chronic Effect of Paracetamol Treatment for 30 days on Rat Body Weight

After 30 days of drug administration, the mean body weight was 347.5 ± 2.99, 298.13 ± 3.56 and 290.63 ± 7.16 g for control and treated groups with paracetamol 300 and 400 mg/kg, respectively. A significant decrease of rat body weight was observed in the rats treated with paracetamol 300 and 400 mg/kg/day ($p < 0.001$). The overall loss of weight was 49 g (15% decrease) and 59 g (16% decrease) for the group of treatment with paracetamol 300 and 400 mg/kg, respectively, as compared to the control group (Table 31 and Fig. 84).

Table 31. Comparison of the rat body weight (g) between control and 30-day treated-rats with paracetamol 300 and 400 mg/kg/day

Group of treatment	rat body weight (g)
Control	347.50 ± 2.99
Para 300	298.13 ± 3.65***
Para 400	290.63 ± 7.16***

Data were shown as means ± S.E.M.of 8 rats per group.

*** indicate significant difference from control group ($p < 0.001$, the non-paired Student's *t*-test) .

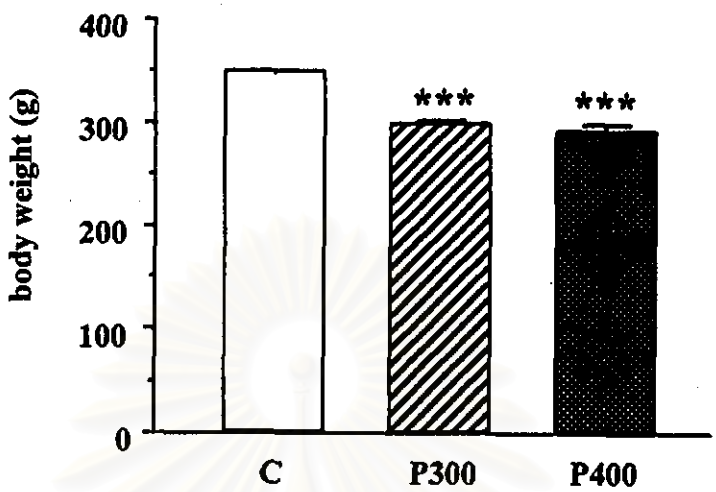

Figure 84. Comparison of body weight of control (C) and 30-day treated rats with paracetamol 300 (P300) and 400 (P400) mg/kg/day. Data were shown as means ± S.E.M. of 8 rats per group.

*** indicate significant difference from control group ($p < 0.001$, the non-paired Student's *t*-test).