CHAPTER 3

RESULTS

3.1 Effects of *Centella asiatica* extract on AFB_1-albumin adduct after a single dose of AFB_1 exposure.

3.1.1 Time course of AFB_1-albumin adducts formation after a single dose of AFB_1 in rats.

As shown in Table 3, AFB_1-albumin adduct levels were detected within 2 hours after AFB_1 treatment. The maximum level (1.06 + 0.05) was detected at 4 hours post-treatment and remained steady until 8 hours. After that the adducts had declined to 0.12 ± 0.06 ng/mg serum albumin at 120 hours (Figure 14) with half-life of the adduct of about 46 hours as calculated assuming a first-order rate constant based on the data in Figure 15.

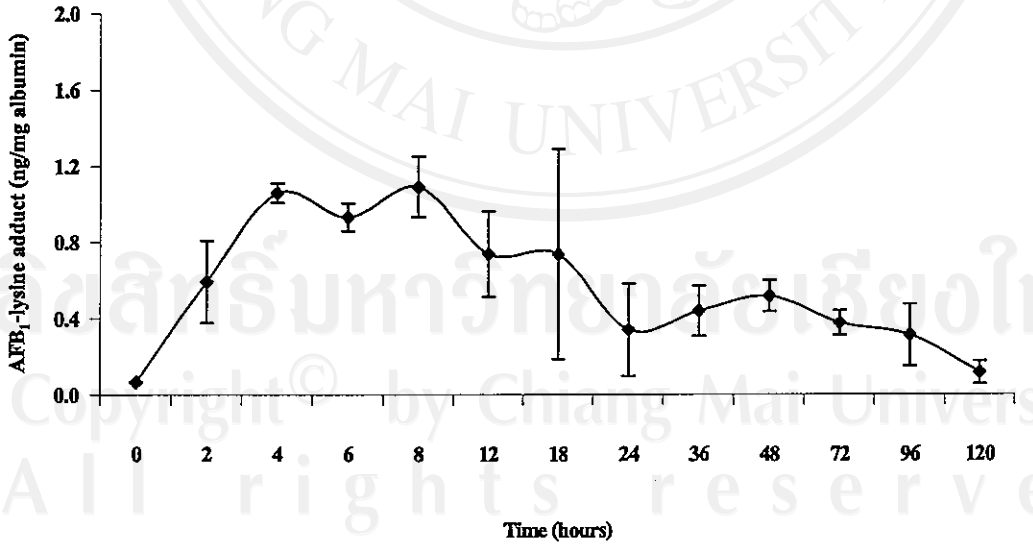

Figure 14 The levels of serum AFB_1-albumin adduct in rats treated with single doses of AFB_1

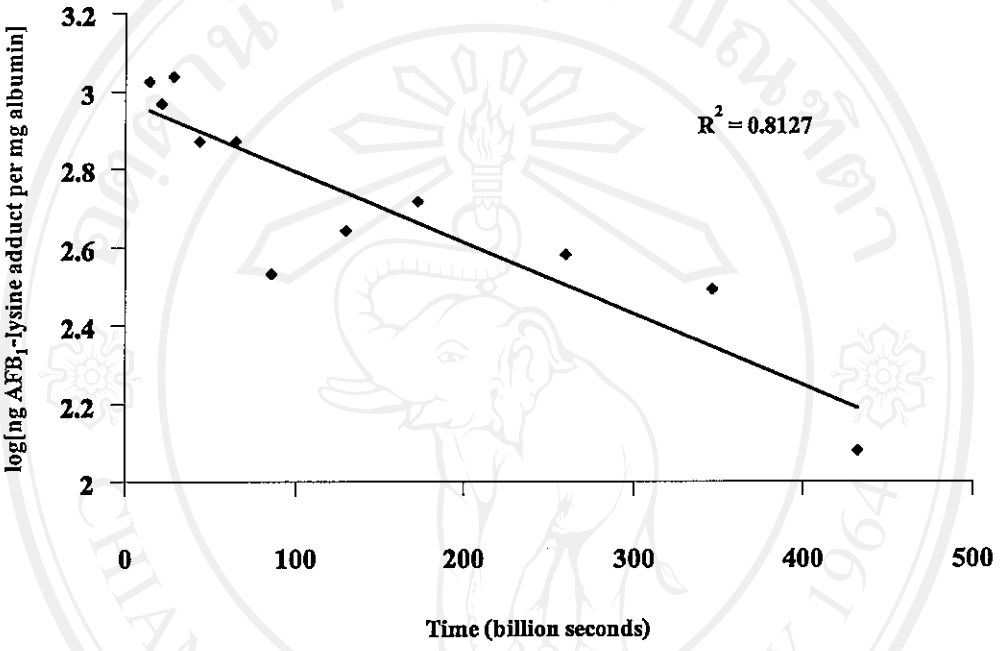

Figure 15 Kinetic of removal of AFB_1-albumin adducts following exposure to AFB_1.

Table 3 The level of AFB_1-albumin adducts in serum after a single dose of AFB_1 exposure

Time(hrs)	Aflatoxin B_1-lysine adducts (ng/mg albumin)[a]				
	Group 1	Group 2	Group 3	Group 4	Group 5
0	0.07 + 0.00	0.09 + 0.01	0.12 + 0.01	0.08 + 0.02	0.05 + 0.03
2	0.59 + 0.22	1.74 + 0.08[c]	1.35 + 0.18[c]	0.11 + 0.08[b]	1.43 + 0.24[c]
4	1.06 + 0.05	1.22 + 0.26	2.47 + 0.09[b]	1.36 + 0.03	1.64 + 0.75
6	0.93 + 0.07	1.18 + 0.22	1.12 + 0.25	0.74 + 0.38	0.97 + 0.33
8	1.09 + 0.16	1.36 + 0.10	1.20 + 0.33	0.34 + 0.06[c]	0.46 +0.19[b]
12	0.74 + 0.22	1.44 + 0.13[c]	1.04 + 0.19[b]	0.31 + 0.11[b]	0.37 + 0.09
18	0.74 + 0.55	0.74 + 0.15	0.94 + 0.26	0.31 + 0.03	0.36 + 0.06
24	0.34 + 0.24	1.11 + 0.18[c]	0.67 + 0.12	0.70 + 0.29	0.33 + 0.03
36	0.44 + 0.13	0.40 + 0.04	0.48 + 0.17	0.55 + 0.13	0.62 + 0.06
48	0.52 + 0.08	0.41 + 0.12	0.50 + 0.14	0.48 + 0.32	0.52 + 0.04
72	0.38 + 0.06	0.43 + 0.06	0.50 + 0.15	0.49 + 0.27	0.30 + 0.08
96	0.31 + 0.16	0.38 + 0.13	0.55 + 0.24	0.18 + 0.01	0.13 + 0.05
120	0.12 + 0.06	0.66 + 0.05[c]	0.55 + 0.12[c]	0.24 + 0.11	0.07 + 0.01

a) Mean + SD

b-c) Significantly different from AFB_1-treated control group (Group 1) by Mann-Whiney U test [(b) < 0.05 and (c) < 0.01]

Group 1 : distilled water + AFB_1+ distilled water

Group 2 : *C. asiatica* (CA) extract (10 mg/kg bw) + AFB_1+ CA

Group 3 : *C. asiatica* (CA) extract (100 mg/kg bw) + AFB_1+CA

Group 4 : distilled water + AFB_1+ CA(10 mg/kg bw)

Group 5 : distilled water + AFB_1+ CA(100 mg/kg bw)

3.1.2 Modulation effect of *C. asiatica* extract on AFB_1-albumin adduct in AFB_1-treated rat serum

The effect of *C. asiatica* extract on AFB_1-albumin adduct formation was shown in Table 3. Administration the low dose (10 mg/kg bodyweight) of extract prior to AFB_1 exposure (group 2) resulted in an early detectable the maximum level of AFB_1-albumin adduct formation with significantly ($p<0.01$) higher levels 2 hours after treatment as compared to 4 hours in the control (group 1) while the maximum level of the adduct observed in the rats received the high dose (100 mg/kg bodyweight) of extract (group 3) was 4 hours after AFB_1 exposure similarly as the control. After that, the adduct levels in both group were slightly higher than in controls, and were maintained at the low steady state level (0.38-0.66 ng/mg albumin) until 36 hours post-treatment throughout the study (Figure 16).

Similar as control group, the levels of AFB_1-albumin adduct in the rats that fed with either low dose or high dose of the extract after AFB_1 exposure (group 4 and group 5, respectively) was peak at 4 hours post-treatment. However, the modulating effect of administration of the extract after treatment with AFB_1 (group 4 and 5) was different from the effect of pretreatment with the extract. As shown in Figure 17, after the adduct level reached the maximum level at 4 hours after treatment, the level rapidly decreased to significantly ($p<0.05$) lower than in the control group at 8 hours post-exposure of AFB_1. After that the level reached a plateau level and was not significantly different from the control.

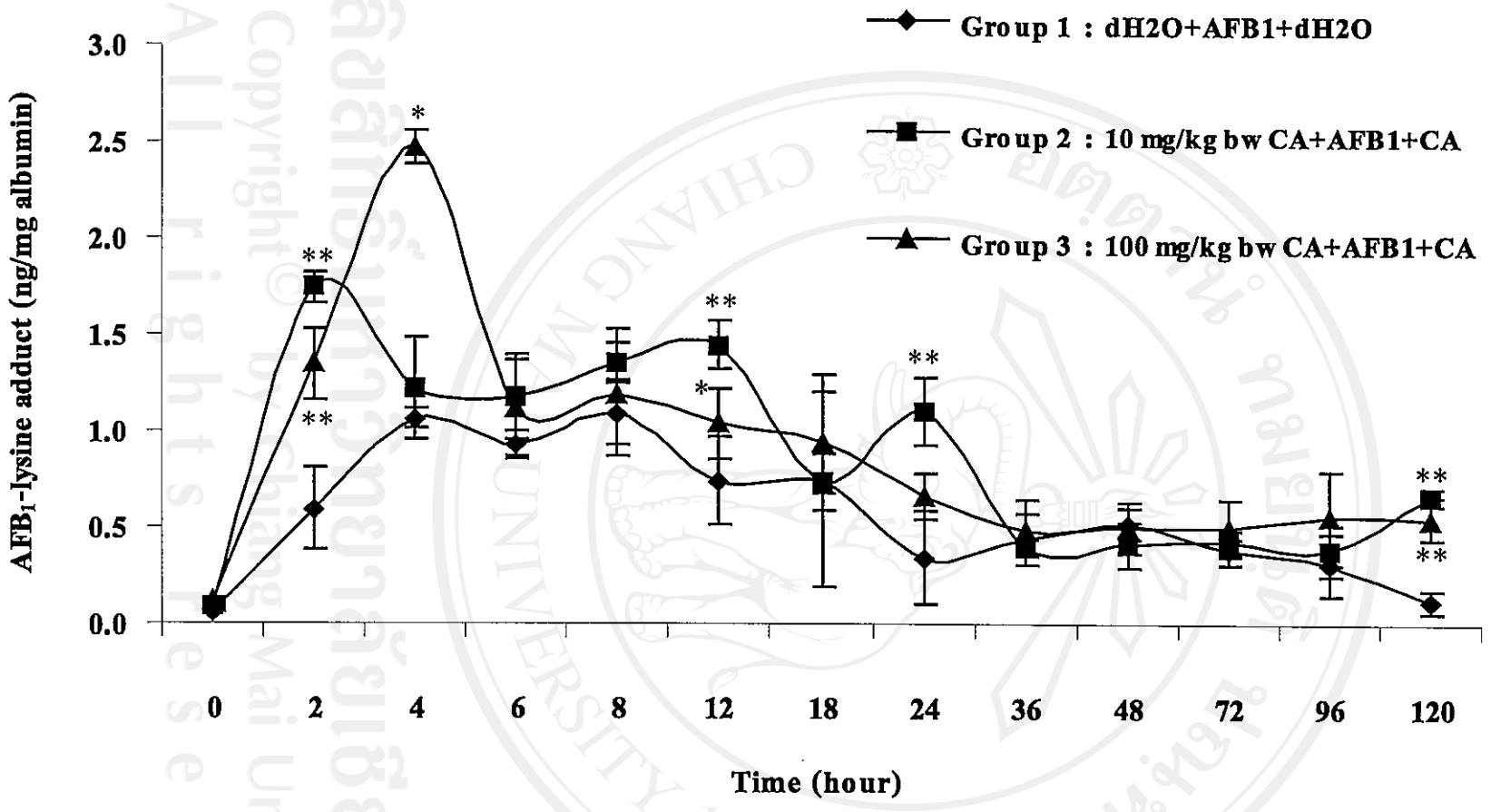

Figure 16 The levels of AFB_1-albumin adduct in serum of the rats received *C. asiatica* extract (CA) before and after treated with single doses of AFB_1 (* , ** Significantly different from treatment with AFB_1 alone (group 1): $p< 0.05$, $p< 0.01$ respectively; Mann-Whitney U test)

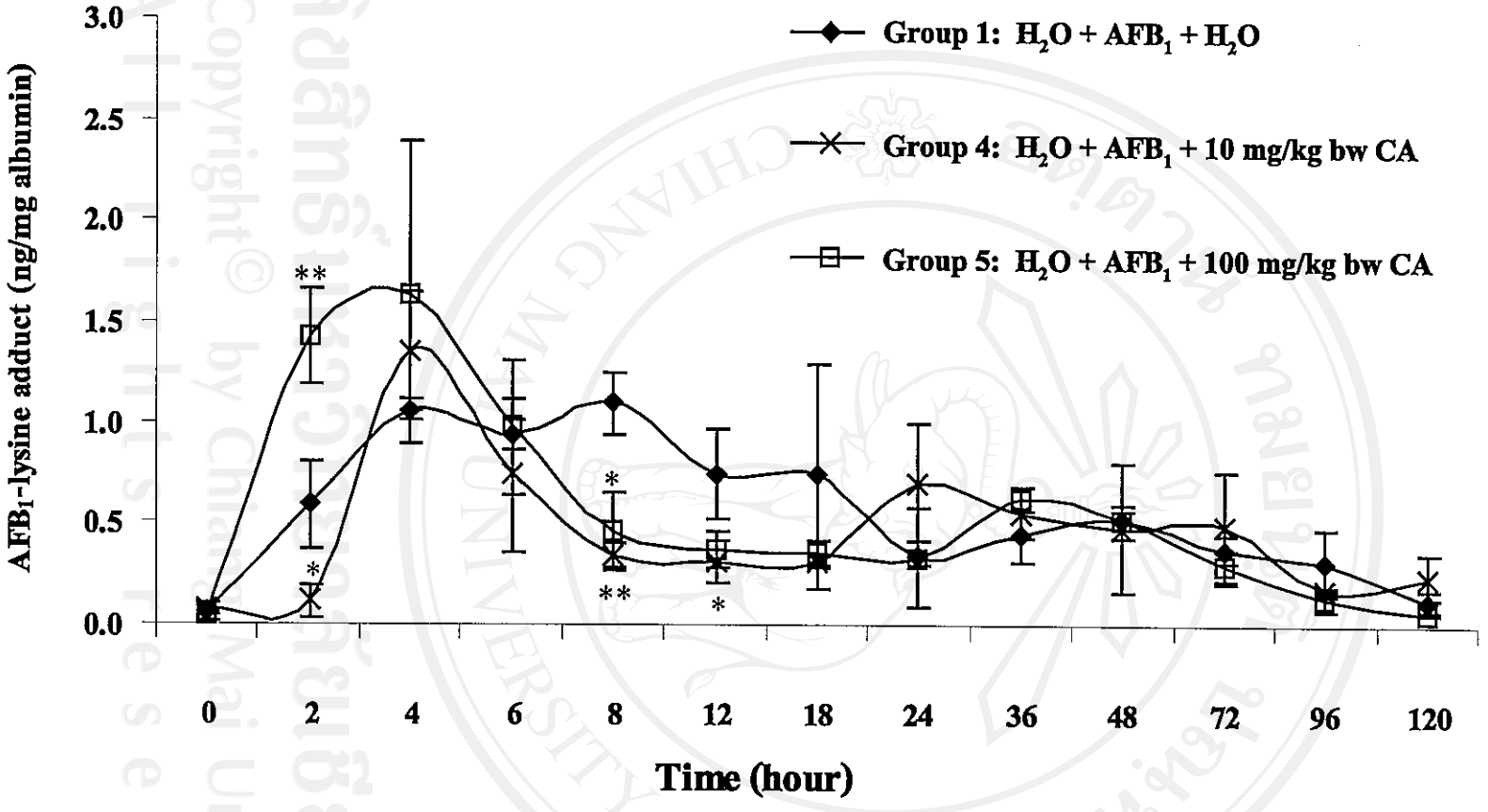

Figure 17 The levels of AFB_1-albumin adduct in serum of the rats received *C. asiatica* extract (CA) after treated with single doses of AFB_1 (* , ** Significantly different from treatment with AFB_1 alone (group 1): $p< 0.05$, $p< 0.01$ respectively; Mann-Whitney U test)

3.2 Effects of *C. asiatica* extract on AFB_1-metabolism in rat after treated with multiple dose of AFB_1 exposure

3.2.1 Effects of *C. asiatica* extract on AFB_1-albumin adduct formation

The mean body weight of rats in each group was shown in Figure 18. The body weight of rats that continuously received *C. asiatica* extract only (group 2) was not significantly difference from the rats that received distilled water only (group 1). Apparently, the body weight of rats both fed with AFB_1 only (group 5) and co-treated with AFB_1 and either high dose or low dose (group 3 or group 4) of *C. asiatica* extract was significantly lower than normal rats (group 1) after 16 weeks of the experiment.

It was noteworthy that neither distilled water (group 1) nor *C. asiatica* extract (group 2) administration was detectable the AFB_1-albumin adduct formation in rat serum, while the multiple dose of AFB_1 exposure (group 3, 4 and 5) resulted in accumulation of AFB_1-albumin adduct (Figure 19). As shown in Figure 20, the accumulation of AFB_1-albumin adduct in AFB_1-treated rats reached a steady state after 20 to 24 doses of AFB_1.

The effects of *C. asiatica* extract on AFB_1-albumin adduct level are shown in Table 4. Receiving the high dose (100 mg/kg bw) of extract (group 3) with post-treatment of four doses of AFB_1 resulted in slightly increased albumin adduct levels but this was not significantly difference from the AFB_1 control group (group 5). In addition, there was a significantly ($p<0.05$) elevated (2.03 folds) level from the AFB_1 control group in rats that co-treatment of the low dose (10 mg/kg bw) of extract with AFB_1 (group 4).

Apparently, administration the high dose of the extract resulted in the significantly difference in adducts level observed after 12 and 16 doses of AFB_1 treatment compared to AFB_1 control group. Although the adduct level was lower than AFB_1 control group, no significant difference could be observed after 24 doses of AFB_1. Conversely, the albumin adduct level observed in rats that received the low dose of extract (group 4) was significantly decreased ($p<0.05$) after 24 doses of AFB_1 as compared to AFB_1 control group, as shown in Figure 20.

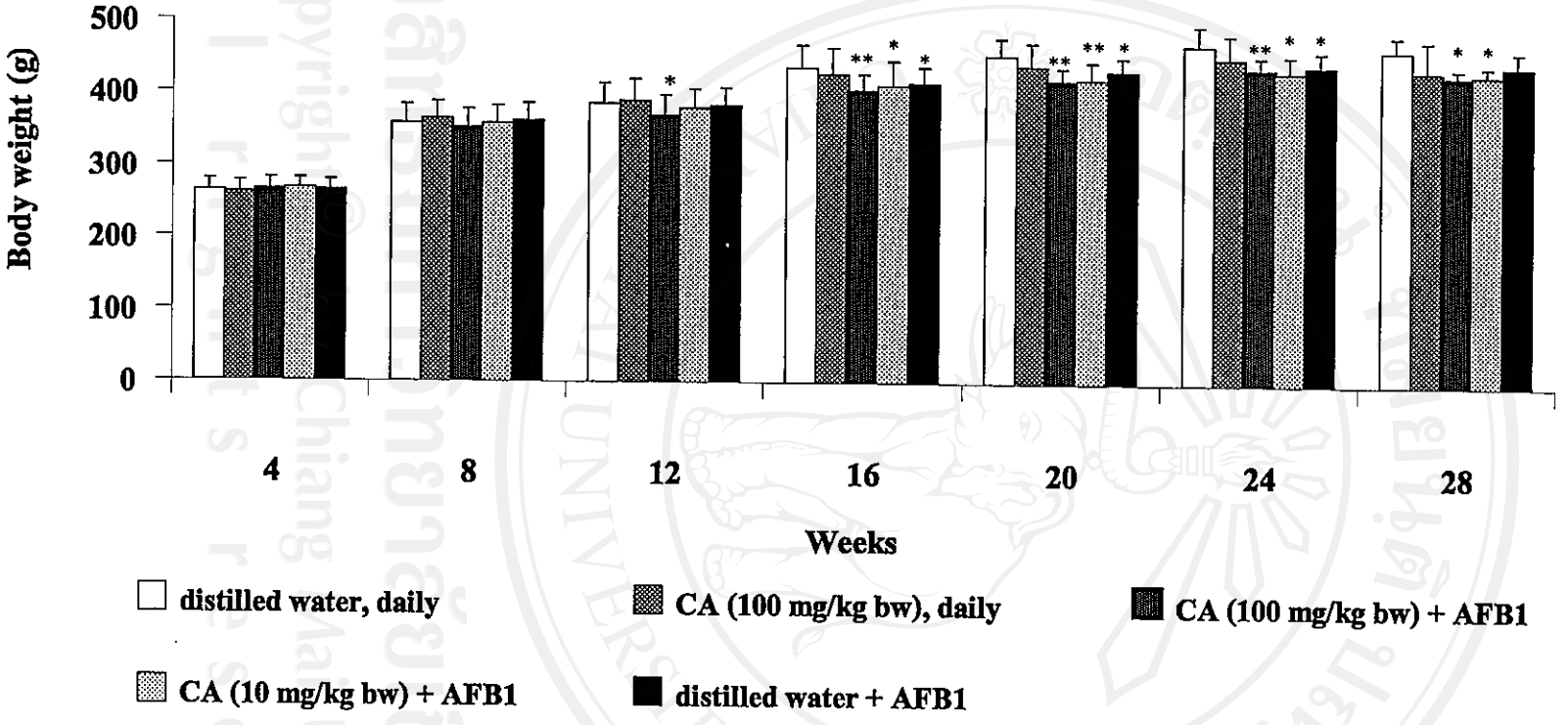

Figure 18 Mean body weight of Wistar rats in the multiple dose of AFB_1 exposure experiment

(*, ** Significantly different from AFB_1-treated control group by Mann-Whiney U test, $p< 0.05$ and $p< 0.01$ respectively)

Table 4 Effects of *C. asiatica* extract on AFB_1-albumin adduct in multiple dose of AFB_1-treated rat

Group	Treatment	AFB_1-lysine adduct (ng/mg albumin)[a] (after dose of AFB_1 administration)					
		4	8	12	16	20	24
1	Distilled water, daily	0.20 ± 0.13	0.67 ± 0.19	0.28 ± 0.19	0.10 ± 0.08	0.17 ± 0.16	0.08 ± 0.06
2	CA extract (100 mg/kg bw), daily	0.55 ± 0.34	0.59 ± 0.17	0.46 ± 0.15	0.08 ± 0.04	0.08 ± 0.04	0.14 ± 0.07
3	CA extract (100 mg/kg bw), daily AFB_1 (400 μg/kg bw), once a week	16.73 ± 2.06	19.40 ± 3.67	10.62 ± 4.24[b]	8.71 ± 1.43[c]	8.01 ± 2.37	4.72 ± 2.38
4	CA extract (10 mg/kg bw), daily AFB_1 (400 μg/kg bw), once a week	24.28 ± 5.90[b]	17.15 ± 4.44	16.35 ± 3.00	7.30 ± 2.57	11.53 ± 1.56	4.57 ± 2.89[b]
5	Distilled water, daily AFB_1 (400 μg/kg bw), once a week	11.98 ± 4.69	17.98 ± 2.18	15.66 ± 2.42	5.25 ± 1.19	10.29 ± 1.40	9.89 ± 3.23

a) Mean ± SD

b-c) Significantly different from AFB_1-treated control group by Mann-Whiney U test [(b) < 0.05 and (c) < 0.01]

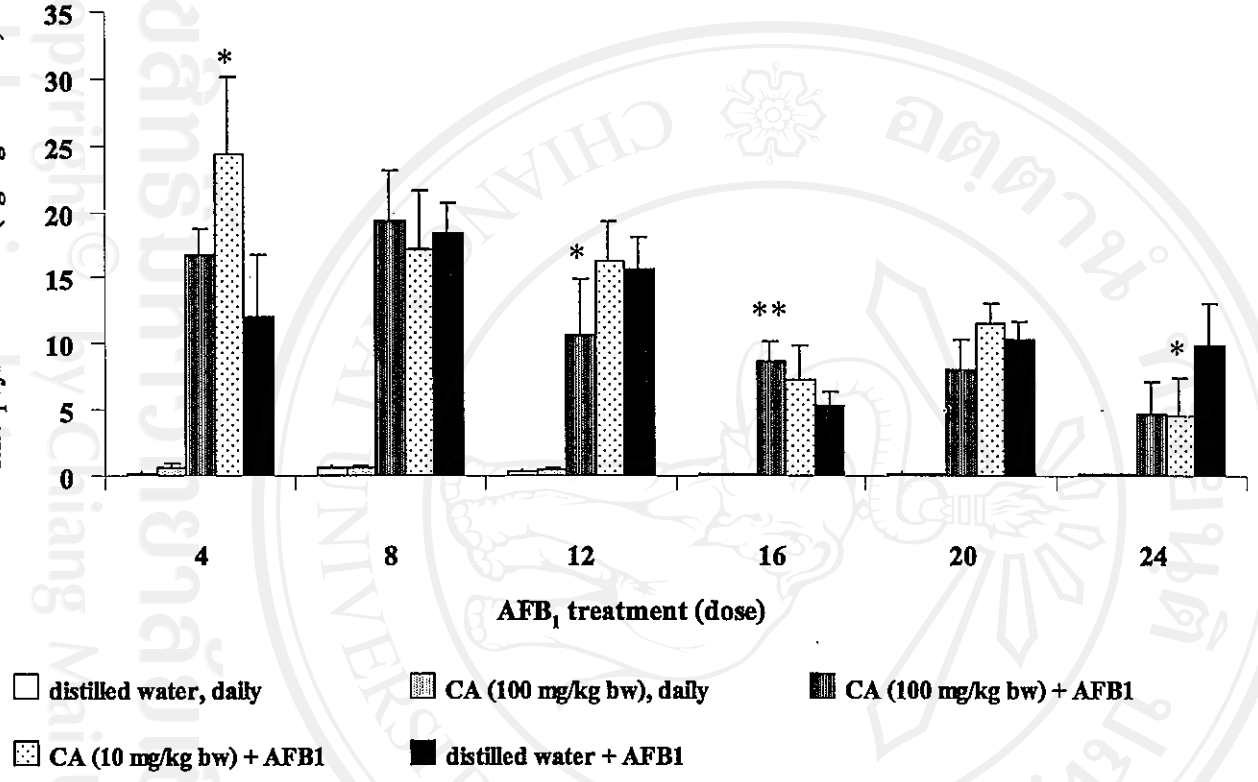

Figure 19 Mean serum AFB_1-albumin adduct levels in rats received *C. asiatica* extract after treated with multiple doses of AFB_1 (*, ** Significantly different from treatment with AFB_1 alone (group 5), $p< 0.05$, $p< 0.01$ respectively; Mann-Whitney Test)

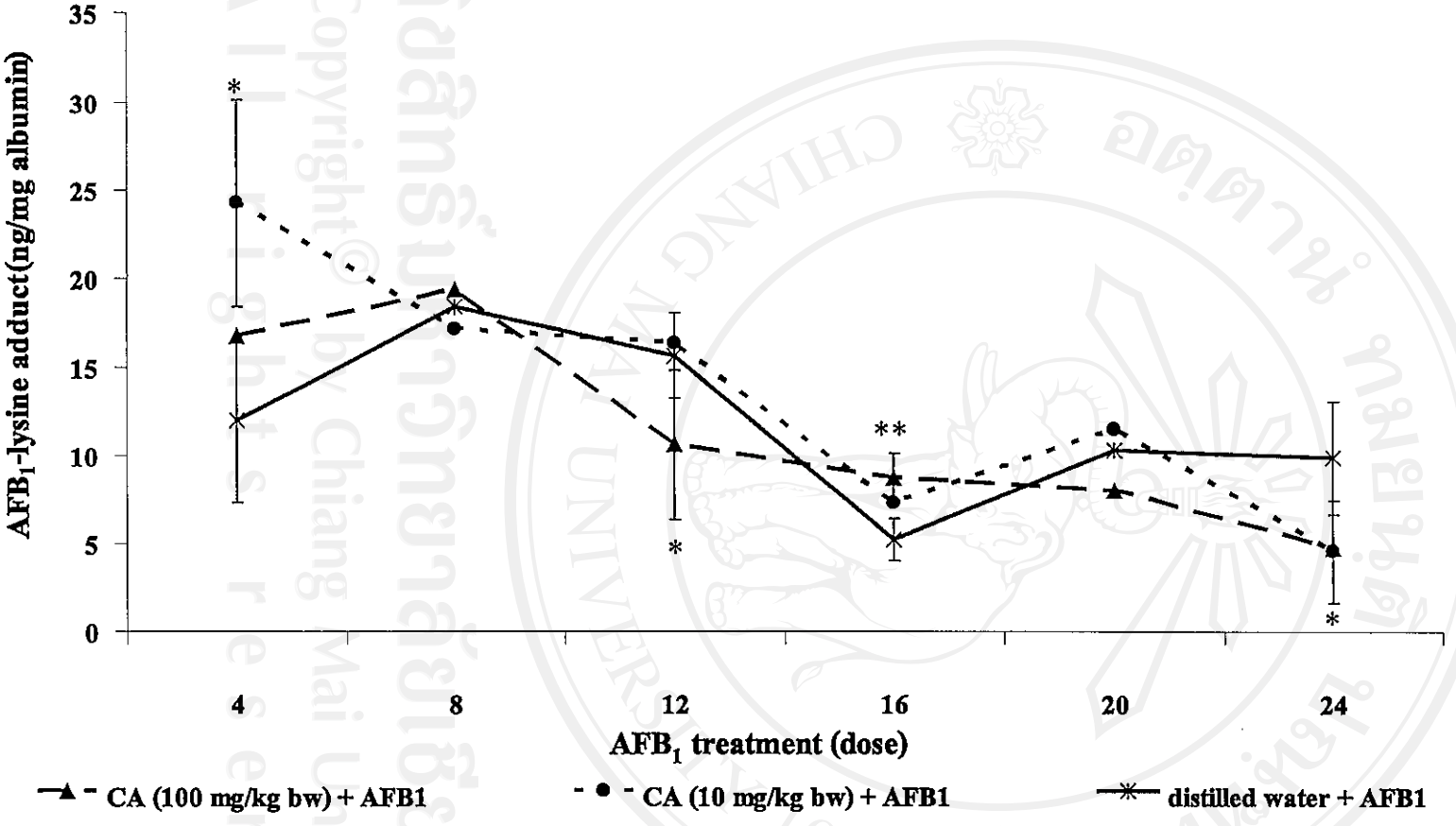

Figure 20 Accumulation of AFB_1 bound to albumin following multiple dose exposure

(*, ** significantly different from treatment with AFB_1 alone (group 5) $p< 0.05$, $p< 0.01$ respectively; Mann-Whitney U Test)

3.2.2 Effects of *C. asiatica* extract on 8-OHdG formation in rat liver

The standard curves for 2'-deoxyguanosine (2'-dG) and 8-hydroxy-2'-deoxyguanosine (8-OHdG) were obtained for concentrations ranging between 20-100 μg/ml and 0.5-7.0 μg/ml, respectively, as shown in Figure 21. In this system the limits of detection of 2'-dG and 8-OHdG were 0.2 μg/ml.

Figure 22 and Figure 23 show the electropherograms of standard and liver DNA samples of 2'-dG and 8-OHdG under described CE conditions. Migration times were 1.5 min and 1.75 min for 2'-dG and 8-OHdG, respectively.

The results (Table 5) showed that after AFB_1 exposure the level of 8-OHdG formation in rat liver was significantly increased in all doses except at 12 and 20 doses of AFB_1 exposure. However, the level of 8-OHdG in rat received *C. asiatica* extract (100 mg/kg bodyweight) only (group 2) was not significantly different from the control group that received distilled water only (group 1).

By administration of the low dose (10 mg/kg bw) of *C. asiatica* extract (group 4), the level of 8-OHdG was significantly increased ($p<0.01$), while the administration of the higher dose (100 mg/kg bw) of *C. asiatica* extract (group 3) was slightly increased after 4 doses of AFB_1 (Figure 24). However, after exposure to 8 doses of AFB_1, the 8-OHdG formation was decreased. The reduction of 8-OHdG level was significant only in rats that received the high dose of extract. It was noteworthy that reduction in 8-OHdG of about 1.4 folds and 1.5 folds was observed in rats that received the high dose and low dose of extract after the 24 doses of AFB_1, respectively, but not significantly (Figure 25).

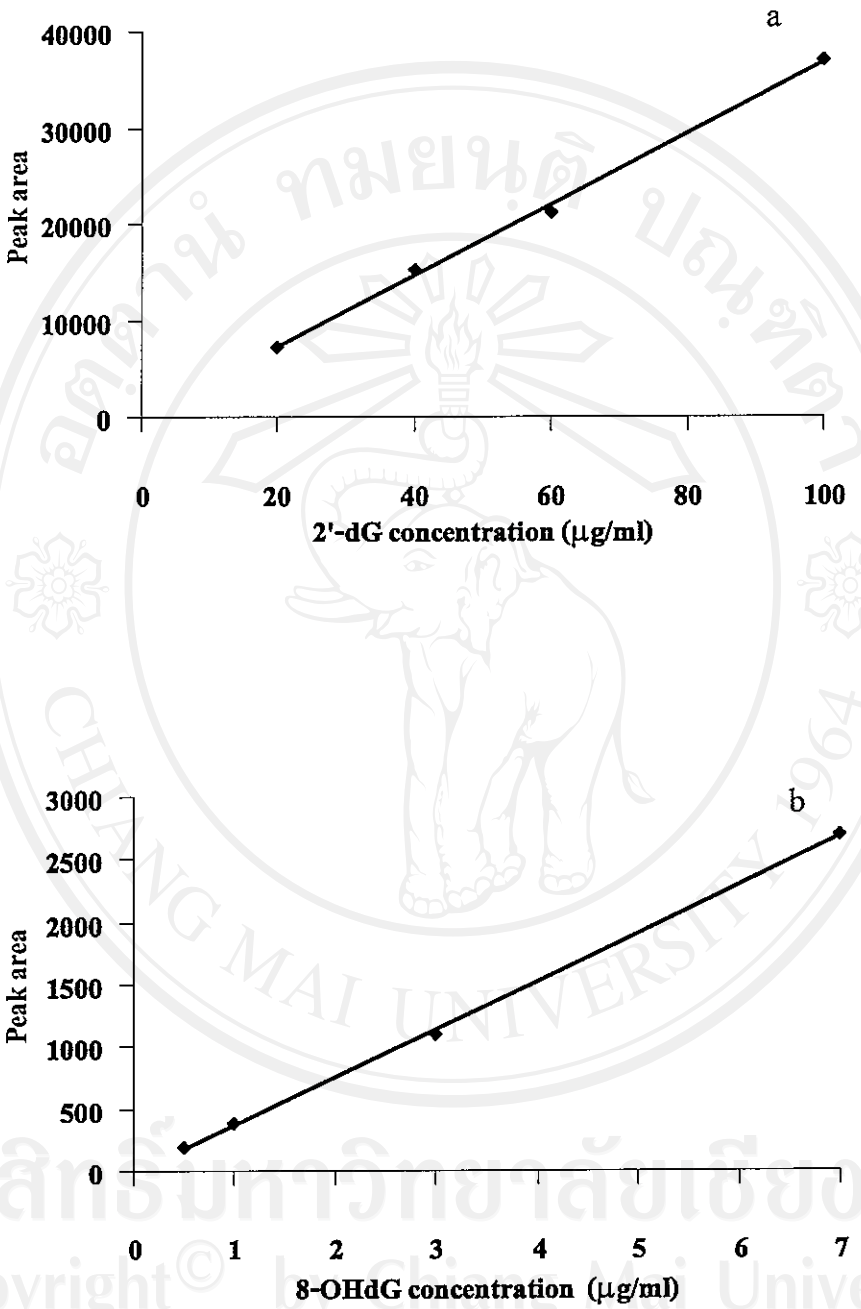

Figure 21 Standard calibration curves of standard (a) 2′-deoxyguanosine (2′-dG), (b) 8-hydroxy-2′-deoxyguanosine (8-OHdG)

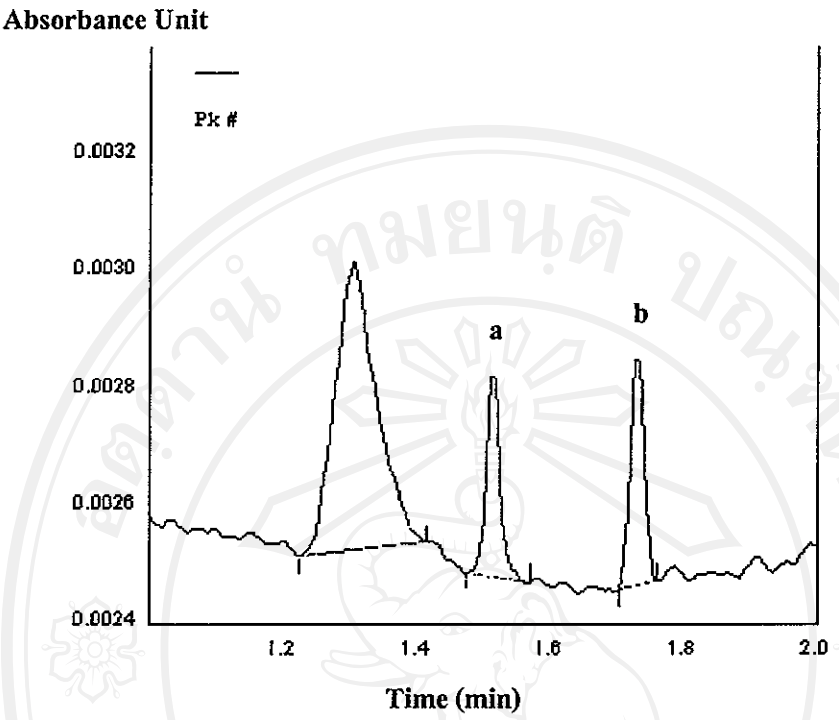

Figure 22 Electropherogram of standard (a) 2'-dG and (b) 8-OHdG

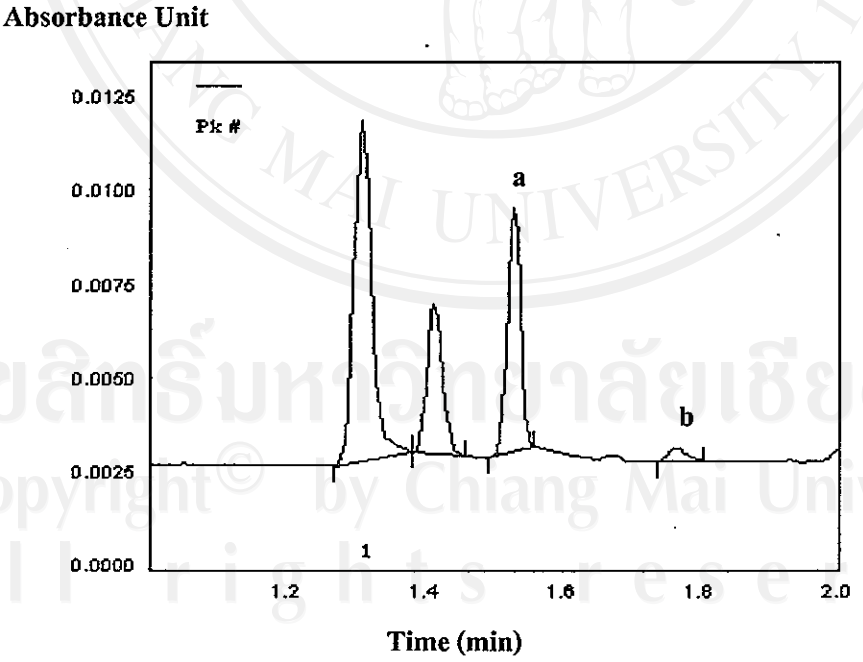

Figure 23 Electropherogram of liver DNA sample of rat treated with AFB_1 (a) 2'-dG and (b) 8-OHdG

Table 5 Effect of *C. asiatica* extract on 8-OHdG formation in multiple dose of AFB_1-treated rat

Group	Treatment	8-OHdG / 10^3 dG[a] (after dose of AFB_1 administration)					
		4	8	12	16	20	24
1	Distilled water	6.06 ± 0.84	9.39 ± 0.64	10.49 ± 4.38	15.18 ± 1.60	11.55 ± 2.80	13.30 ± 1.47
2	CA extract (100 mg/kg bw)	7.54 ± 3.04	11.20 ± 2.34	8.54 ± 2.21	17.98 ± 3.57	14.34 ± 4.07	16.50 ± 4.61
3	CA extract (100 mg/kg bw) AFB_1	9.48 ± 3.29	10.29 ± 2.21[d]	15.27 ± 6.03	21.21 ± 4.82	17.86 ± 2.32	18.09 ± 5.94
4	CA extract (10 mg/kg bw) AFB_1	13.89 ± 3.94[e]	12.77 ± 2.24	15.71 ± 5.85	20.76 ± 4.05	17.77 ± 3.01	16.31 ± 6.51
5	Distilled water AFB_1	8.22 ± 0.52[c]	15.64 ± 2.59[b]	15.33 ± 2.98	25.47 ± 4.31[c]	14.60 ± 1.93	24.47 ± 2.99[b]

a) Mean ± SD

b-c) Significantly different from non-treated group (Group 1) by Mann-Whiney U test [(b) < 0.05 and (c) < 0.01]

d-e) Significantly different from AFB_1-treated control group (Group 5) by Mann-Whiney U test [(d) < 0.05 and (e) < 0.01]

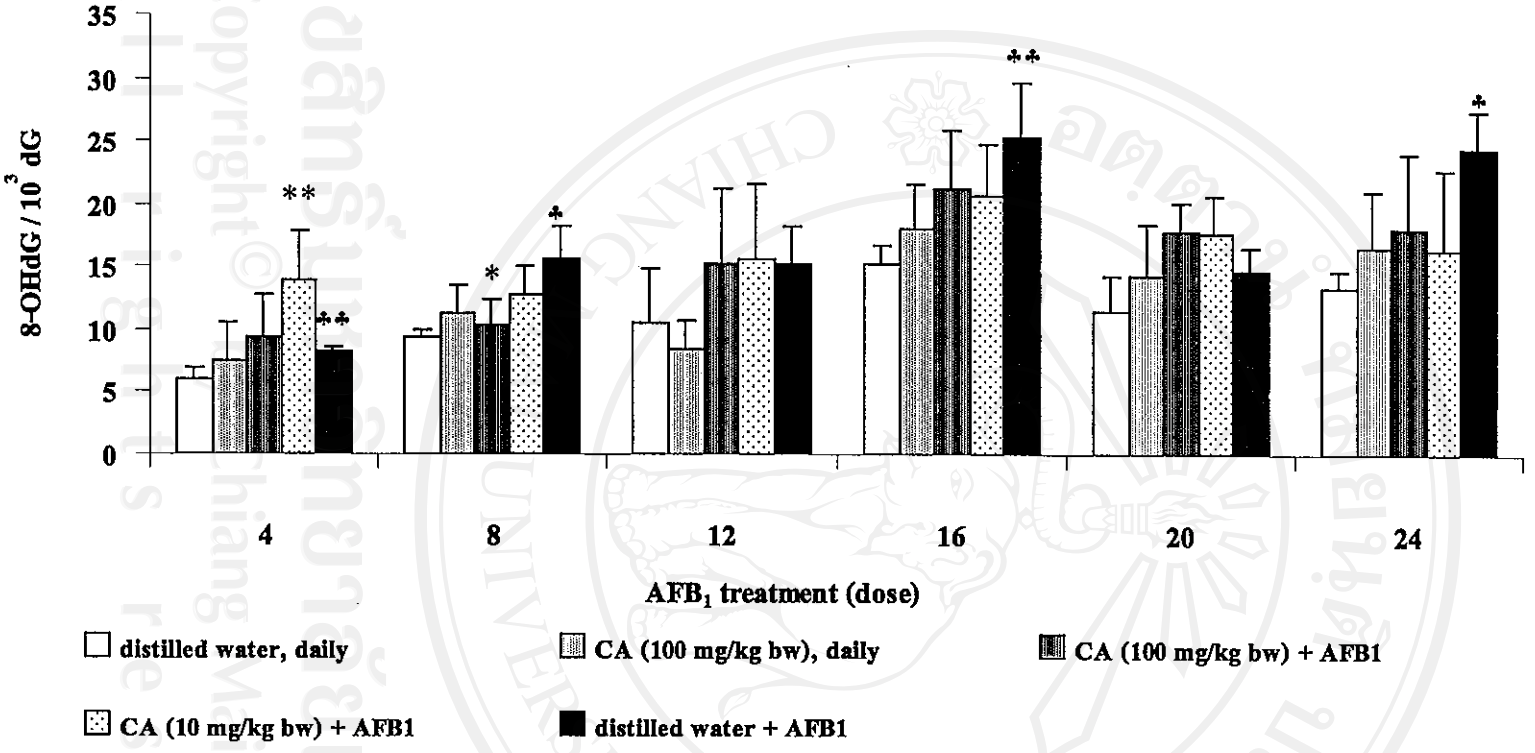

Figure 24 Mean 8-OHdG levels in rats received *C. asiatica* extract after treated with multiple doses of AFB_1

(*, ** significantly different from treatment with AFB_1 alone (group 5), $p< 0.05$, $p< 0.01$ respectively; Mann-Whitney U Test)

(♣, ♣♣ significantly different from non-treated rats (group 1), $p< 0.05$, $p< 0.01$ respectively; Mann-Whitney U Test)

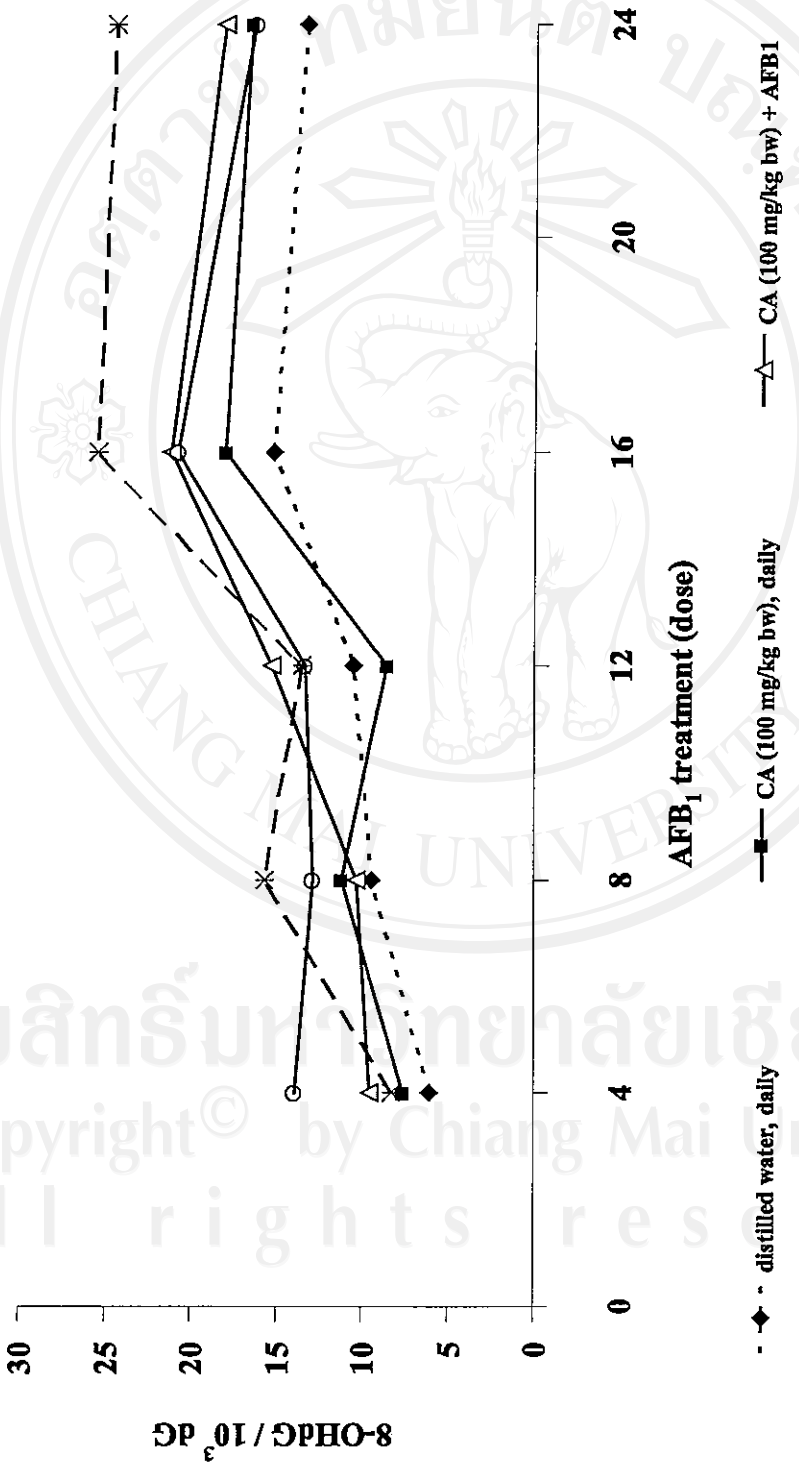

Figure 25 Effects of *C. asiatica* extract on 8-OHdG formation in rats treated with multiple doses of AFB_1

3.3.3 Effects of *C. asiatica extract* on GGT activity in rat serum

As shown in Table 6 and Figure 26, GGT activity in non-treated rats (Group 1) was 3.00-5.00 IU/L. A significant (1.6 folds) induction of GGT activity in AFB_1 control rat serum (Group 5) was demonstrated after the 24 doses of AFB_1 as compared to non-treated rat (Group 1).

The results showed that the GGT activity was reduced ($p<0.01$) in rats that received the high dose (100 mg/kg bw) of *C.asiatica* extract after 24 doses of AFB_1 administration. Conversely, administration of low dose (10 mg/kg bw) of *C. asiatica* extract resulted in significantly increased GGT activity after 16 doses of AFB_1 exposure. It was apparently that GGT activity was slightly decreased after 24 doses of AFB_1, but not significantly (Figure 26).

Table 6 Effect of *C. asiatica* extract induced GGT in multiple dose of AFB_1-treated rat

Group	Treatment	GGT activity (IU/L) (after dose of AFB_1 administration)					
		4	8	12	16	20	24
1	Distilled water	3.67 ± 2.08	5.00 ± 1.41	3.00 ± 0.00	3.80 ± 1.10	3.00 ± 0.00	3.50 ± 1.00
2	CA extract (100 mg/kg bw)	2.80 ± 1.30	2.75 ± 0.50	7.40 ± 4.34	4.60 ± 0.89	4.33 ± 1.16	3.00 ± 0.00
3	CA extract (100 mg/kg bw) AFB_1	2.50 ± 0.71	4.75 ± 3.10	11.25 ± 8.66	5.50 ± 2.89	3.00 ± 0.00	3.00 ± 0.00[c]
4	CA extract (10 mg/kg bw) AFB_1	3.00 ± 1.73	5.00 ± 2.12	5.40 ± 2.51	8.00 ± 2.12[d]	3.00 ± 0.00	4.33 ± 1.16
5	Distilled water AFB_1	2.50 ± 0.58	4.25 ± 2.50	4.67 ± 2.89	3.00 ± 0.00	3.50 ± 1.00	5.75 ± 1.50[b]

a) Mean ± SD

b) Significantly different from non-treated group (Group 1) by Mann-Whiney U test [(b) < 0.05]

c-d) Significantly different from AFB_1-treated group (Group 5) by Mann-Whiney U test [(c) < 0.05, (d) <0.01]

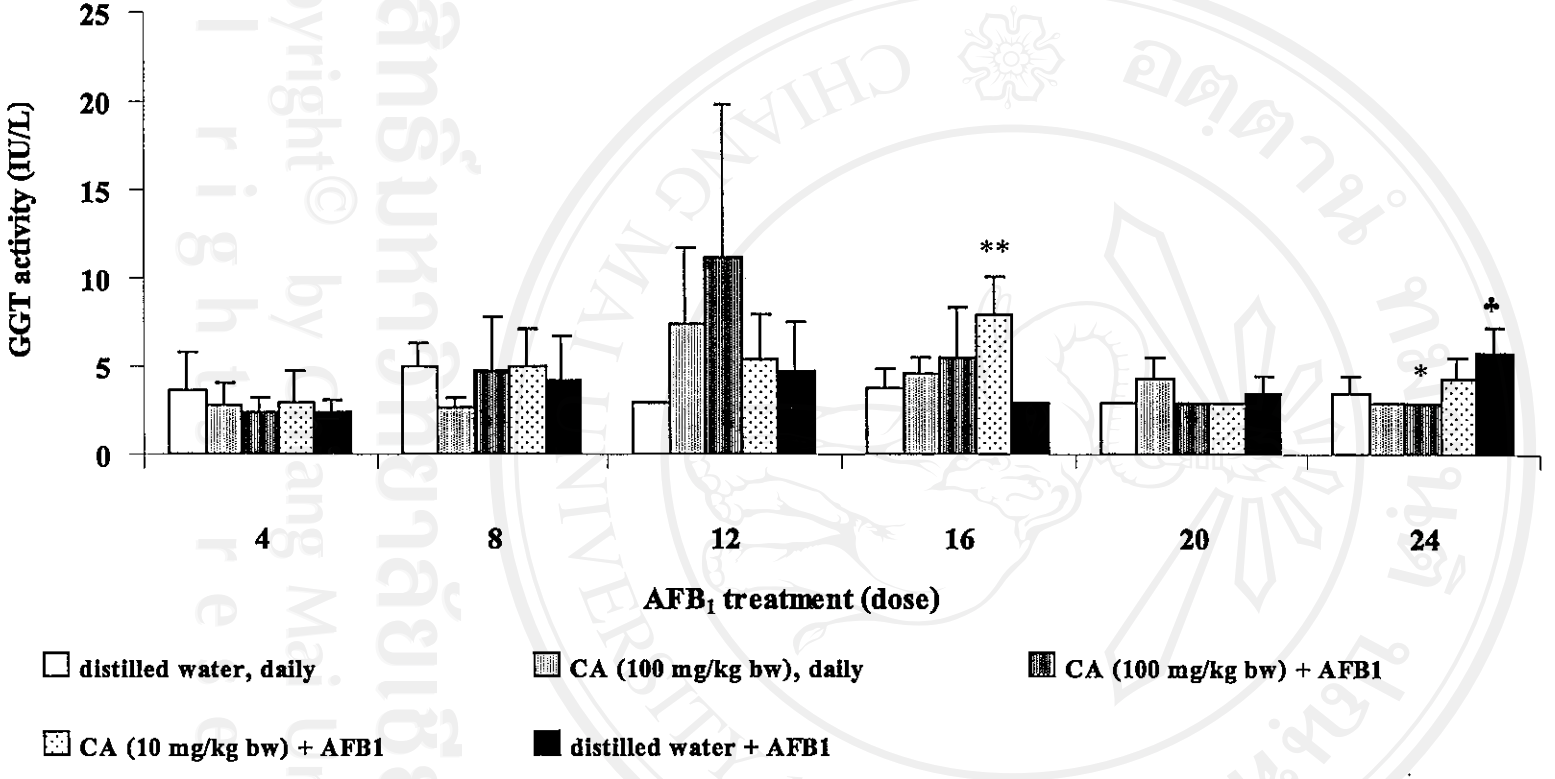

Figure 26 Effect of *C. asiatica* extract on γ-glutamyl transpeptidase activity

(*, ** Significantly different from treatment with AFB_1 alone (group 5), $p< 0.05$, $p< 0.01$ respectively; Mann-Whitney Test)

(♣ significantly different from non-treated rats (group 1), $p< 0.05$; Mann-Whitney U Test)