Chapter 3

Results

3.1 Effect of *M. loriformis* extract on AOM-induced Aberrant crypt foci formation

3.1.1 Effect of *M. loriformis* extract on AOM-induced Aberrant crypt foci formation at initiation stage

Table 3 shows the effect of *M. loriformis* extract on AOM-induced aberrant crypt foci formation at the initiation stage. The number of ACF was not observed in vehicle-treated groups (group 5,6 and 7). AOM treatment greatly increased the incidence of ACF (115 aberrant crypts/colon in group 1) while *M. loriformis* extract reduced the number of ACF in groups 2 and 3 to 94 and 70 aberrant crypts/colon at doses of 1.0 and 0.1 g/kg bw., respectively. The rats in group 4 which received *M. loriformis* extract for only 1 week before the first AOM treatment, had lower ACF than other rats that received the extract thoughout the experimental period. The extract also inhibited rectal ACF formation, with the most effective inhibition observed in rats treated with 1.0 g/kg bw. for 1 week (group 4). It is important to note that body weights were not significantly different between the control and treated groups at either the initiation stage or promotion stage of the experiment (Figure 17a, b). Morphology of aberrant crypt foci shows in appendix 4.

3.1.2 Effect of *M. loriformis* extract on AOM-induced Aberrant crypt foci formation at promotion stage

Table 4 shows the effect of *M. loriformis* extract on AOM-induced aberrant crypt foci formation at the promotion stage. The *M. loriformis* extract inhibited ACF formation by 11.5 to 17.2% at dose of 1.0 g and 0.1 g/kg bw., respectively. With an extract dose at 0.1 g/kg bw., a significant reduction in the number of crypt/focus was observed. In addition, at the same dose (0.1 g/kg bw.) the number of ACF with four or more crypt/focus ($\geq$ 4 crypt/focus; shows in appendix 4.) was significantly reduced.

The extract inhibited larger ACF formation (12.2% and 27.0%) at doses of 1.0g and 0.1 g/kg bw.

3.2 Effect of *M. loriformis* extract on AOM-induced DNA adduct formation

The data obtained from experiment 2.5 showed that an 80% alcoholic extract of *M. loriformis* inhibited DNA methylation of both O^6-methylguanine and N^7-methylguanine in the colonic mucosa and muscular layer of AOM-treated rats. The extract at various concentrations inhibited O^6-methylguanine without a dose-dependency of about 10-20% and 30-60% in the colonic mucosa and muscular layer. The most effective dose was observed at 1.0 g/kg bw. (group 2); here only inhibitory effects in the muscular layer were significant, but there were not observed in the colonic mucosa. The inhibition of N^7-methylguanine adduct, was also found in group 2. The inhibition of N^7-methylguanine adduct formation either in colonic mucosa or muscular layer was observed, but without a significant effect. A correlation between the number of ACF and O^6-methylguanine and N^7-methylguanine adduct formation was observed (Figure 18a,b). HPLC profile of DNA adduct shows in appendix 5.

Table 3 Inhibition effect of *M. loriformis* extract on AOM-induced aberrant crypt foci in male F344 rats in initiation stage

Group	Treatment	No. of rat	Colon		Rectum		Total	
			Focus[a]	crypt/focus	Focus	crypt/focus	Focus (%inh)	crypt/focus
1	25% DMSO + AOM	8	115.4 ± 29.6	1.32 ± 0.11	14.4 ± 5.8	1.49 ± 0.41	129.8 ± 31.6 (0)	1.35 ± 0.15
2	Extract 1.0 g/kg wt. + AOM	6	94.2 ± 25.2	1.28 ± 0.09	8.5 ± 4.0[b]	1.08 ± 0.10[c]	102.7 ± 27.4 (20.9)	1.27 ± 0.09
3	Extract 0.1 g/kg wt. + AOM	8	70.5 ± 24.5[c]	1.31 ± 0.08	8.8 ± 4.4[b]	1.23 ± 0.19[b]	79.2 ± 24.8 (39.0)[d]	1.30 ± 0.08
4	Extract 1.0 g/kg wt., 1 wk + AOM	8	48.9 ± 13.6[d]	1.27 ± 0.11	8.2 ± 2.3[c]	1.18 ± 0.15[b]	57.1 ± 14.9 (56.0)[d]	1.25 ± 0.11
5	25% DMSO	6	0	0	0	0	0	0
6	Extract 1.0 g/kg wt.	6	0	0	0	0	0	0
7	Extract 0.1 g/kg wt.	6	0	0	0	0	0	0

[a] Mean ± SD [b] Significantly difference from 25% DMSO + AOM $p < 0.05$ [c] $p < 0.005$ [d] $p < 0.0005$

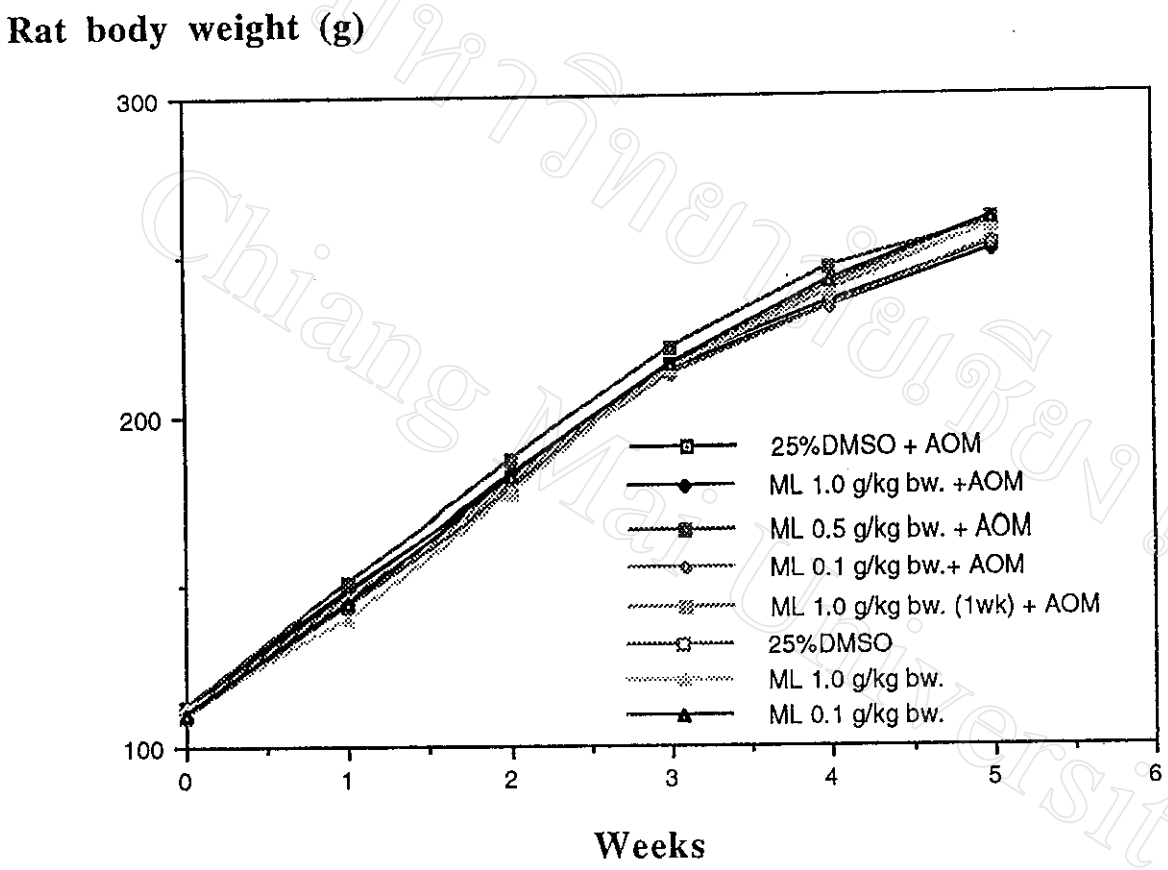

Figure 17a. Rat body weight of AOM-induced ACF at initiation stage

Table 4 Inhibition effect of *M. loriformis* extract on AOM-induced aberrant crypt foci in male F344 rats in promotion stage

Group	Treatment	No.	Colon			Rectum			Total		
			Focus	crypt/focus	≥ 4 C/f	Focus	crypt/focus	≥ 4 C/f	Focus (%inh)	crypt/focus	≥ 4 C/f (%inh)
1	25% DMSO + AOM	10	257.4 ± 65.6	2.71 ± 0.27	52.1 ± 23.0	57.4 ± 23.0	2.34 ± 0.25	7.8 ± 3.5	324.8 ± 78.0 (0)	2.64 ± 0.26	59.9 ± 12.3 (0)
2	Extract 1.0 g/kg wt. + AOM	5	236.0 ± 38.3	2.33 ± 0.09[c]	43.2 ± 8.3	51.6 ± 11.1	2.39 ± 0.40	9.4 ± 6.6	287.6 ± 47.1 (11.5)	2.34 ± 0.11[b]	52.6 ± 11.6 (12.2)
3	Extract 0.1 g/kg wt. + AOM	7	223.0 ± 35.6	2.35 ± 0.25[c]	38.3 ± 11.9[b]	45.8 ± 11.0	2.16 ± 0.15	5.4 ± 4.2	268.8 ± 44.2 (17.2)	2.32 ± 0.22[c]	43.7 ± 14.5 (27.0)[b]
4	25% DMSO	6	0	0	0	0	0	0	0	0	0
5	Extract 1.0 g/kg wt.	4	0	0	0	0	0	0	0	0	0
6	Extract 0.1 g/kg wt.	5	0	0	0	0	0	0	0	0	0

[a] Mean ± SD [b] Significantly difference from 25% DMSO + AOM $p < 0.005$ [c] $p < 0.001$

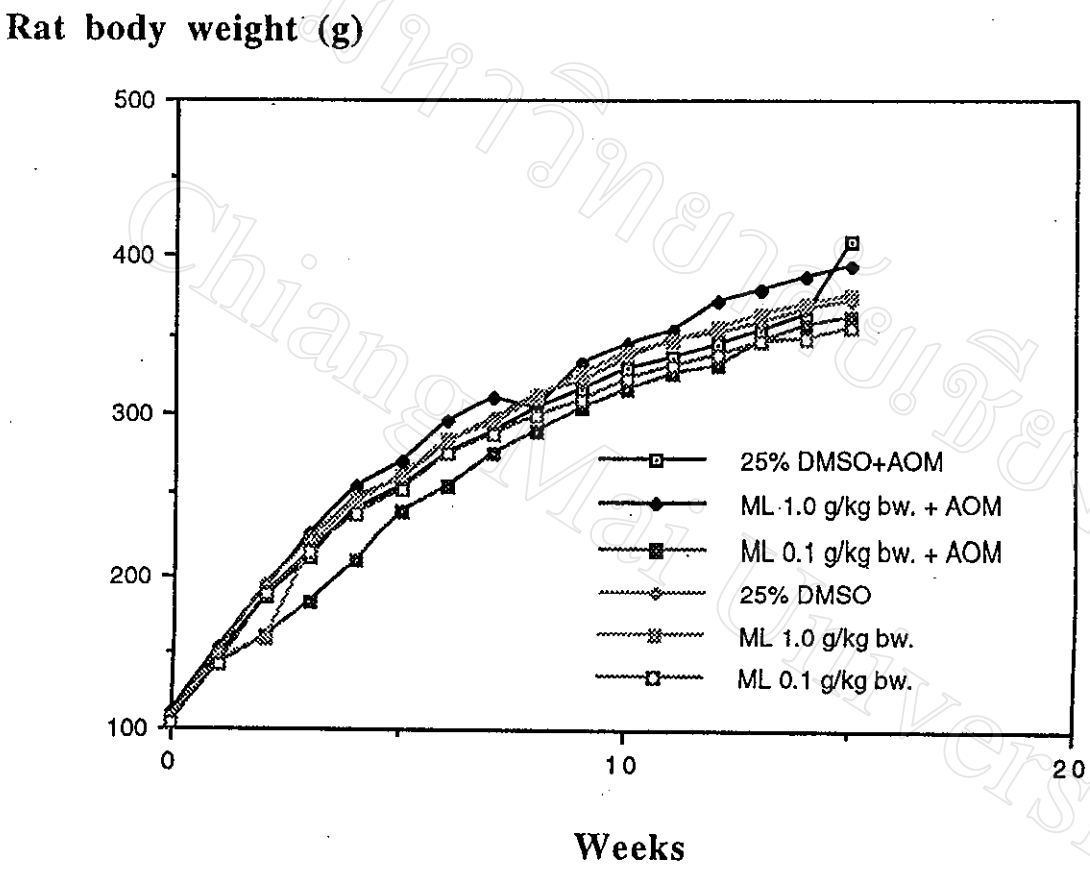

Figure 17b. Rat body weight of AOM-induced ACF at promotion stage

Table 5 Effect of *M. loriformis* extract on level of O^6-Methylguanine in AOM-induced rats

Group	Treatment	O^6-Methylguanine (μmole/mole guanine)[a]		
		n	Colonic mucosa	Muscular layer
1	25% DMSO + AOM	5	91.39 ± 27.21	40.96 ± 30.91
2	Extract 1.0 g/kg wt. + AOM	5	79.18 ± 12.43	16.75 ± 9.27[b]
3	Extract 0.1 g/kg wt. + AOM	5	82.79 ± 18.06	27.90 ± 9.04
4	Extract 1.0 g/kg wt.(1 wk) + AOM	5	73.02 ± 14.63	23.03 ± 12.29
5	25% DMSO	4	< 3.86 ± 1.43	< 5.85 ± 4.31
6	Extract 1.0 g/kg wt.	4	< 4.76 ± 1.24	< 3.86 ± 0.47
7	Extract 0.1 g/kg wt.	3	< 4.21 ± 0.94	< 4.08 ± 1.57

[a] Mean ± SD

[b] Significantly difference from 25% DMSO + AOM $p < 0.05$

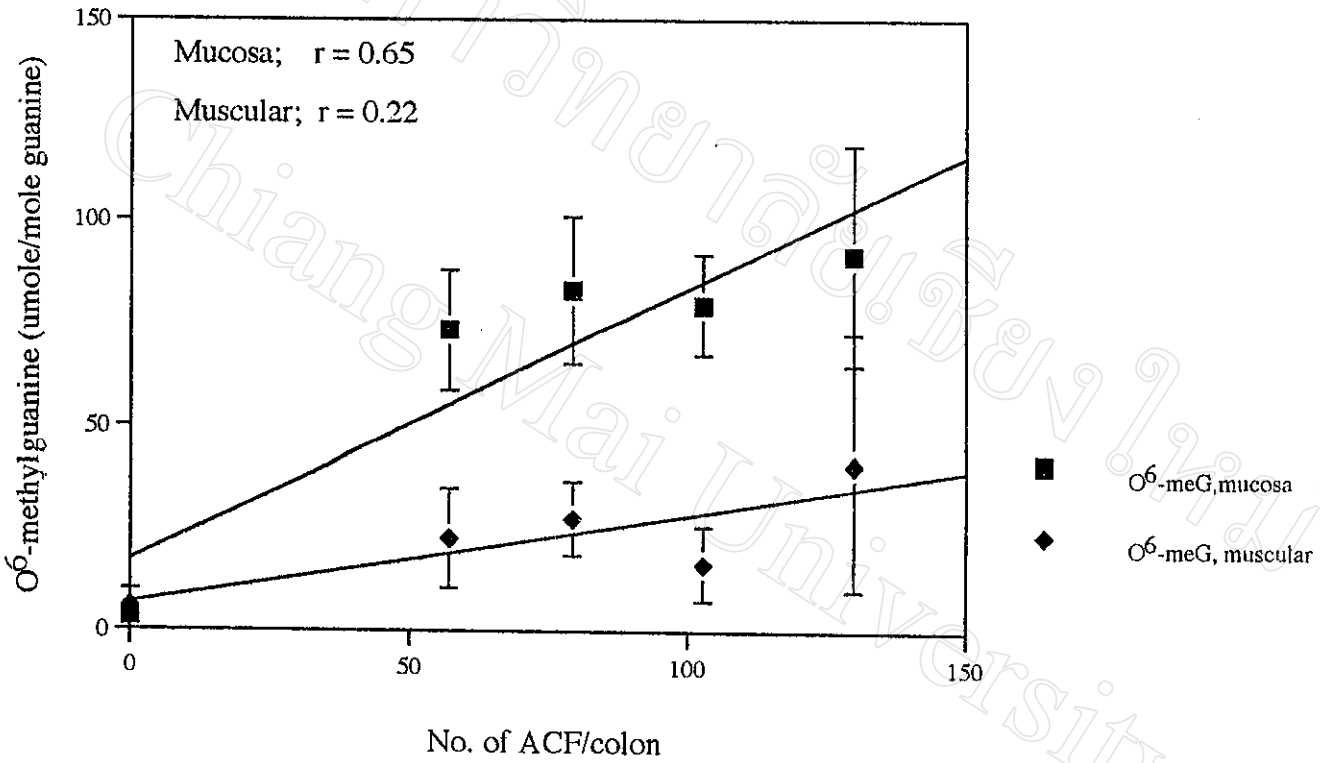

Figure 18a. Correlation curve between No. of ACF/colon and O^6-methylguanine

Table 6 Effect of *M. loriformis* extract on level of N^7-methylguanine in AOM-induced rats

Group	Treament	N^7-Methylguanine (mmole/mole guanine)[a]		
		n	Colonic mucosa	Muscular layer
1	25% DMSO + AOM	5	1.67 ± 0.50	1.69 ± 0.75
2	Extract 1.0 g/kg wt. + AOM	5	1.45 ± 0.34	1.85 ± 1.11
3	Extract 0.1 g/kg wt. + AOM	5	1.59 ± 0.72	1.13 ± 0.22
4	Extract 1.0 g/kg wt.(1 wk) + AOM	5	0.43 ± 0.26[d]	0.29 ± 0.21[c]
5	25% DMSO	4	< 0.13 ± 0.05	< 0.20 ± 0.14
6	Extract 1.0 g/kg wt.	4	< 0.16 ± 0.04	< 0.13 ± 0.02
7	Extract 0.1 g/kg wt.	3	< 0.14 ± 0.03	< 0.14 ± 0.05

[a] Mean ± SD

[b] Significantly difference from 25% DMSO + AOM $p < 0.0005$

[c] $p < 0.0001$

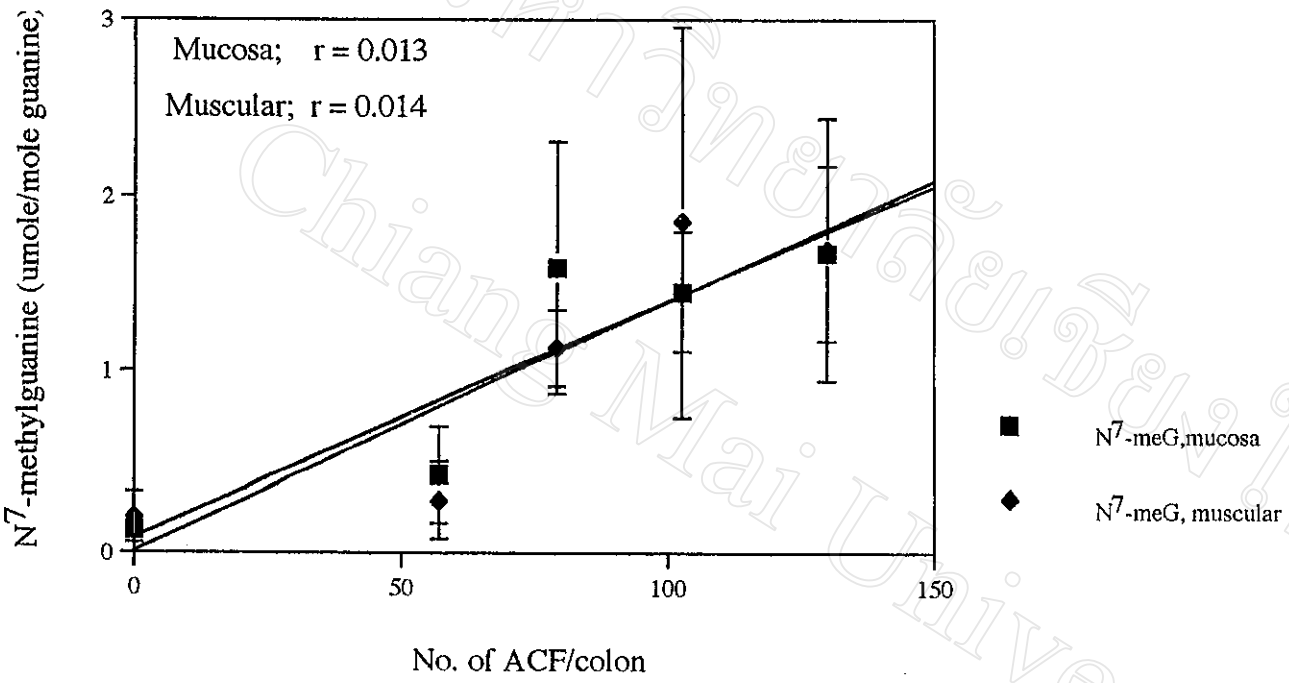

Figure 18b. Correlation curve between No. of ACF/colon and N^7-methylguanine

3.3 Antioxidant activity of *M. loriformis* extract

Antioxidant activity of the *M. loriformis* extract against malondialdehyde (MDA) formation induced by *t*-BHP in rabbit erythrocyte ghost membrane was examined from data in experiment 2.6. The data shown in Figure 18. The result shows that the extract inhibited MDA formation from 15 to 44% without dose-dependency; the most effective dose was shown at dose 500 µg/ml.

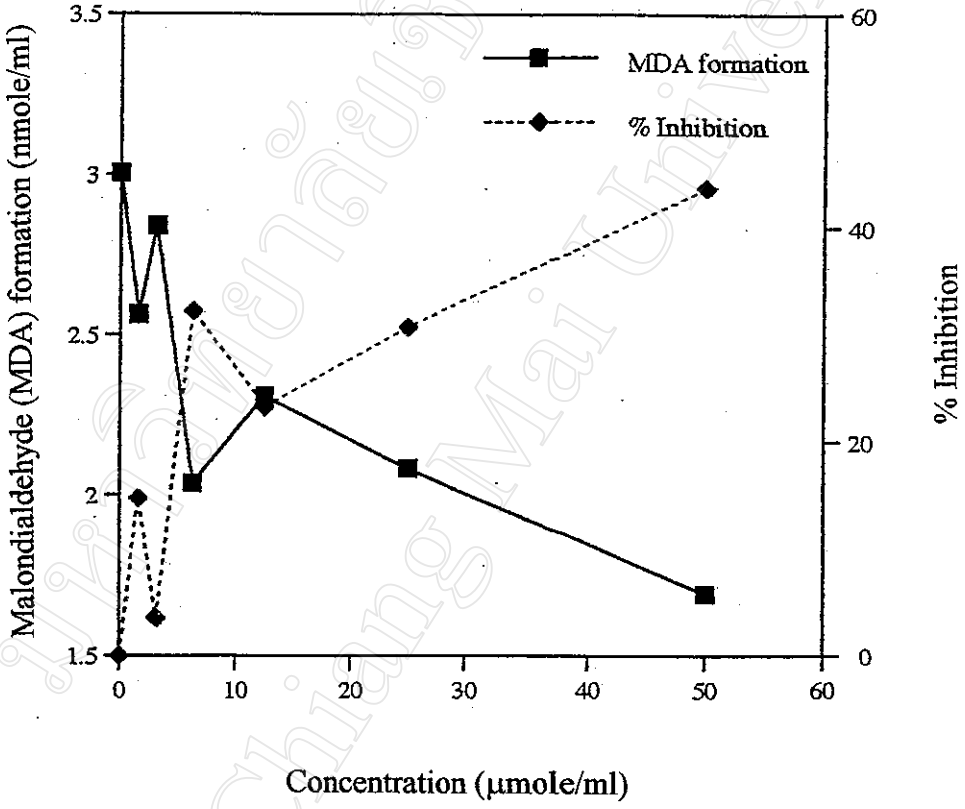

Figure 19. Antioxidant activity of *M. loriformis* extract

3.4 Effect of *M. loriformis* extract on Phase I and Phase II enzymatic activity

3.4.1 Total P450 Content

The total content of cytochrome P450 was determined by the method of Omura and Sato (Omura and Sato, 1964). After exposure for 10 days, the total P450 content in microsomal fraction was reduced from 1.44 (control group) to 1.26 and 0.95 nmole/min/mg protein (treated group; exposed to the extract at 1.0 g and 0.1 g/kg bw. respectively). The rats which received the extract only one week, had a total P450 content that was significantly reduced to 0.85 nmole/min/mg protein at $p< 0.05$.

After 30 days of exposure, the total P450 content increased from 1.47 (control group) to 2.35 nmole/min/mg protein in rats that received the extract only one week. But the total P450 content in rats that received the extract continuously for 30 days was decrease by 45%.

3.4.2 Aminopyrene Demethylase (APD) Activity

Aminopyrene demethylase was a P450-dependent enzyme in the microsomal fraction. The result were similar with P450 activity. APD activity was significantly reduced from 29.02 (control group) to 22.26, 17.77 and 17.06 nmole/min/mg protein (treated group; exposed to the extract 1.0, 0.1 and 1.0 g/kg bw. only 1 week respectively) 10 days after exposure. While with 30 days treated-rats, APD activities were significantly increased from 26.70 (control group) to 42.96 nmole/min/mg protein (received the extract 1.0g/kg bw. for only 1 week).

3.4.3. Glutathione *S*-transferase (GST) Activity

Using glutathione and chlorodinitrobenzene as substrates, GST activity was measured in the cytosolic fraction. The results show that GST activity increased after 30 days of the exposure and their was a significant difference between the control (25% DMSO) and the treated group (exposed of the extract 0.1 and 1.0 g/kg bw. only 1 week, respectively). This contrasts with the 10 day treated rats where no effect on GST activity was observed. Interestingly, the extract at lower doses increased GST activity more than that at high doses.

3.4.4. DT-Diaphorase Activity

The result show that 10 days after extract exposure, DT-diaphorase activity increased significantly from 1.59 (control group) to 2.66 μmole/min/mg protein (exposed the extract 1.0 g/kg bw. only 1 week). After 30 days exposure to the extract, DT-diaphorase activity was reduced from 2.36 (control group) to 2.04 and 2.05 μmole/min/mg protein (exposed extract 0.1 and 1.0 g/kg bw. only for 1 week respectively).

3.4.5. UDP-Glucuronyl Transferase (UDP-GT) Activity

The result show that 10 days after extract exposure, the UDP-GT activity was reduced significantly from 75.44 (control group) to 51.38 nmole/min/mg protein (extract 0.1g/kg bw.). Thirty days treated-rats show UDP-GT activity increased significantly from 97.73 (control group) to 130.41 nmole/min/mg protein (extract 1.0 g/kg bw. only for 1 week). UDP-GT activity was reduced from 97.73 (control group) to 59.64 and 78.24 nmole/min/mg protein (treated group; extract 1.0 and 0.1 g/kg bw. respectively).

Table 7. Effect of *M. loriformis* extract on P450 content

Treatment	P450 content[a] (nmole/min/mg protein)	
	10 days	30 days
25% DMSO	1.44 ± 0.33	1.47 ± 0.43
Extract 1.0 g/kg bw.	1.26 ± 0.19	0.81 ± 0.14
Extract 0.1 g/kg bw.	0.95 ± 0.28	1.85 ± 0.40
Extract 1.0 g/kg bw. (1 wk.)	0.85 ± 0.32	2.35 ± 0.60[b]

[a] Mean ± SD [b] Significantly difference from 25%DMSO $p < 0.05$

Table 8. Effect of *M. loriformis* extract on APD activity

Treatment	APD activity[a] (nmole/min/mg protein)	
	10 days	30 days
25% DMSO	29.02 ± 3.22	26.70 ± 3.36
Extract 1.0 g/kg bw.	22.26 ± 2.91	19.70 ± 5.30
Extract 0.1 g/kg bw.	17.77 ± 3.34[c]	32.78 ± 7.68[b]
Extract 1.0 g/kg bw. (1 wk.)	17.06 ± 3.16[c]	46.96 ± 7.82[c]

[a] Mean ± SD [b] Significantly difference from 25%DMSO $p < 0.05$

[c] $p < 0.005$

Table 9. Effect of *M. loriformis* extract on GST activity

Treatment	GST activity[a] (nmole/min/mg protein)	
	10 days	30 days
25% DMSO	165.38 ± 27.74	184.35 ± 29.45
Extract 1.0 g/kg bw.	192.41 ± 26.66	186.77 ± 38.72
Extract 0.1 g/kg bw.	161.74 ± 28.16	242.16 ± 44.56[b]
Extract 1.0 g/kg bw. (1 wk.)	177.79 ± 22.51	242.11 ± 16.29[c]

[a] Mean ± SD [b] Significantly difference from 25%DMSO $p < 0.05$

[c] $p < 0.005$

Table10 Effect of *M. loriformis* extract on DT-diaphorase activity

Treatment	DT-diaphorase activity[a] (μmole/min/mg protein)	
	10 days	30 days
25% DMSO	1.59 ± 0.41	2.36 ± 0.57
Extract 1.0 g/kg bw.	1.39 ± 0.27	2.35 ± 0.50
Extract 0.1 g/kg bw.	2.08 ± 0.59	2.04 ± 0.45
Extract 1.0 g/kg bw. (1 wk.)	2.66 ± 0.51[b]	2.05 ± 0.56

[a] Mean ± SD [b] Significantly difference from 25%DMSO $p < 0.005$

Table11 Effect of *M. loriformis* extract on UDP-GT activity

Treatment	UDP-GT activity[a] (nmole/min/mg protein)	
	10 days	30 days
25% DMSO	75.44 ± 18.35	97.73 ± 26.04
Extract 1.0 g/kg bw.	64.41 ± 4.82	59.64 ± 12.95[c]
Extract 0.1 g/kg bw.	51.38 ± 11.64[b]	78.24 ± 19.52[b]
Extract 1.0 g/kg bw. (1 wk.)	72.63 ± 19.81	130.41 ± 12.21[c]

[a] Mean ± SD [b] Significantly difference from 25%DMSO $p < 0.05$

[c] $p < 0.005$