CHAPTER IV

RESULTS

1. Preparation of *M. loriformis* ethanolic extract

Four kilograms of dry powder of *M. loriformis* stem and leaves were used in this study. Following the extraction process, 653.43 g of the ethanolic extract was obtained. Thus, percentage yield of the extract was 16.34% w/w.

2. Preliminary identification of *M. loriformis* ethanolic extract

2.1 Color reaction test (Jiratchariyakul W. and Soonthornchareonnon N.,1995)

The ethanolic extract of *M. loriformis* produced green color in Libermann-Burchard's test and dark green color in ferric chloride TS. These results indicated that *M. loriformis* ethanolic extract was composed of phenolic compound and steroidal moiety.

2.2 Thin layer chromatography (วีณา จิรัจฉริยากูล, 2536)

Figure 4 showed the TLC chromatograms of *M. loriformis* ethanolic extract. The specific characteristics of spots on TLC plates conformed the reference standard, contain β-sitosterol and sitosteryl glucoside (3-β-D-glucopyranosyl-24ξ-ethyl-cholest-5-ene).

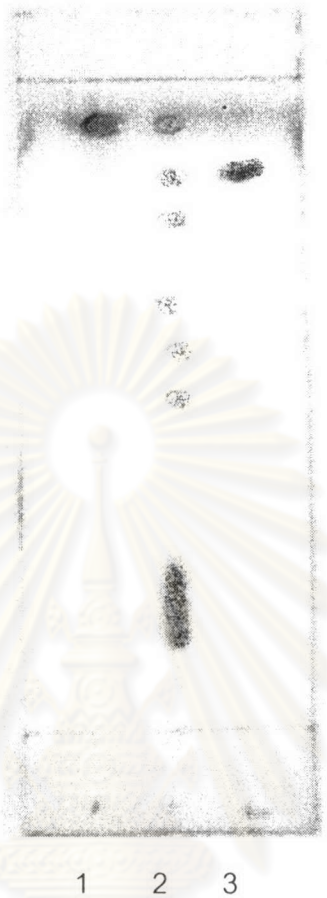


Figure 4 TLC chromatograms of *M. loriformis* ethanolic extract

Adsorbent : silica gel GF254 (Merck)

Solvent system : chloroform : methanol : water (15: 7: 1)

Detection : sprayed with 10% aqueous sulfuric acid and activated at 110°C for 2-3 min

The violet-red color appearance and Rf value were compared to those of the reference standards.

Reference standards : lane 1 = β-sitosterol

: lane 3 = sitosteryl glucoside (3-β-D-glucopyranosyl-24ξ-ethyl-cholest-5-ene)

Sample : lane 2 = *M. loriformis* ethanolic extract

Effects of *M. loriformis* ethanolic extract on body weight, food & water consumption, liver weight and % relative liver weight

M. loriformis ethanolic extract at both dosages regimens (0.1 and 1.0 g/kg/days orally for 30 days) used in this study did not affect body weight (Figure 5), body weight gain, liver weight and % relative liver weight (Table 7), water consumption (Figure 6), and food consumption (Figure 7). All rats were alive till the end of the experiment and exhibited no apparent signs of toxicity.

Effects of *M. loriformis* ethanolic extract on clinical blood chemistry and hematology

Subacute exposure (30 days) of oral 0.1 and 1.0 g/kg/day of *M. loriformis* ethanolic extract did not cause any significant effects on clinical blood chemistry parameters as compared to the control group. These parameters in serum included AST (Figure 8), ALT (Figure 9), ALP (Figure 10), total and direct bilirubin (Figure 11), BUN and SCr (Figure 12), total cholesterol and TG (Figure 13), LDL-C and HDL-C (Figure 14), glucose (Figure 15), uric acid (Figure 16), sodium (Figure 17), potassium (Figure 18), chloride (Figure 19). Likewise, no effect of *M. loriformis* ethanolic extract on these following hematological parameters : Hb and Hct (Figure 20), platelet count and WBC count (Figure 21), RBC count (Figure 22), % differential WBCs (Figure 23), RBC indices (MCV, MCH, MCHC) (Figure 24) and RBC morphology.

Effects of *M. loriformis* ethanolic extract on hepatic CYPs

Subacute exposure (30 days) of *M loriformis* ethanolic extract at 0.1 and 1.0 g/kg/day to rats did not cause any significant changes of total CYP contents (Figure 25). *M. loriformis* at both dosages also did not demonstrate any significant effects on activities of ethoxyresorufin O-dealkylase (EROD) which represented the activity of CYP1A1, methoxyresorufin O-dealkylase (MROD) which represented the activity of CYP1A2, benzyloxyresorufin O-dealkylase (BROD) & pentoxyresorufin O-dealkylase (PROD) which represented the activity of CYP2B1/2 (Figure 26-29) as well as aniline 4-hydroxylase which represented the activity of CYP2E1 (Figure 30). CYP3A activity was examined using the rate of erythromycin N-demethylation reaction. No significant effects of *M. loriformis* ethanolic extract were found on CYP3A activity (Figure 31) in both treatment groups as compared to the control group.

Table 7 Effects of *M. loriformis* ethanolic extract on body weight, body weight gain, liver weight, and % relative liver weight

	Treatment group		
	Control group	*M. loriformis* group I 0.1 g/kg/day	*M. loriformis* group II 1.0 g/kg/day
Initial body weight [a] (g)	355.83 ± 14.99	340.37 ± 13.12	352.36 ± 16.64
Final body weight [b] (g)	413.49 ± 11.93	398.08 ± 9.95	420.02 ± 10.95
Body weight gain (g)	57.66 ± 10.58	57.71 ± 11.09	67.66 ± 12.69
Liver weight (g)	12.78 ± 0.79	12.80 ± 0.52	13.42 ± 0.58
% relative liver weight (g/100 g of body weight)	3.07 ± 0.13	3.21 ± 0.09	3.18 ± 0.10

Data expressed as mean ± SEM

[a] Body weight at the beginning of *M. loriformis* administration

[b] Body weight at the time of animal sacrification

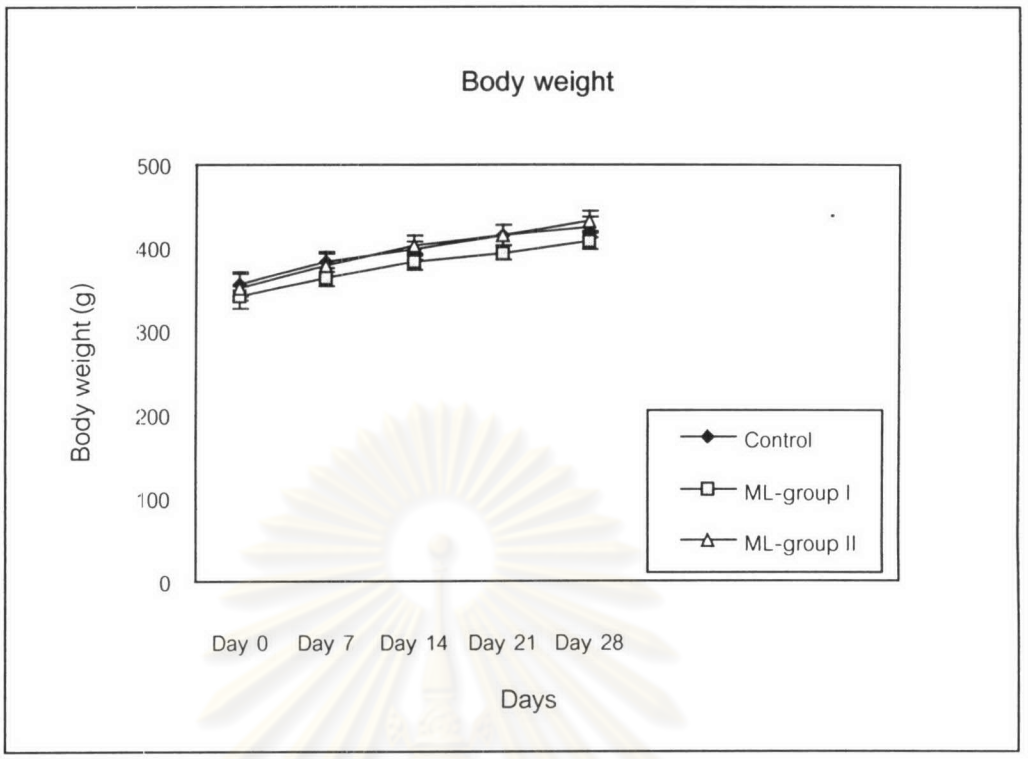


Figure 5 Subacute effects of *M. loriformis* ethanolic extract on body weight of rats 1 ml/kg/day distilled water (Control), 0.1 and 1 g/kg/day of *M. loriformis* ethanolic extract (ML-group I & ML-group II, respectively) were given orally to rats for 30 days. The individual mark represented the mean of body weight with standard error of mean (SEM) (n = 10). One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.

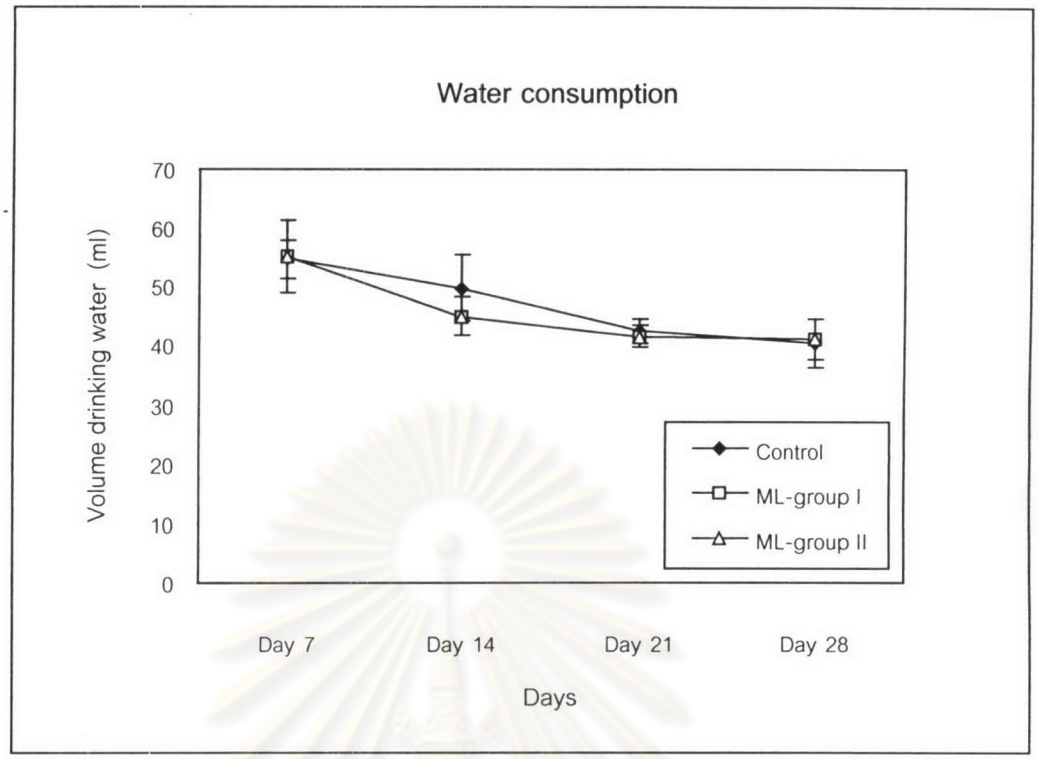


Figure 6 Subacute effects of *M. loriformis* ethanolic extract on water consumption of rats

1 ml/kg/day distilled water (Control), 0.1 and 1 g/kg/day of *M. loriformis* (ML-group I & ML-group II, respectively) were given orally to rats for 30 days. Water consumption of each rat was recorded every 7 days. The individual mark represented the mean of volume of drinking water per day with standard error of mean (SEM) (n = 10). One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.

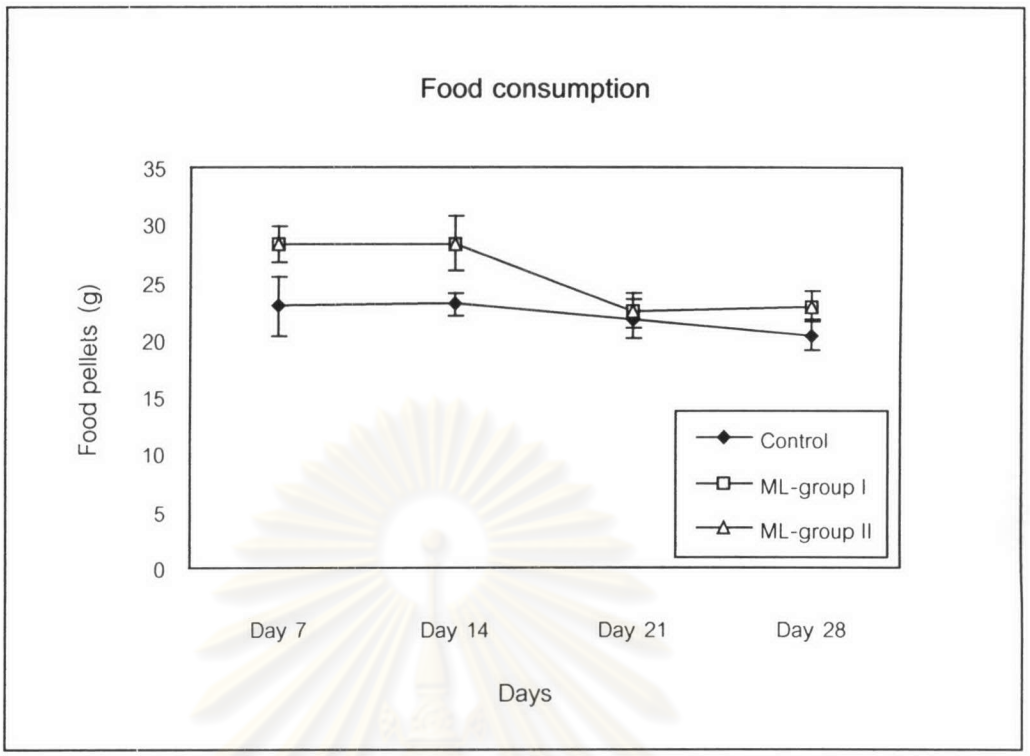


Figure 7 Subacute effects of *M. loriformis* ethanolic extract on food consumption of rats 1 ml/kg/day distilled water (Control), 0.1 and 1 g/kg/day of *M. loriformis* (ML-group I & ML-group II, respectively) were given orally to rats for 30 days. Food consumption of each rat was recorded every 7 days. The individual mark represented the mean of food consumption per day with standard error of mean (SEM) (n = 10). One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.

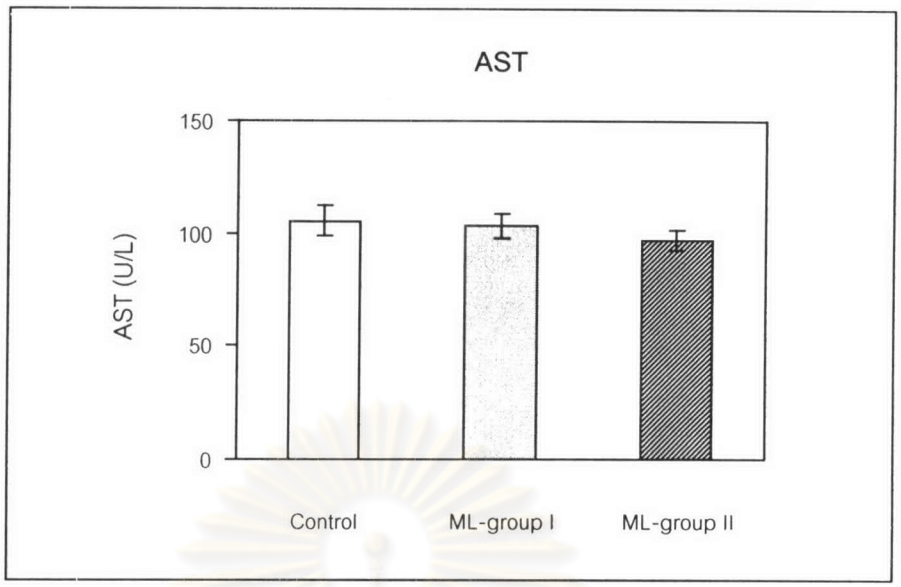


Figure 8 Subacute effects of *M. loriformis* ethanolic extract on serum AST

1 ml/kg/day distilled water (Control, n = 9), 0.1 and 1 g/kg/day of *M. loriformis* (ML-group I, n = 9 & ML-group II, n = 10, respectively) were given orally to rats for 30 days. Serum samples were determined for AST concentrations. The individual bar represented the mean of AST with standard error of mean (SEM). One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.

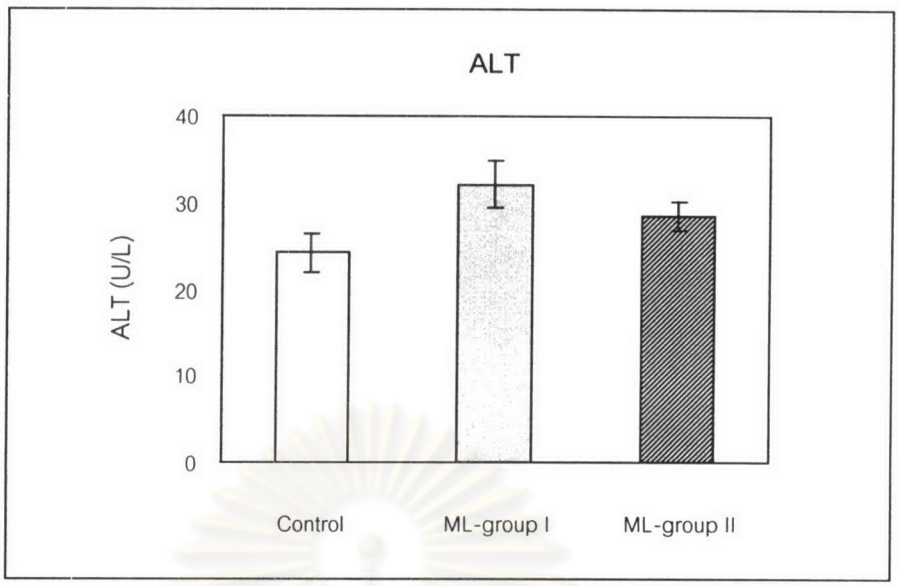


Figure 9 Subacute effects of *M. loriformis* ethanolic extract on serum ALT

1 ml/kg/day distilled water (Control, n = 9), 0.1 and 1 g/kg/day of *M. loriformis* (ML-group I & ML-group II, n = 10 respectively) were given orally to rats for 30 days. Serum samples were determined for ALT concentrations. The individual bar represented the mean of ALT with standard error of mean (SEM). One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.

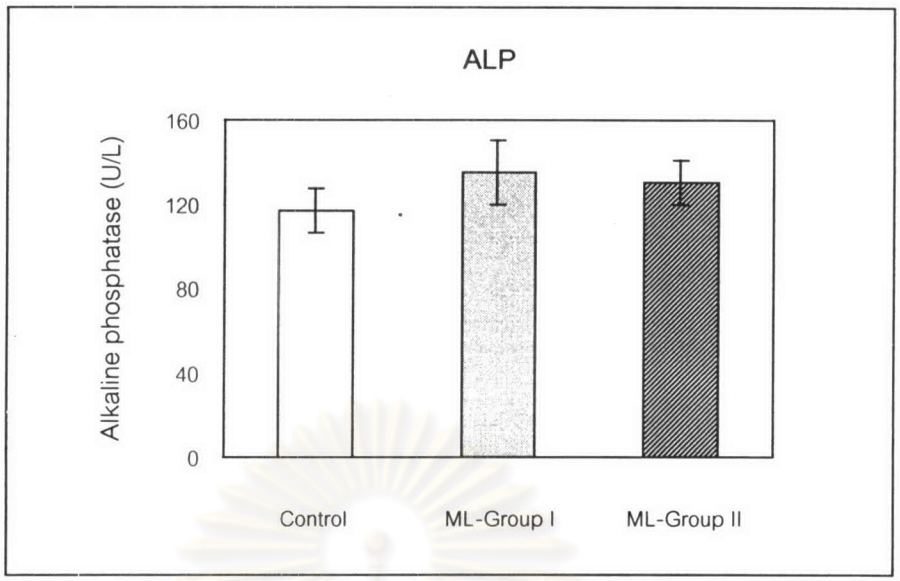


Figure 10 Subacute effects of *M. loriformis* ethanolic extract on serum ALP

1 ml/kg/day distilled water (Control, n = 9), 0.1 and 1 g/kg/day of *M. loriformis* (ML-group I & ML-group II, n = 10 respectively) were given orally to rats for 30 days. Serum samples were determined for ALP concentrations. The individual bar represented the mean of ALP with standard error of mean (SEM). One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.

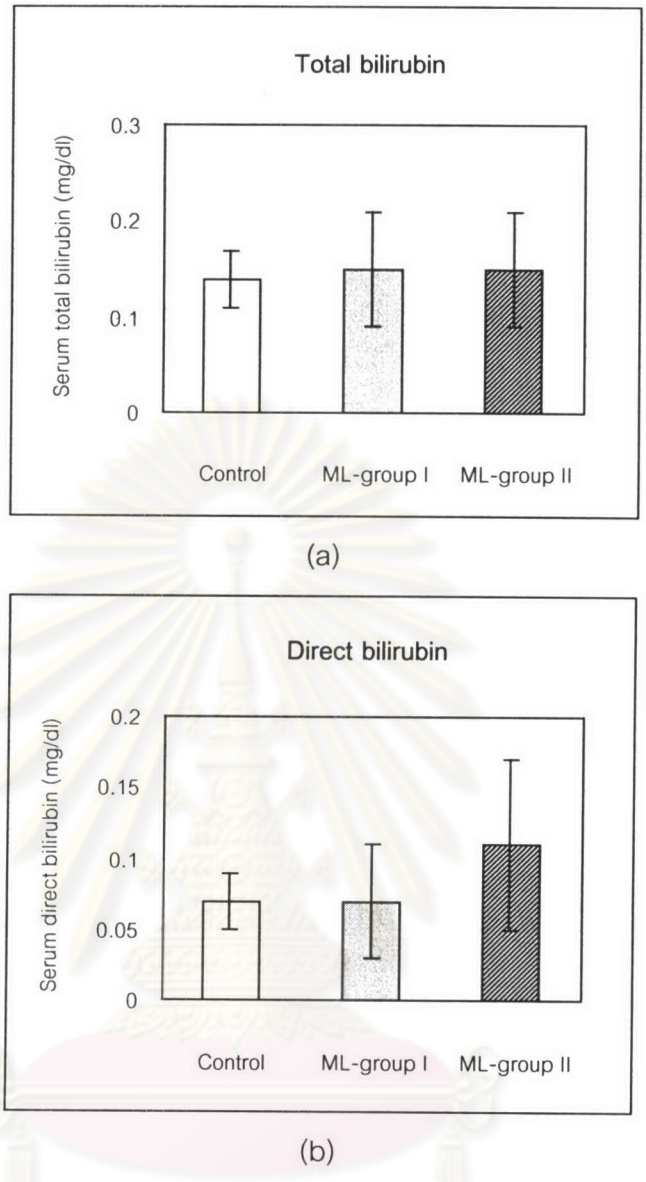
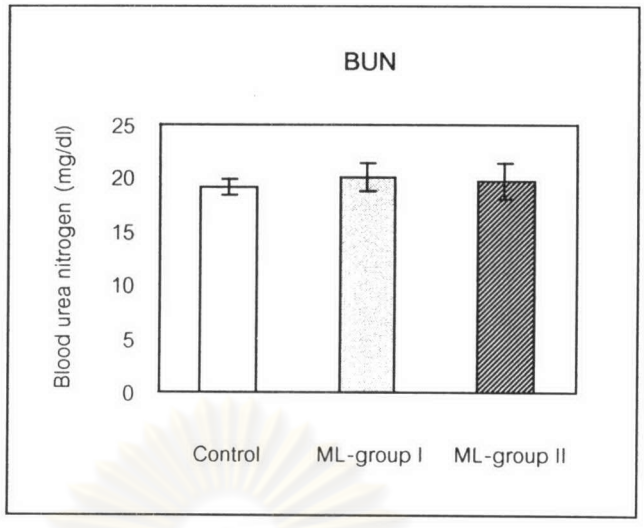
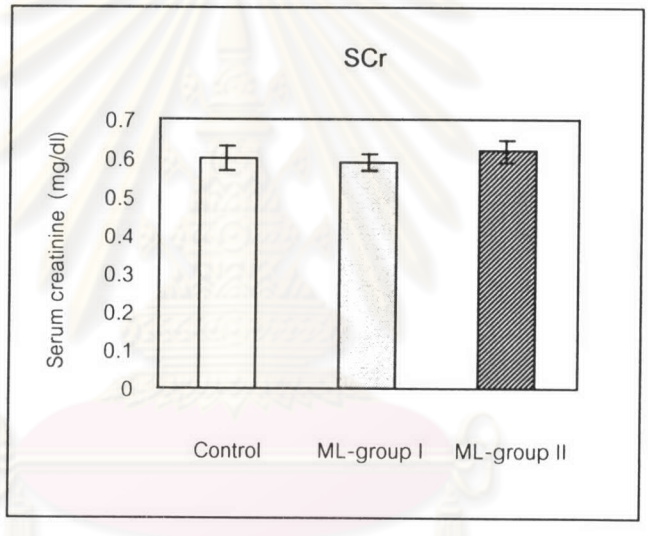


Figure 11 Subacute effects of *M. loriformis* ethanolic extract on serum total bilirubin (a) and direct bilirubin (b)

1 ml/kg/day distilled water (Control, n = 9), 0.1 and 1 g/kg/day of *M. loriformis* (ML-group I & ML-group II, n = 10, respectively) were given orally to rats for 30 days. Serum samples were determined for total bilirubin (a) and direct bilirubin (b) concentrations. The individual bar represented the mean of serum bilirubin with standard error of mean (SEM). One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.

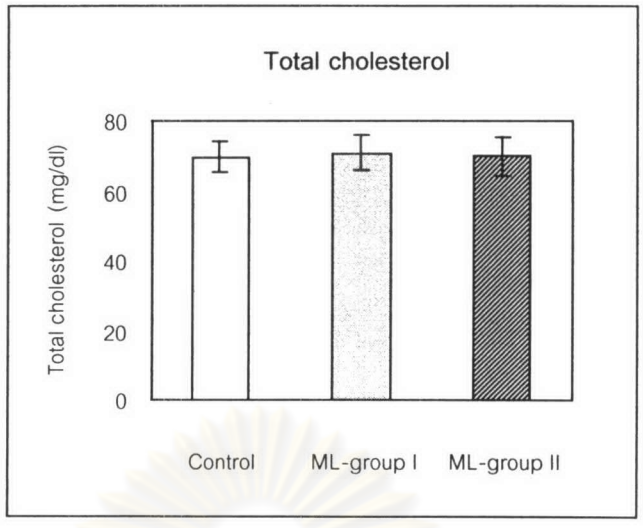


(a)



(b)

Figure 12 Subacute effects of *M. loriformis* ethanolic extract on BUN (a) and SCr (b) 1 ml/kg/day distilled water (Control, n = 9), 0.1 and 1 g/kg/day of *M. loriformis* (ML-group I & ML-group II, n = 10, respectively) were given orally to rats for 30 days. Serum samples were determined for BUN (a) and SCr (b) concentrations. The individual bar represented the mean of BUN (a) and SCr (b) with standard error of mean (SEM). One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.



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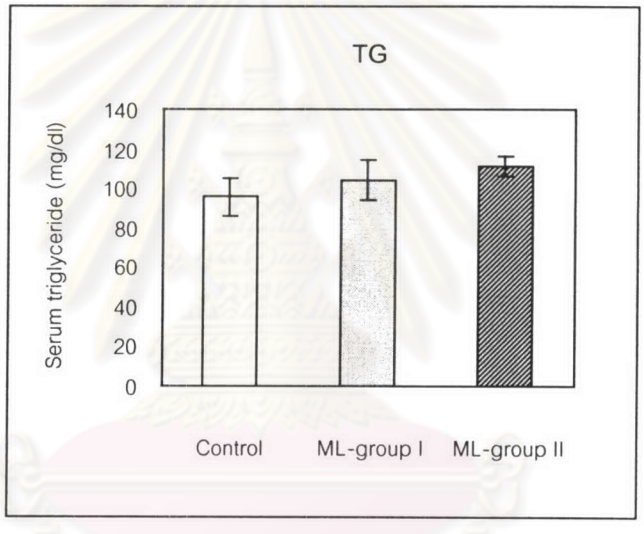
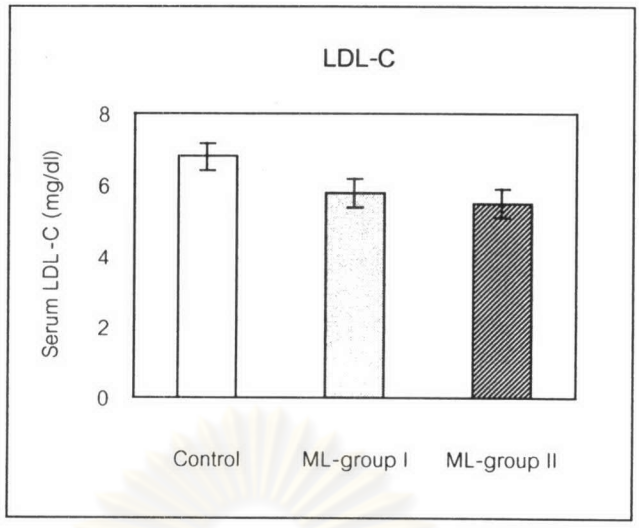


(b)

Figure 13 Subacute effects of *M. loriformis* ethanolic extract on serum total cholesterol (a) and triglyceride (b)

1 ml/kg/day distilled water (Control, n =9), 0.1 and 1 g/kg/day of *M. loriformis* (ML-group I & ML-group II, n = 10 respectively) were given orally to rats for 30 days. Serum samples were determined for total cholesterol (a) and triglyceride (b) concentrations. The individual bar represented the mean of total cholesterol (a) and triglyceride (b) with standard error of mean (SEM). One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.



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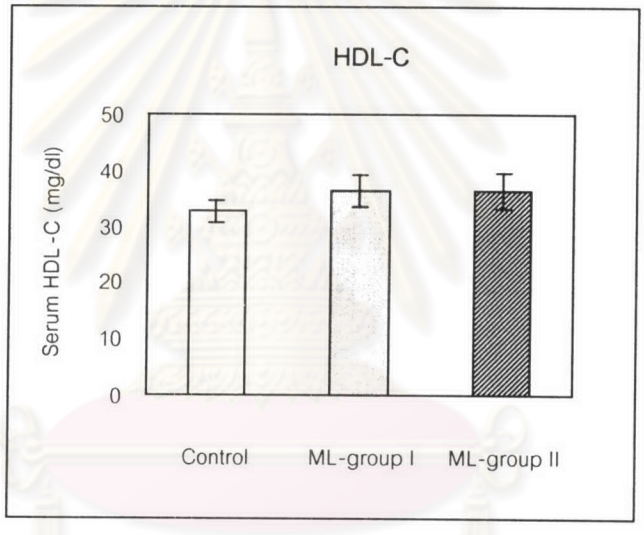


(b)

Figure 14 Subacute effects of *M. loriformis* ethanolic extract on serum LDL-C (a) and HDL-C (b)

1 ml/kg/day distilled water (Control, n= 9), 0.1 and 1 g/kg/day of *M. loriformis* (ML-group I & ML-group II, n= 10, respectively) were given orally to rats for 30 days. Serum samples were determined for LDL-C (a) and HDL-C (b) concentrations. The individual bar represented the mean of LDL-C (a) and HDL-C (b) with standard error of mean (SEM). One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.

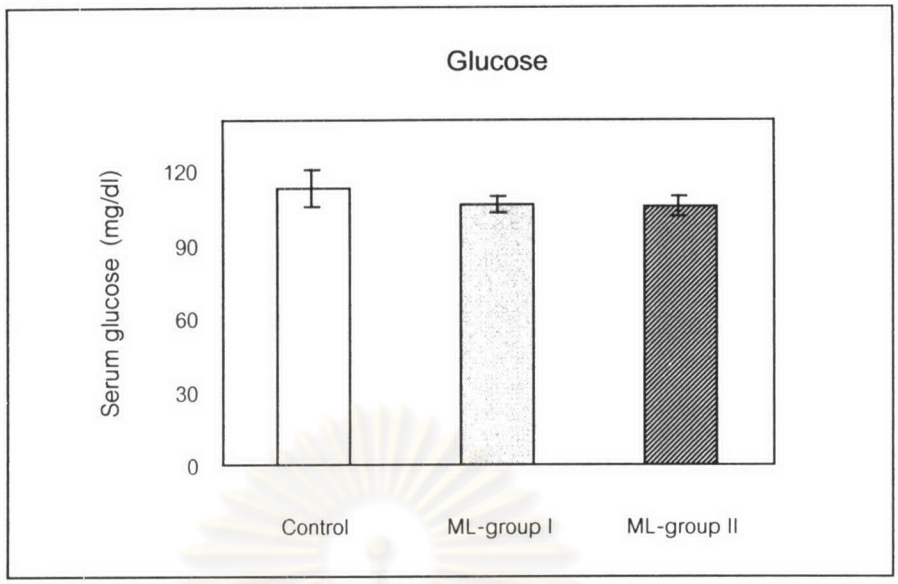


Figure 15 Subacute effects of *M. loriformis* ethanolic extract on serum glucose

1 ml/kg/day distilled water (Control, n= 10), 0.1 and 1 g/kg/day of *M. loriformis* (ML-group I & ML-group II, n= 10, respectively) were given orally to rats for 30 days. Blood samples were determined for glucose concentrations. The individual bar represented the mean of glucose concentrations with standard error of mean (SEM). One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.

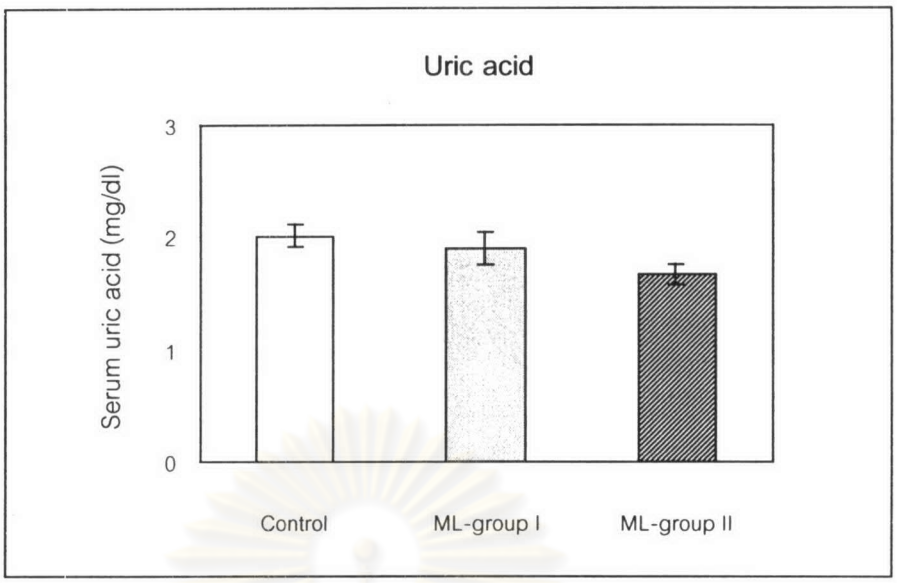


Figure 16 Subacute effects of *M. loriformis* ethanolic extract on serum uric acid

1 ml/kg/day distilled water (Control, n = 9), 0.1 and 1 g/kg/day of *M. loriformis* (ML-group I & ML-group II, n = 10, respectively) were given orally to rats for 30 days. Serum samples were determined for uric acid concentrations. The individual bar represented the mean of uric acid concentrations with standard error of mean (SEM). One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.

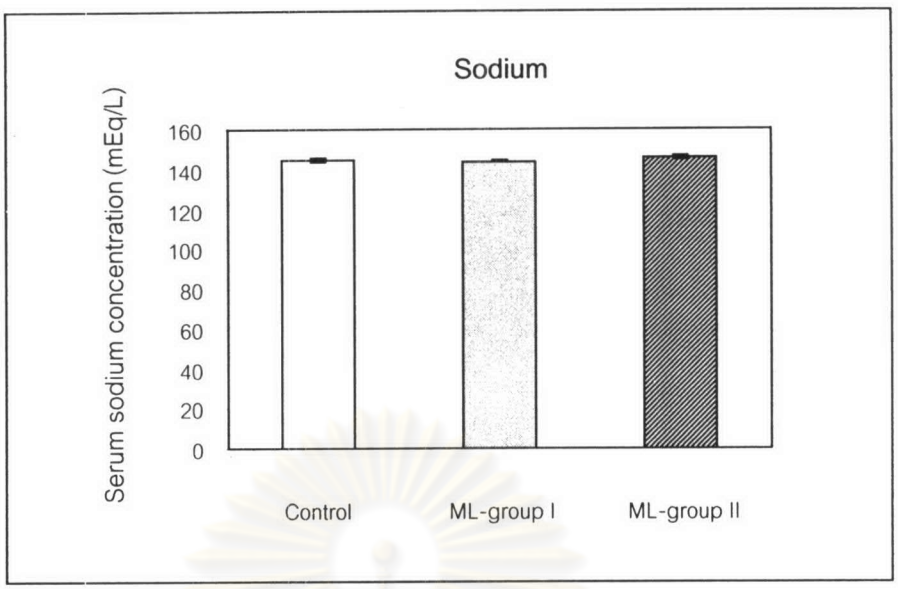


Figure 17 Subacute effects of *M. loriformis* ethanolic extract on serum sodium

1 ml/kg/day distilled water (Control, n =9), 0.1 and 1 g/kg/day of *M. loriformis* (ML-group I & ML-group II, n = 10, respectively) were given orally to rats for 30 days. Serum samples were determined for sodium concentrations. The individual bar represented the mean of sodium concentrations with standard error of mean (SEM). One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.

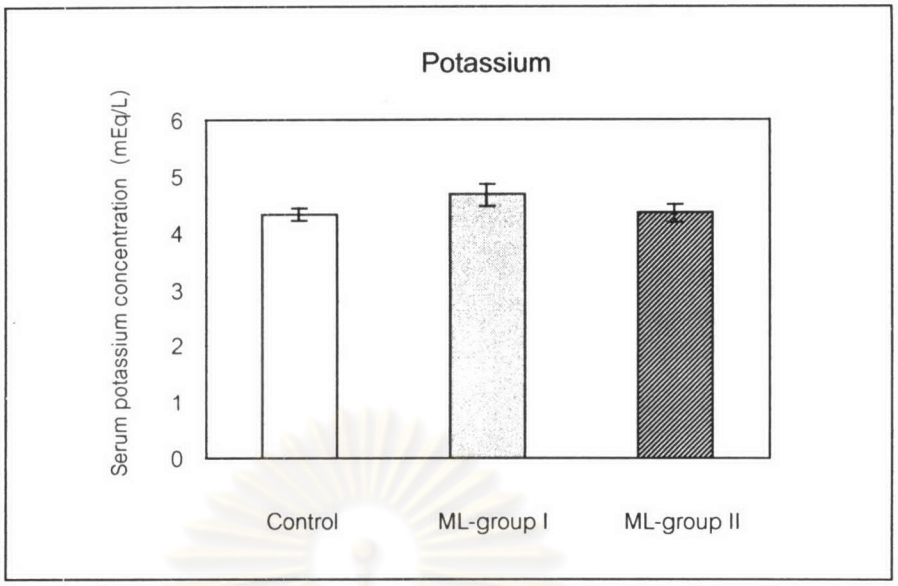


Figure 18 Subacute effects of *M. loriformis* ethanolic extract on serum potassium

1 ml/kg/day distilled water (Control, n = 9), 0.1 and 1 g/kg/day of *M. loriformis* (ML-group I, n = 10 & ML-group II, n = 9, respectively) were given orally to rats for 30 days. Serum samples were determined for potassium concentrations. The individual bar represented the mean of potassium concentrations with standard error of mean (SEM). One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.

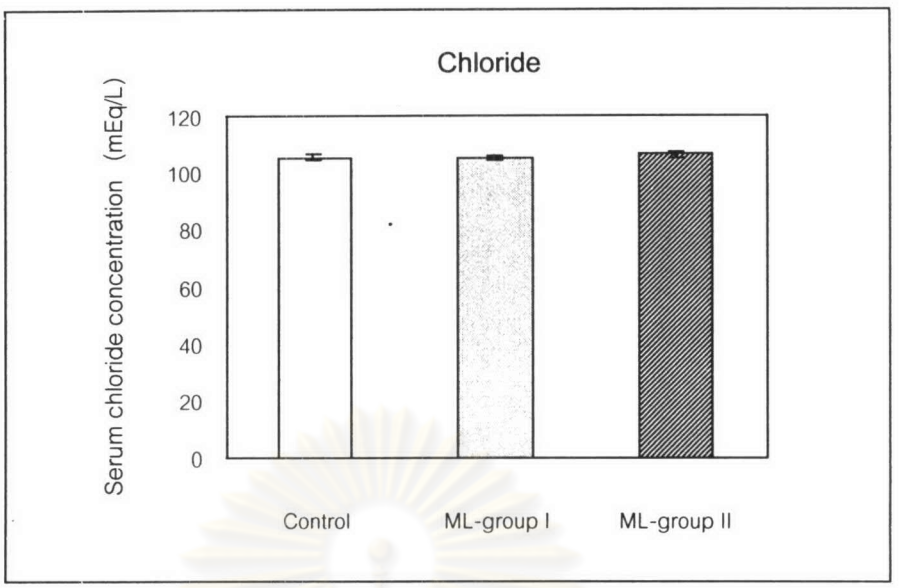
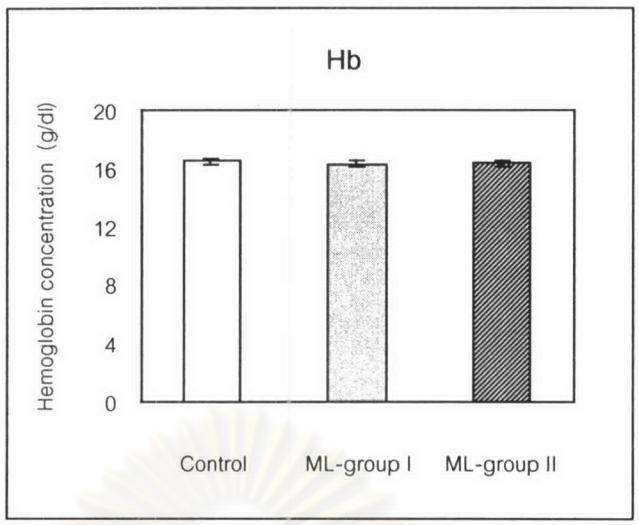


Figure 19 Subacute effects of *M. loriformis* ethanolic extract on serum chloride

1 ml/kg/day distilled water (Control, n = 9), 0.1 and 1 g/kg/day of *M. loriformis* (ML-group I & ML-group II, n = 10, respectively) were given orally to rats for 30 days. Serum samples were determined for chloride concentrations. The individual bar represented the mean of chloride concentrations with standard error of mean (SEM). One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.



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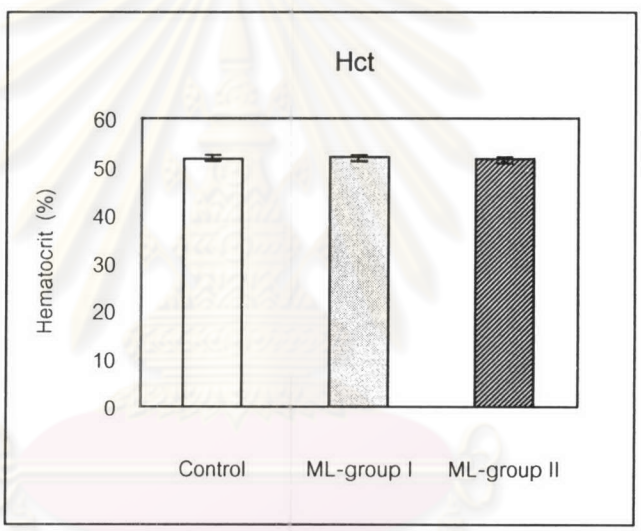
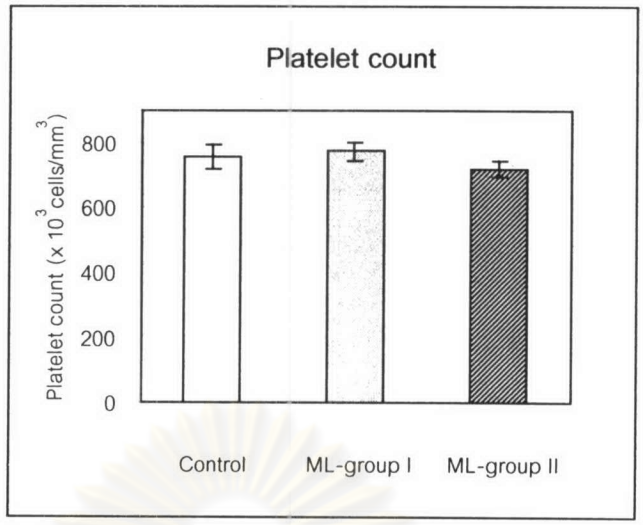
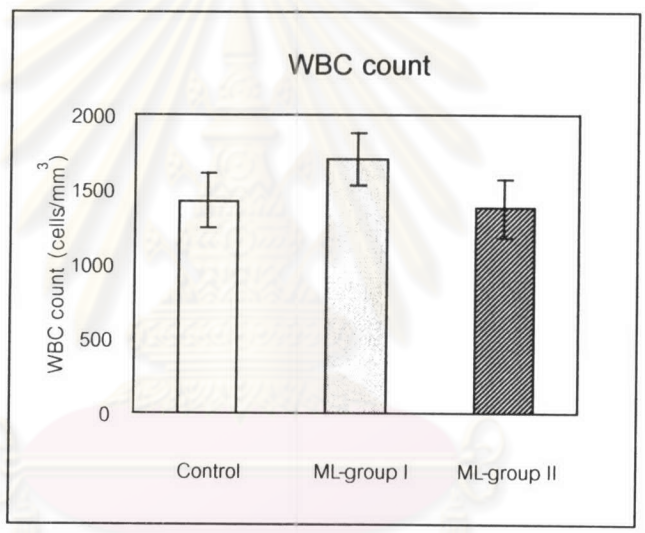


(b)

Figure 20 Subacute effects of *M. loriformis* ethanolic extract on Hb (a) and Hct (b) 1 ml/kg/day distilled water (Control, n= 9), 0.1 and 1 g/kg/day of *M. loriformis* (ML-group I & ML-group II, n= 9, respectively) were given orally to rats for 30 days. Blood samples were determined for Hb (a) and Hct (b) concentration. The individual bar represented the mean of Hb (a) and Hct (b) concentrations with standard error of mean (SEM). One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.



(a)



(b)

Figure 21 Subacute effects of *M. loriformis* ethanolic extract on platelet count (a) and WBC count (b)

1 ml/kg/day distilled water (Control, n= 9), 0.1 and 1 g/kg/day of *M. loriformis* (ML-group I & ML-group II, n= 9, respectively) were given orally to rats for 30 days. Blood samples were determined for platelet count (a) and WBC count (b). The individual bar represented the mean of platelet count (a) and WBC count (b) with standard error of mean (SEM). One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.

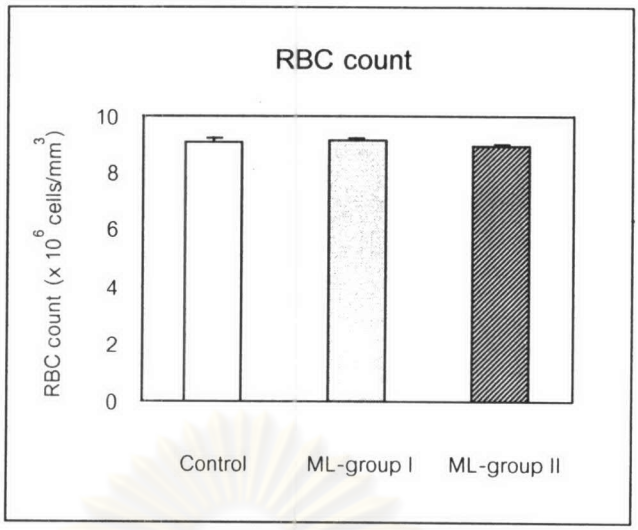


Figure 22 Subacute effects of *M. loriformis* ethanolic extract on RBC count

1 ml/kg/day distilled water (Control, n= 9), 0.1 and 1 g/kg/day of *M. loriformis* (ML-group I & ML-group II, n= 9, respectively) were given orally to rats for 30 days. Blood samples were determined for RBC count. The individual bar represented the mean of RBC count with standard error of mean (SEM). One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.

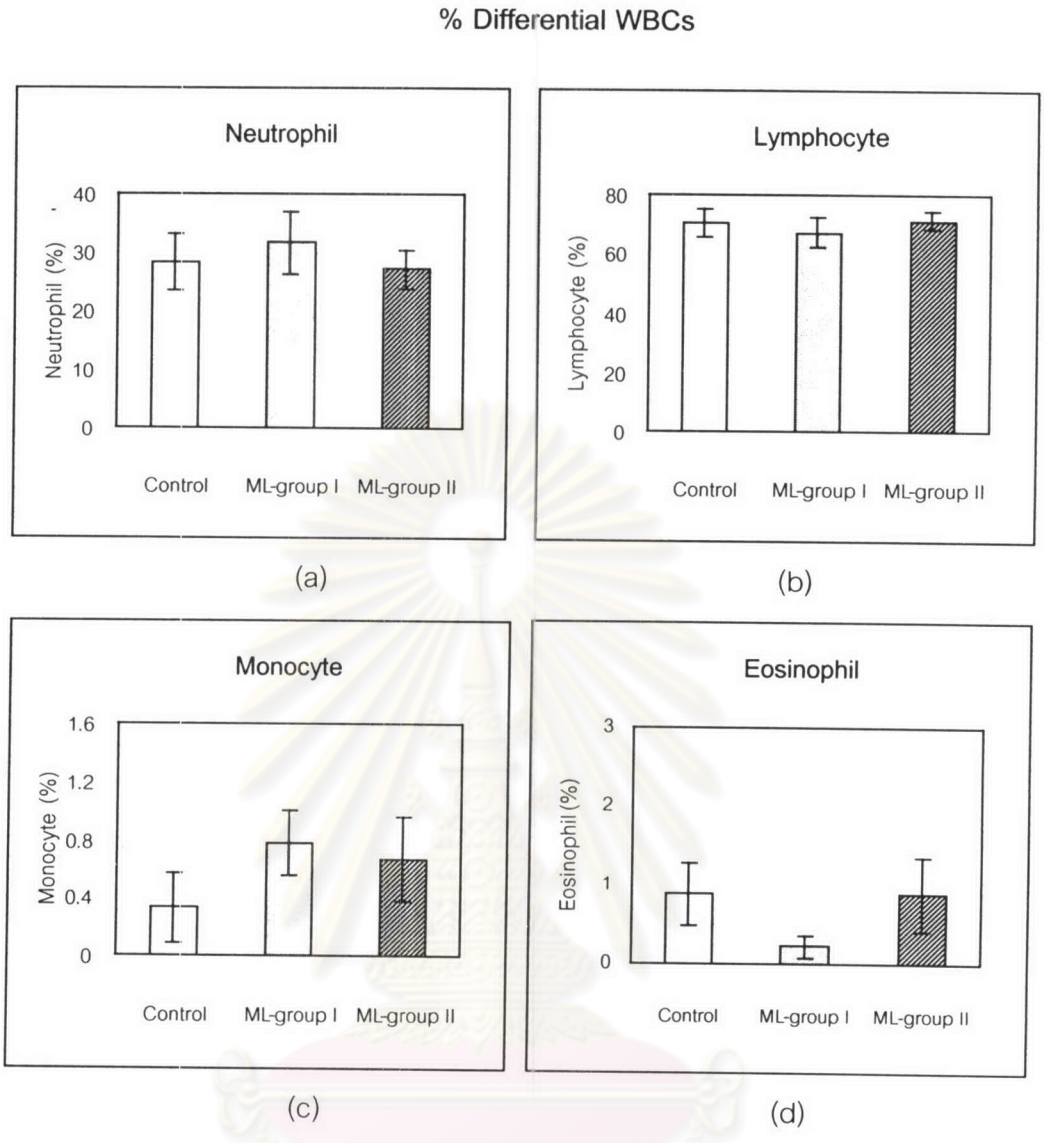


Figure 23 Subacute effects of *M. loriformis* ethanolic extract on % differential WBCs 1 ml/kg/day distilled water (Control, n= 9), 0.1 and 1 g/kg/day of *M. loriformis* (ML-group I & ML-group II, n= 9, respectively) were given orally to rats for 30 days. Blood samples were determined for % differential WBCs. The individual bar represented the mean of % differential WBCs that included neutrophil (a), lymphocyte (b), monocyte (c) and eosinophil (d) with standard error of mean (SEM). One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.

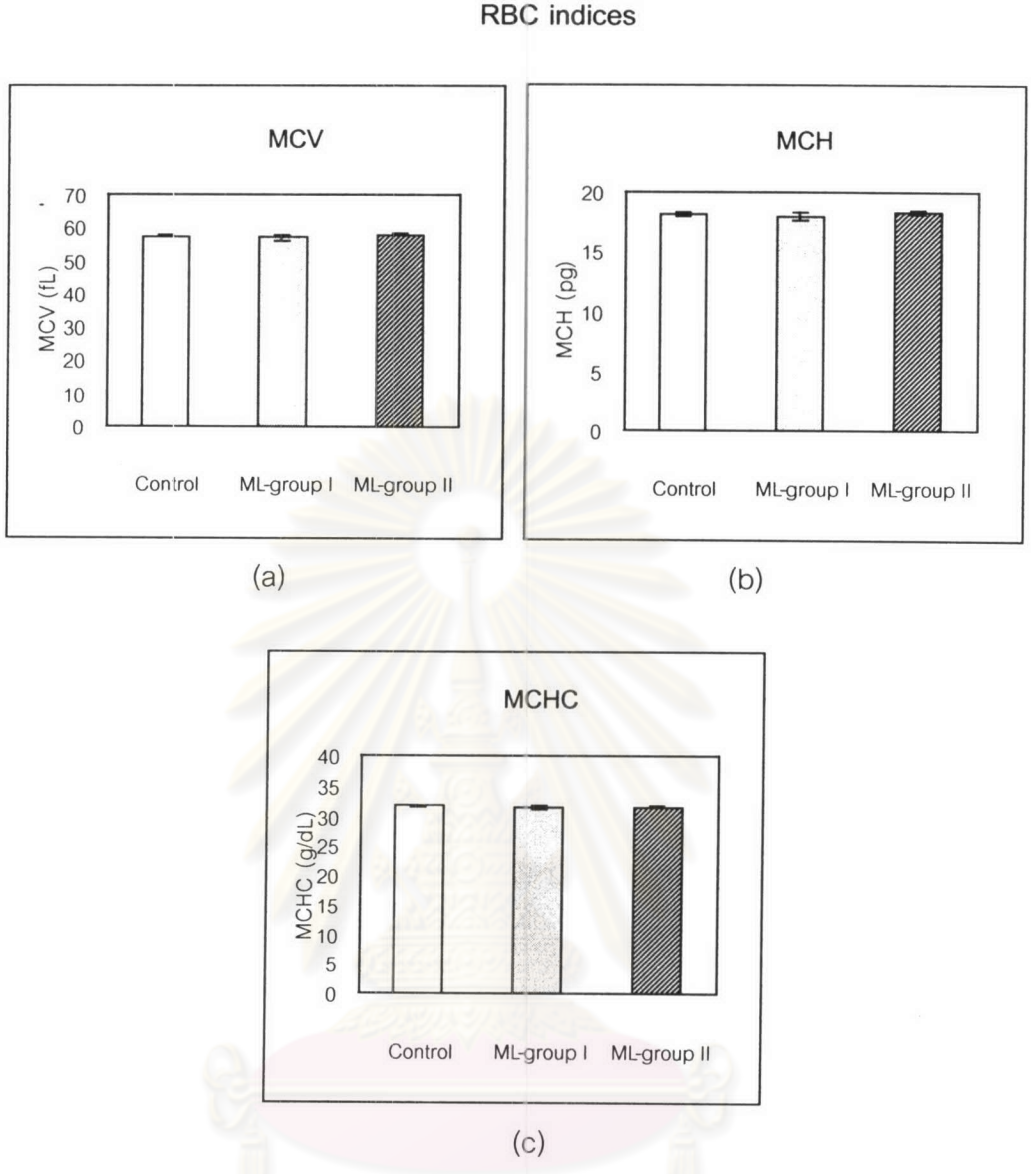


Figure 24 Subacute effects of *M. loriformis* ethanolic extract on RBC indices

1 ml/kg/day distilled water (Control, n= 9), 0.1 and 1 g/kg/day of *M. loriformis* (ML-group I & ML-group II, n= 9, respectively) were given orally to rats for 30 days. Blood samples were determined for MCV (a), MCH (b), MCHC (c). The individual bar represented the mean of MCV (a), MCH (b), MCHC (c) with standard error of mean (SEM). One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.

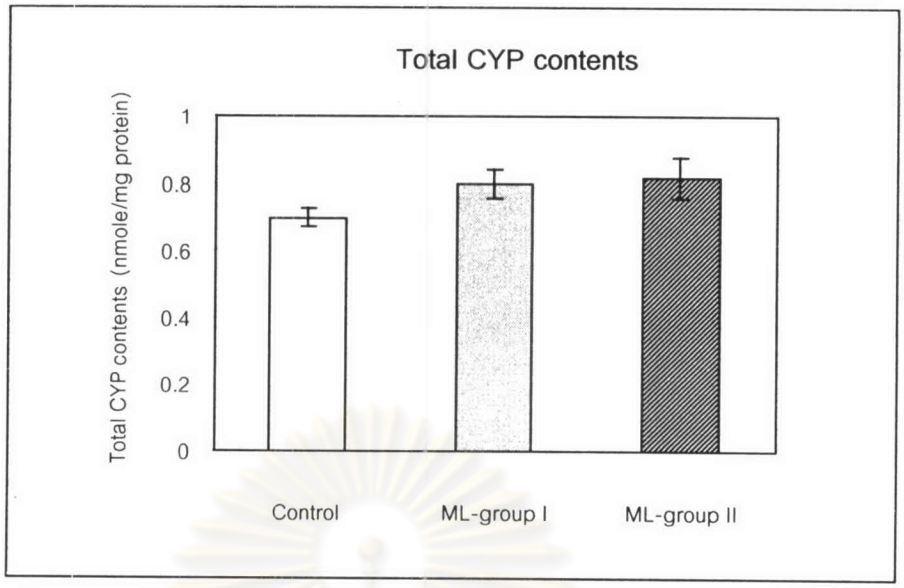


Figure 25 Subacute effects of *M. loriformis* ethanolic extract on rat hepatic total CYP contents.

1 ml/kg/day distilled water (Control, n= 10), 0.1 and 1 g/kg/day of *M. loriformis* (ML-group I & ML-group II, n= 10, respectively) were given orally to rats for 30 days. Liver microsomes were determined for total CYP contents. The individual bar represented the mean of total CYP content with standard error of mean (SEM). One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.

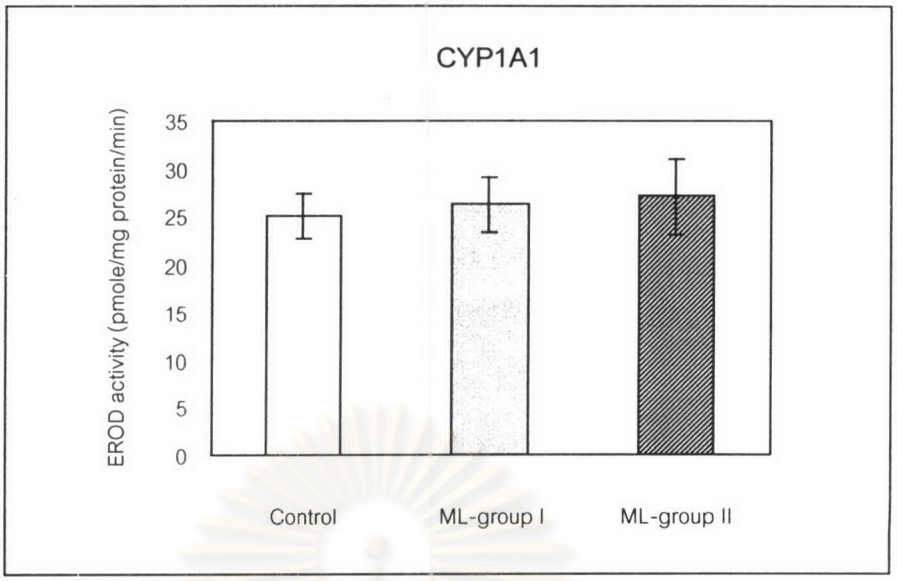


Figure 26 Subacute effects of *M. loriformis* ethanolic extract on rat hepatic CYP1A1 activity

1 ml/kg/day distilled water (Control, n= 10), 0.1 and 1 g/kg/day of *M. loriformis* (ML-group I & ML-group II, n= 10, respectively) were given orally to rats for 30 days. Liver microsomes were determined for EROD activity. The individual bar represented the mean of EROD activity with standard error of mean (SEM). One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.

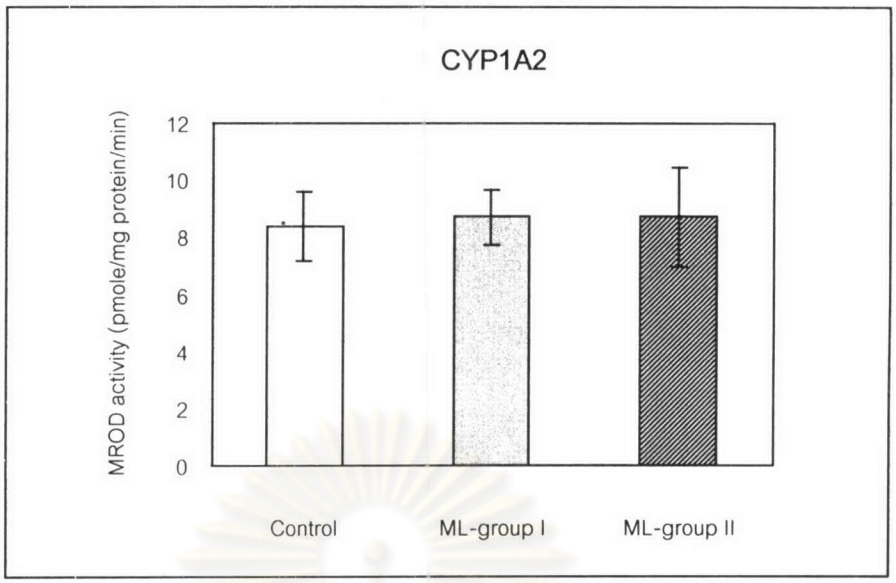


Figure 27 Subacute effects of *M. loriformis* ethanolic extract on rat hepatic CYP1A2 activity

1 ml/kg/day distilled water (Control, n= 10), 0.1 and 1 g/kg/day of *M. loriformis* (ML-group I & ML-group II, n= 10, respectively) were given orally to rats for 30 days. Liver microsomes were determined for MROD activity. The individual bar represented the mean of MROD activity with standard error of mean (SEM). One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.

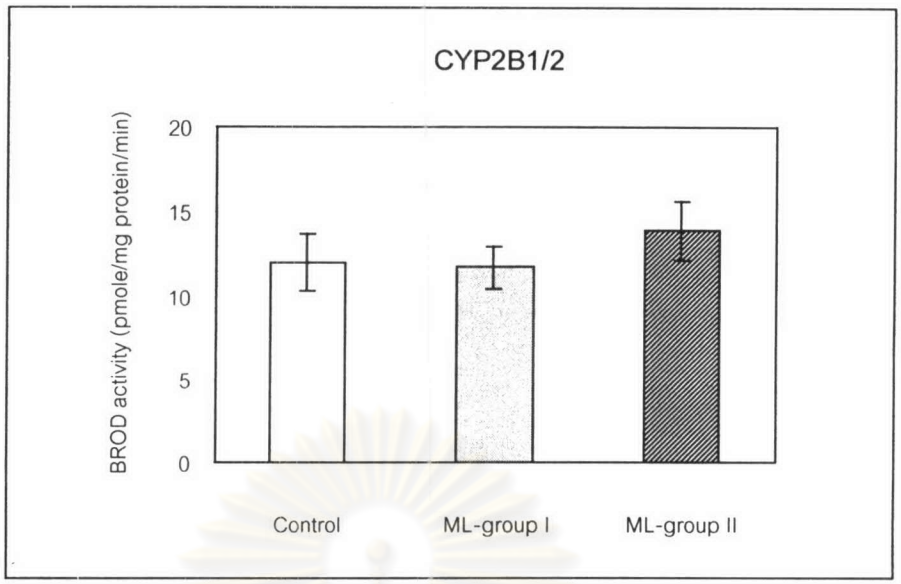


Figure 28 Subacute effects of *M. loriformis* ethanolic extract on rat hepatic CYP2B1/2 (BROD) activity

1 ml/kg/day distilled water (Control, n= 10), 0.1 and 1 g/kg/day of *M. loriformis* (ML-group I & ML-group II, n= 10, respectively) were given orally to rats for 30 days. Liver microsomes were determined for BROD activity. The individual bar represented the mean of BROD activity with standard error of mean (SEM). One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.

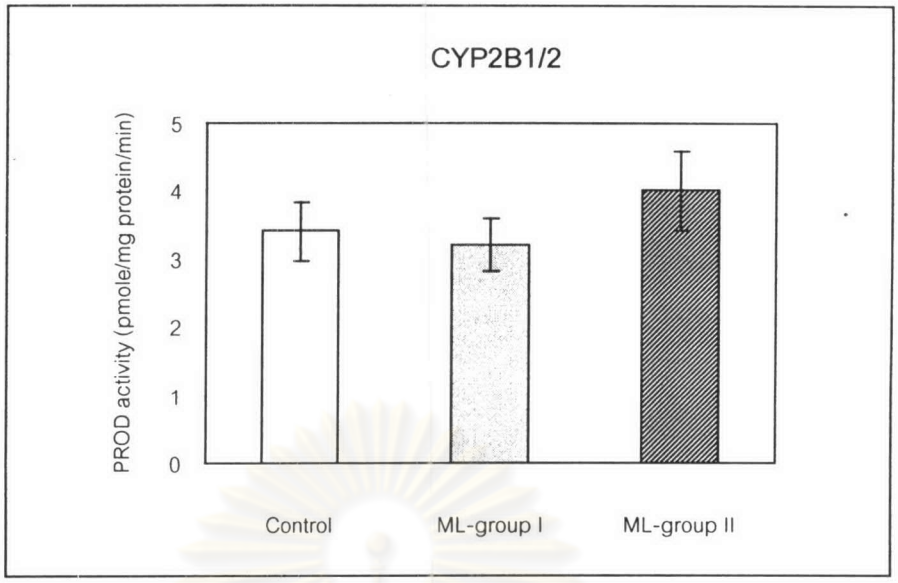


Figure 29 Subacute effects of *M. loriformis* ethanolic extract on rat hepatic CYP2B1/2 (PROD) activity

1 ml/kg/day distilled water (Control, n= 10), 0.1 and 1 g/kg/day of *M. loriformis* (ML-group I & ML-group II, n= 10, respectively) were given orally to rats for 30 days. Liver microsomes were determined for PROD activity. The individual bar represented the mean of PROD activity with standard error of mean (SEM). One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.

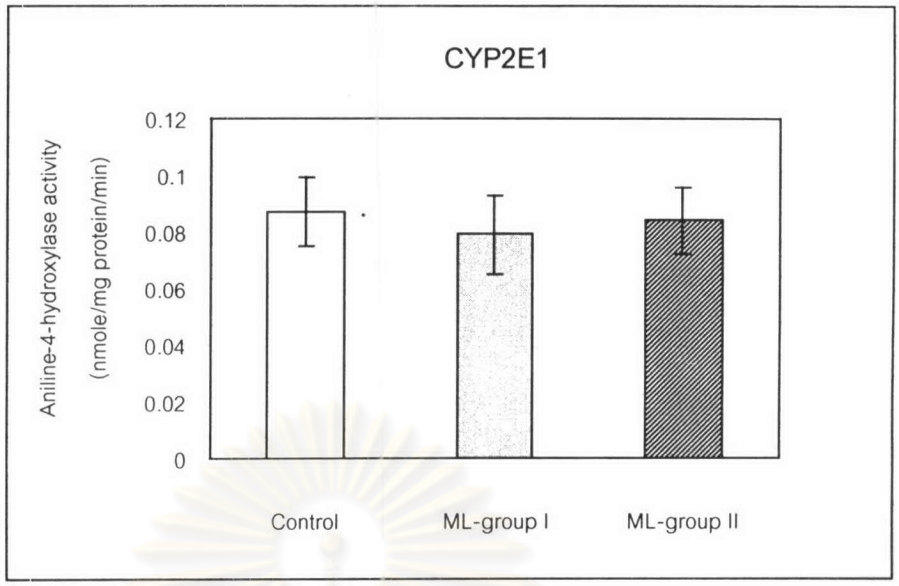


Figure 30 Subacute effects of *M. loriformis* ethanolic extract on rat hepatic CYP2E1 activity

1 ml/kg/day distilled water (Control, n= 10), 0.1 and 1 g/kg/day of *M. loriformis* (ML-group I & ML-group II, n= 10, respectively) were given orally to rats for 30 days. Liver microsomes were determined for aniline-4-hydroxylase activity. The individual bar represented the mean of aniline-4-hydroxylase activity with standard error of mean (SEM). One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.

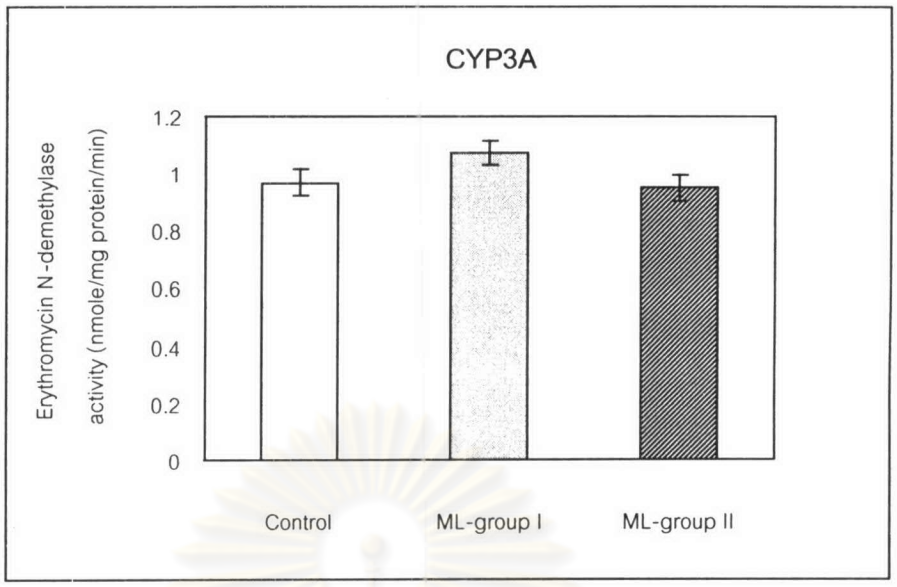


Figure 31 Subacute effects of *M. loriformis* ethanolic extract on rat hepatic CYP3A activity

1 ml/kg/day distilled water (Control, n= 10), 0.1 and 1 g/kg/day of *M. loriformis* (ML-group I & ML-group II, n= 10, respectively) were given orally to rats for 30 days. Liver microsomes were determined for erythromycin N-demethylase activity. The individual bar represented the mean of erythromycin N-demethylase activity with standard error of mean (SEM). One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.