CHAPTER IV

RESULTS

Subacute effects of *M. citrifolia* were investigated on hepatic CYPs and clinical blood chemistry in rats using an *ex vivo* model. During the time of treatment, body weight, food consumption and volume of drinking water were recorded every 5 days. Thirty male Wistar rats completed the 30-day treatment. At the time of sacrifice, liver weighs of all rats were recorded before preparation of microsomes.

Effects of *M. citrifolia* on body weight, food & water consumption, liver weight and relative liver weight

M. citrifolia at both dosages regimens (600 and 1200 mg/kg/days orally for 30 days) used in this study demonstrated no effects on body weight (Figure 4.1), food consumption (Figure 4.2), water consumption (Figure 4.3), terminal body weight, liver weight and relative liver weight (Table 4.1).

Effects of *M. citrifolia* on clinical blood chemistry and hematology

Serum and whole blood of individual rat were measured for clinical blood chemistry and hematology, respectively. Subacute exposure (30 days) of oral 600 and 1200 mg/kg/day of *M. citrifolia* in rats did not cause any significant effects on these following parameters: SGOT(Figure 4.4), SGPT (Figure 4.5), ALP (Figure 4.6), total and direct bilirubin (Figure 4.7), BUN (Figure 4.8), SCr (Figure 4.9), total chloresterol (Figure 4.10), HDL-C (Figure 4.11), TG (Figure 4.12), glucose (Figure 4.13), sodium (Figure 4.14), potassium (Figure 4.15), chloride (Figure 4.16), Hb (Figure 4.17), Hct (Figure 4.18), platelet count (Figure 4.19), WBC count (Figure 4.20), and % differential WBCs (Figure 4.21) and RBC morphology.

Effects of *M. citrifolia* on hepatic CYPs

Subacute exposure (30 days) of *M. citrifolia* at 600 and 1200 mg/kg/day to rats did not cause any significant changes of total CYP contents (Figure 4.22). *M. citrifolia* at the dosage of 1200 mg/kg/day demonstrated a significant ($p<0.05$) reduction of ethoxyresorufin O-dealkylase (EROD) activity which represented the activity of CYP1A1 (Figure 4.23). *M. citrifolia* at both dosages used in this study

demonstrated no significant effects on activities of methoxyresorufin O-dealkylase (MROD) which represented the activity of CYP1A2, benzyloxyresorufin O-dealkylase (BROD) & pentoxyresorufin O-dealkylase (PROD) which represented the activity of CYP2B1/2B2 (Figure 4.24-4.26) as well as aniline 4-hydroxylase which represented the activity of CYP2E1 (Figure 4.27). CYP3A activity was examined using the rate of erythromycin N-demethylation reaction. No significant effects of *M. citrifolia* were found on CYP3A activity (Figure 4.28) in both treatment groups as compared to the control group.

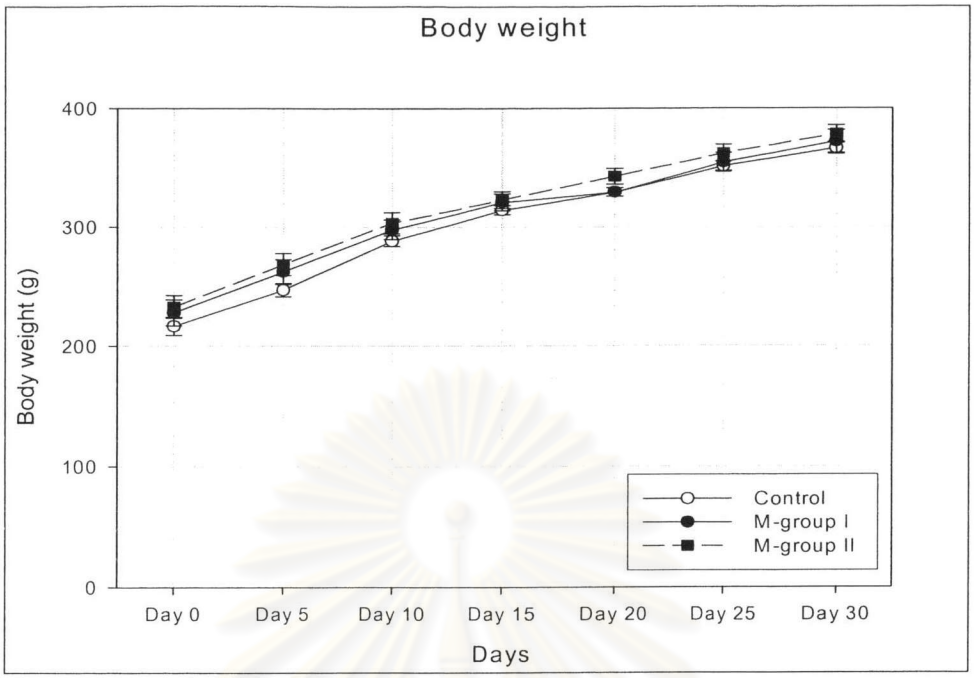

Figure 4.1 Subacute effects of *M. citrifolia* fruit extract on body weight of rats 1 ml/kg/day distilled water (Control), 600 and 1200 mg/kg/day of *M. citrifolia* (M-group I & M-group II, respectively) were given orally to rats for 30 days. The individual mark represented the mean of body weight with an error bar of SEM (n = 10). One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.

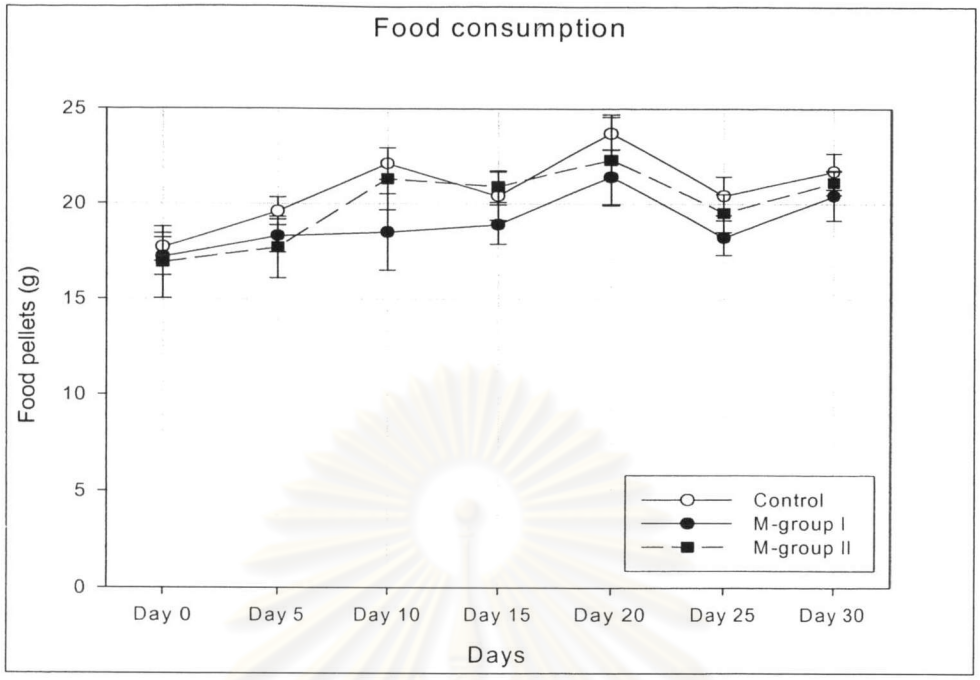

Figure 4.2 Subacute effects of *M. citrifolia* fruit extract on food consumption of rats 1 ml/kg/day distilled water (Control), 600 and 1200 mg/kg/day of *M. citrifolia* (M-group I & M-group II, respectively) were given orally to rats for 30 days. Food consumption of each rat was recorded every 5 days. The individual mark represented mean of food consumption per day with an error bar of SEM (n=10). One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.

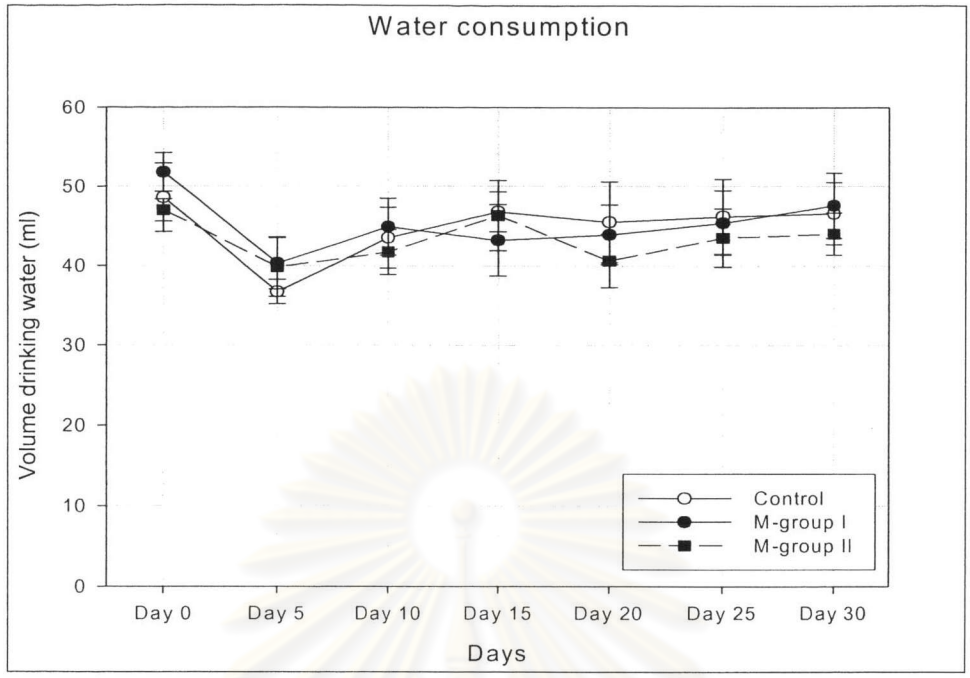

Figure 4.3 Subacute effects of *M. citrifolia* fruit extract on water consumption of rats 1 ml/kg/day distilled water (Control), 600 and 1200 mg/kg/day of *M. citrifolia* (M-group I & M-group II, respectively) were given orally to rats for 30 days. Water consumption of each rat was recorded every 5 days. The individual mark represented mean of volume of drinking water per day with an error bar of SEM (n=10). One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.

Table 4.1 Subacute effects of *M. citrifolia* fruit extract on terminal body weight, liver weight and relative liver weight

1 ml/kg/day distilled water (Control), 600 and 1200 mg/kg/day of *M. citrifolia* (M-group I & M-group II, respectively) were given orally to rats for 30 days. Each rat was recorded for its body weight and liver weight at the end of the experiment. Relative liver weight denoted percent of liver weight per terminal body weight. Values performed as mean ± SEM. One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.

	Group		
	Control	M-group I	M-group II
Terminal Body Weight (g)	358.80 ± 5.47	356.10 ± 9.37	372.80 ± 9.78
Liver weight (g)	12.21 ± 0.48	12.05 ± 0.80	12.86 ± 0.66
Relative liver weight (% of body weight)	3.40 ± 0.12	3.39 ± 0.22	3.44 ± 0.12

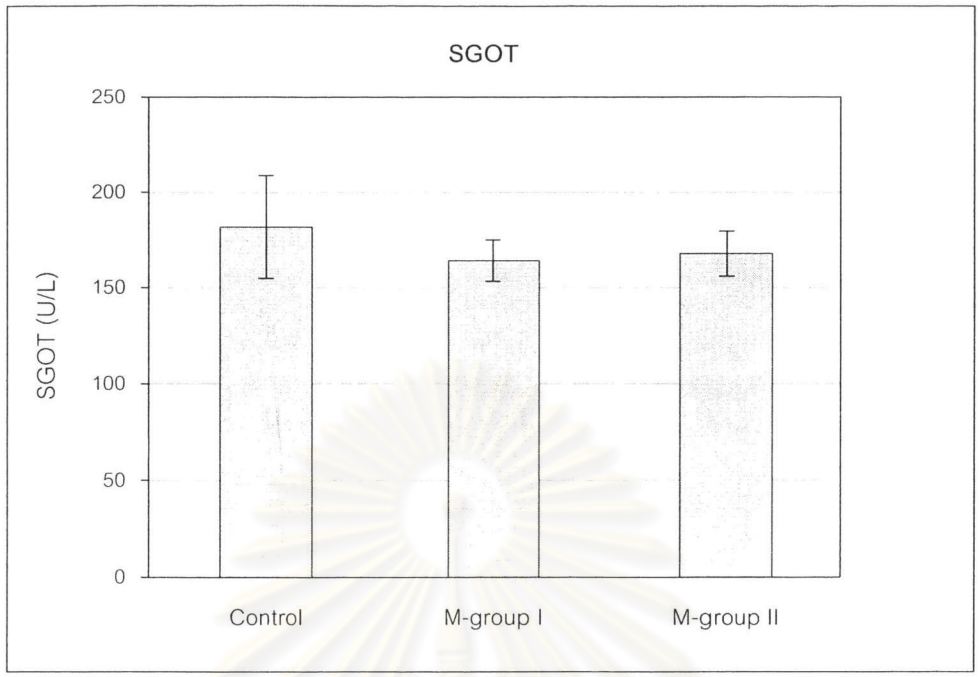

Figure 4.4 Subacute effects of *M. citrifolia* fruit extract on SGOT

1 ml/kg/day distilled water (Control), 600 and 1200 mg/kg/day *M. citrifolia* (M-group I & M-group II, respectively) were given orally to rats for 30 days. Serum samples were determined for SGOT concentrations. The individual bar represented mean of SGOT concentrations with an error bar of SEM (n=10). One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.

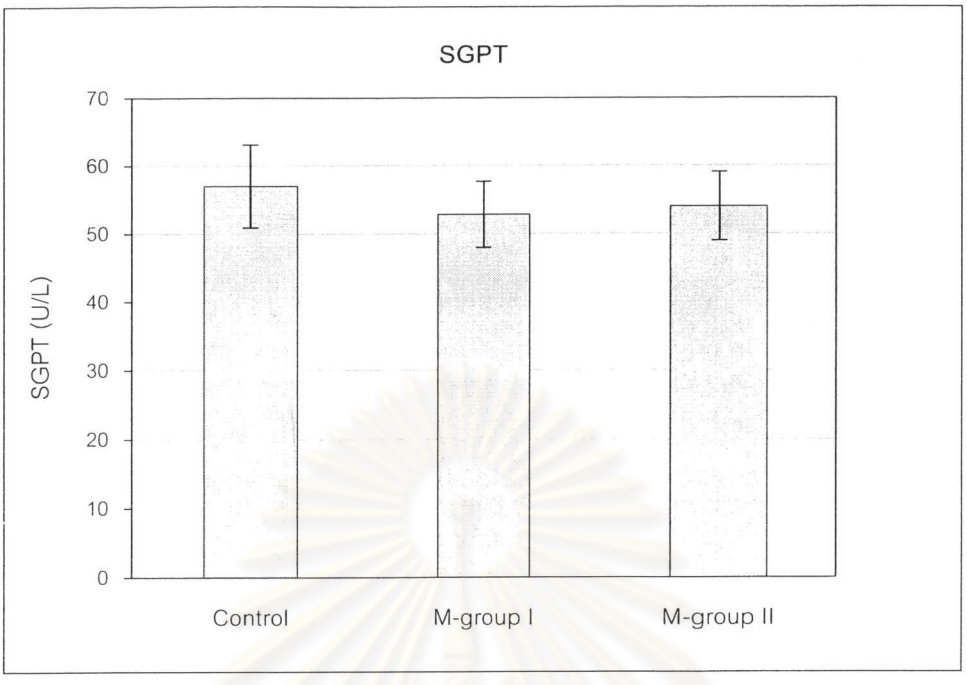

Figure 4.5 Subacute effects of *M. citrifolia* fruit extract on SGPT

1 ml/kg/day distilled water (Control), 600 and 1200 mg/kg/day *M. citrifolia* (M-group I & M-group II, respectively) were given orally to rats for 30 days. Liver microsomes were determined for SGPT concentrations. The individual bar represented mean of SGPT concentrations with an error bar of SEM (n=10). One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.

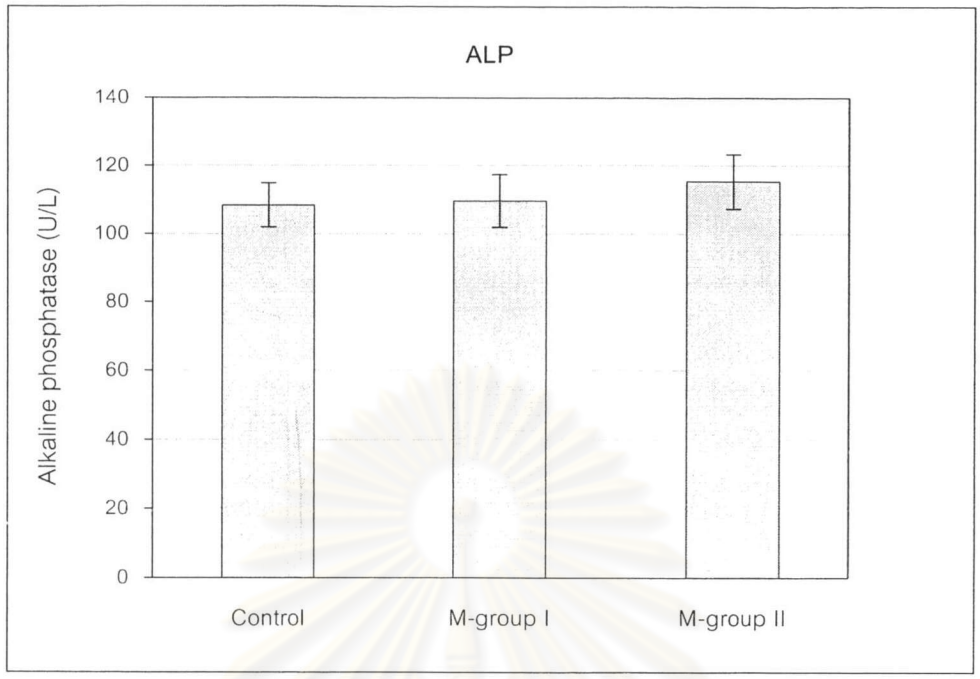

Figure 4.6 Subacute effects of *M. citrifolia* fruit extract on ALP

1 ml/kg/day distilled water (Control), 600 and 1200 mg/kg/day *M. citrifolia* (M-group I & M-group II, respectively) were given orally to rats for 30 days. Liver microsomes were determined for ALP concentrations. The individual bar represented mean of ALP concentrations with an error bar of SEM (n=10). One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.

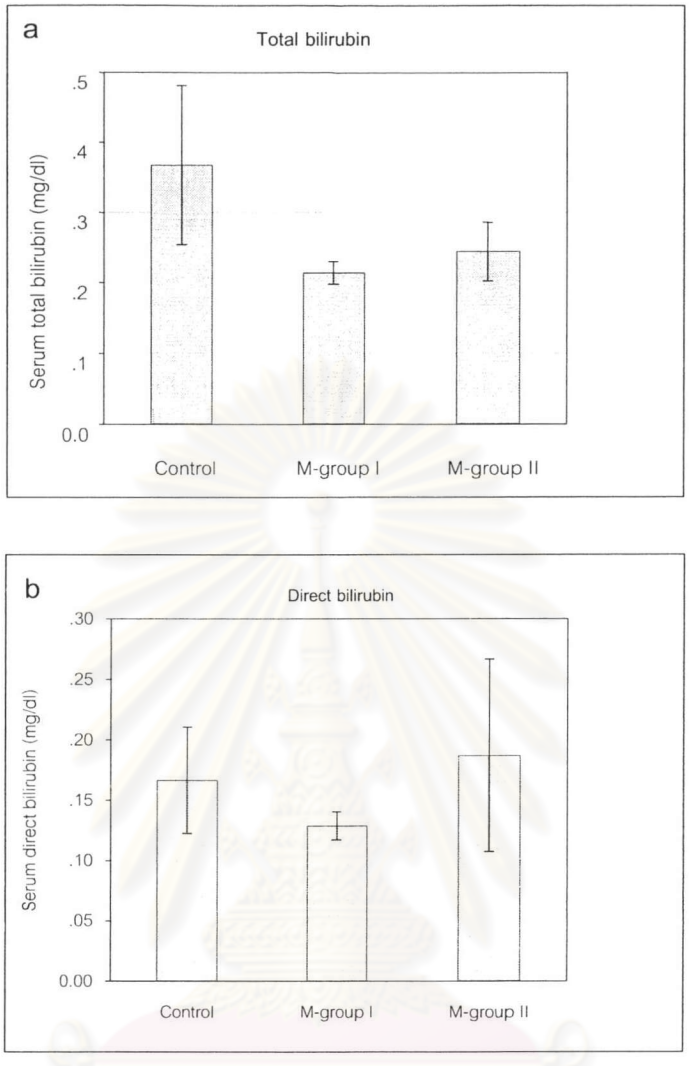

Figure 4.7 Subacute effects of *M. citrifolia* fruit extract on total and direct bilirubin 1 ml/kg/day distilled water (Control), 600 and 1200 mg/kg/day *M. citrifolia* (M-group I & M-group II, respectively) were given orally to rats for 30 days. Serum samples were determined for total bilirubin (a) and direct bilirubin (b) concentrations. The individual bar represented mean of serum bilirubin concentrations with an error bar of SEM (n=10). One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.

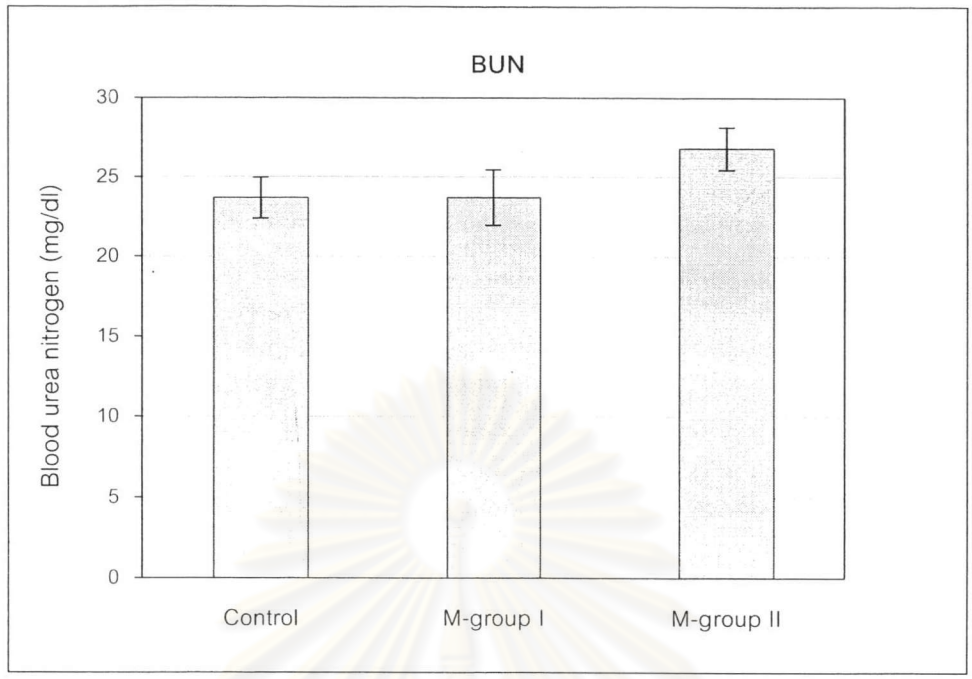

Figure 4.8 Subacute effects of *M. citrifolia* fruit extract on BUN

1 ml/kg/day distilled water (Control), 600 and 1200 mg/kg/day *M. citrifolia* (M-group I & M-group II, respectively) were given orally to rats for 30 days. Serum samples were determined for BUN concentrations. The individual bar represented mean of BUN concentrations with an error bar of SEM (n=10). One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.

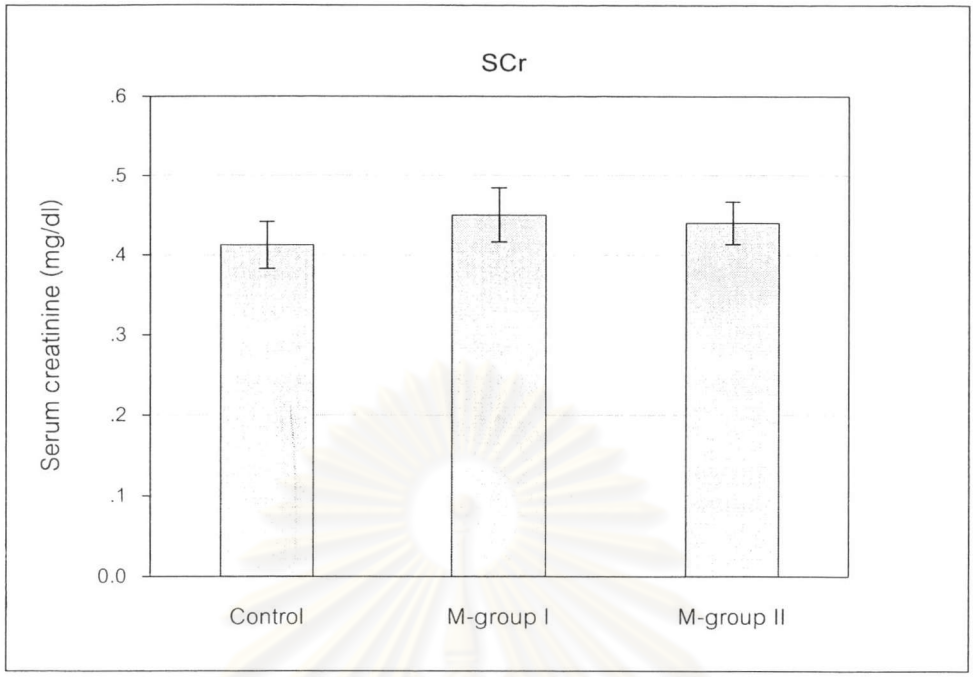

Figure 4.9 Subacute effects of *M. citrifolia* fruit extract on SCr

1 ml/kg/day distilled water (Control), 600 and 1200 mg/kg/day *M. citrifolia* (M-group I & M-group II, respectively) were given orally to rats for 30 days. Serum samples were determined for SCr concentrations. The individual bar represented mean of SCr concentrations with an error bar of SEM (n=10). One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.

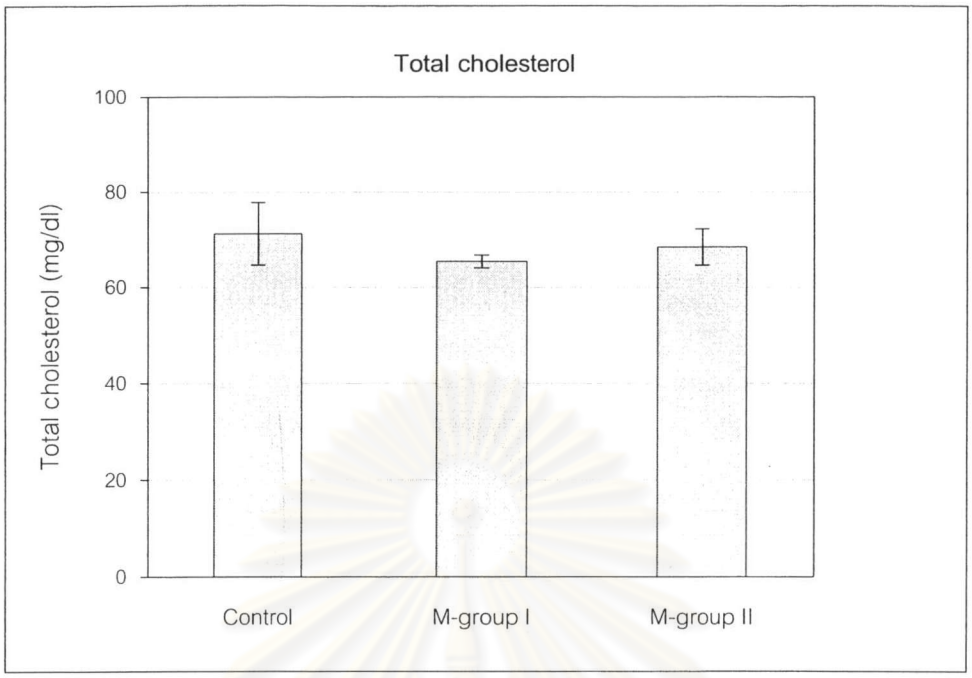

Figure 4.10 Subacute effects of *M. citrifolia* fruit extract on total cholesterol

1 ml/kg/day distilled water (Control), 600 and 1200 mg/kg/day *M. citrifolia* (M-group I & M-group II, respectively) were given orally to rats for 30 days. Serum samples were determined for total cholesterol concentrations. The individual bar represented mean of total cholesterol concentrations with an error bar of SEM (n=10). One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.

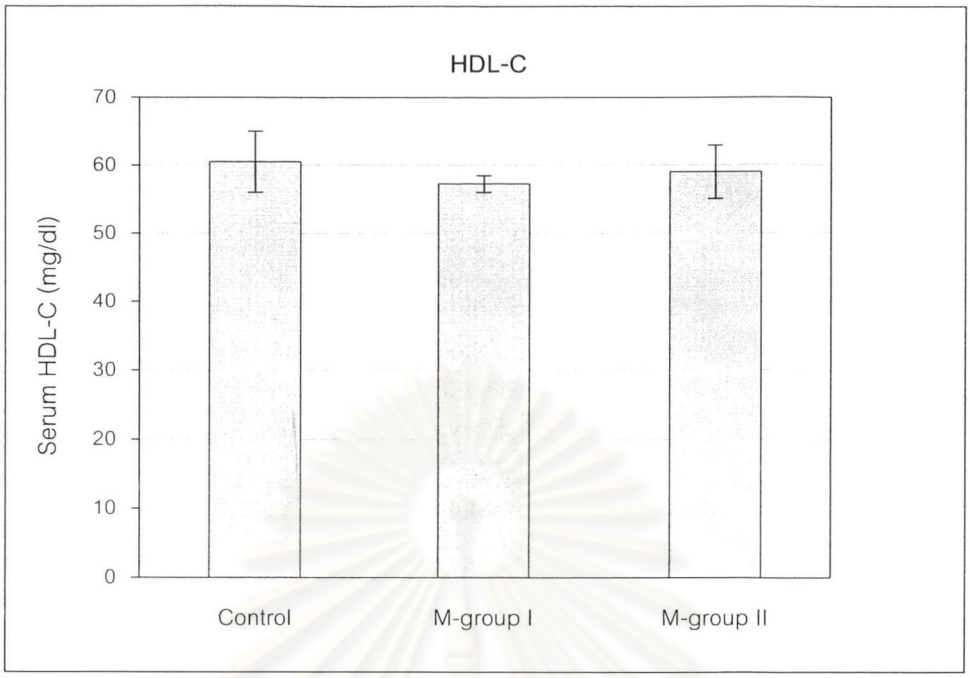

Figure 4.11 Subacute effects of *M. citrifolia* fruit extract on HDL-C

1 ml/kg/day distilled water (Control), 600 and 1200 mg/kg/day *M. citrifolia* (M-group I & M-group II, respectively) were given orally to rats for 30 days. Serum samples were determined for HDL-C concentrations. The individual bar represented mean of HDL-C concentrations with an error bar of SEM (n=10). One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.

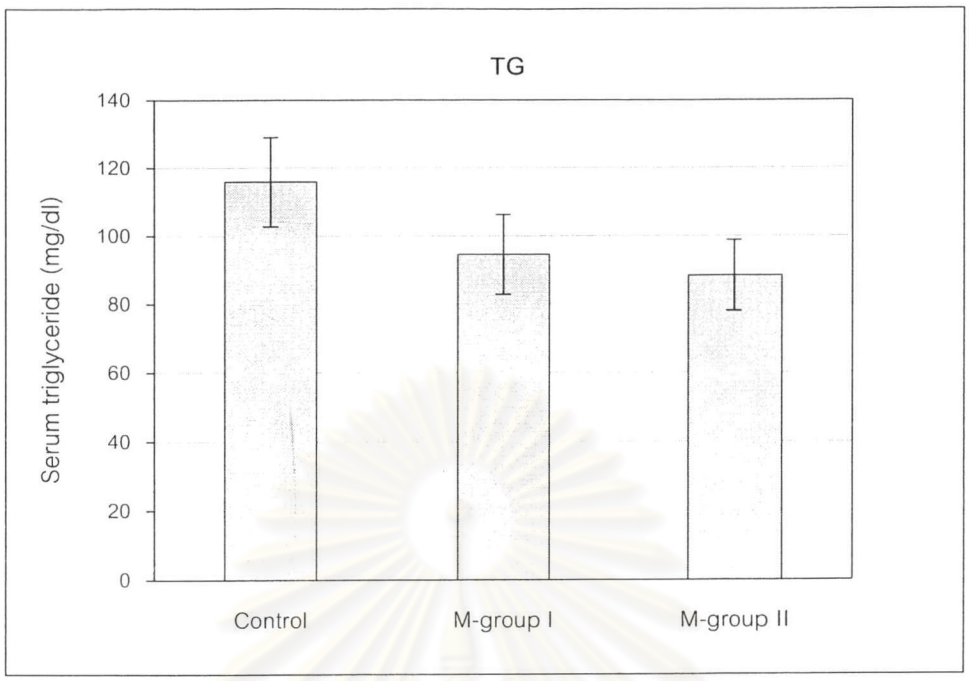

Figure 4.12 Subacute effects of *M. citrifolia* fruit extract on TG

1 ml/kg/day distilled water (Control), 600 and 1200 mg/kg/day *M. citrifolia* (M-group I & M-group II, respectively) were given orally to rats for 30 days. Serum samples were determined for TG concentrations. The individual bar represented mean of TG concentrations with an error bar of SEM (n=10). One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.

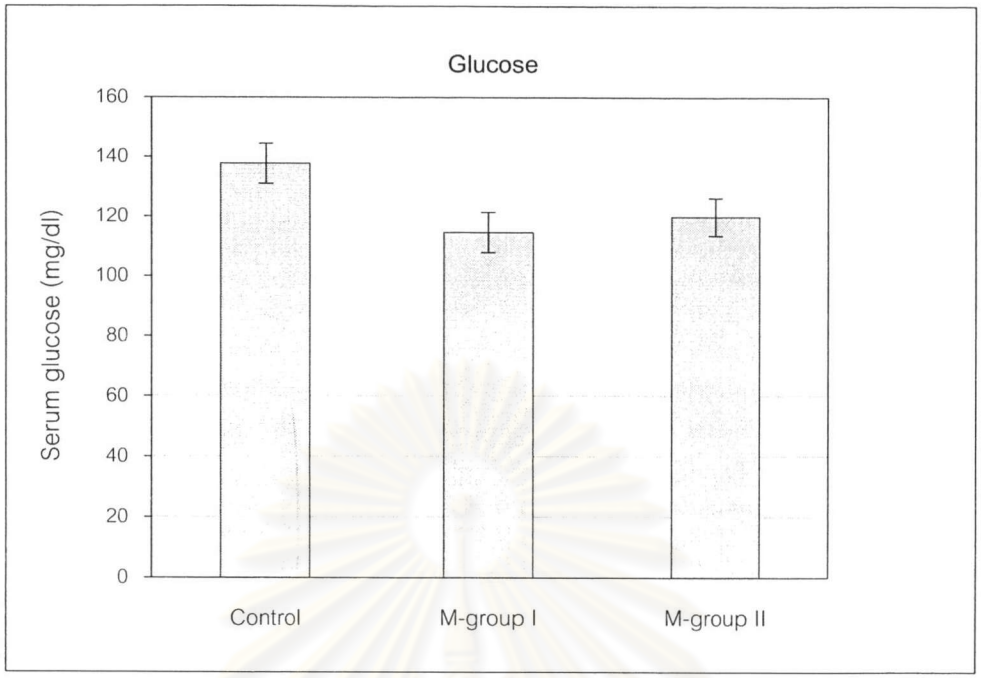

Figure 4.13 Subacute effects of *M. citrifolia* fruit extract on serum glucose

1 ml/kg/day distilled water (Control), 600 and 1200 mg/kg/day *M. citrifolia* (M-group I & M-group II, respectively) were given orally to rats for 30 days. Serum samples were determined for glucose concentrations. The individual bar represented mean of glucose concentrations with an error bar of SEM (n=10). One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.

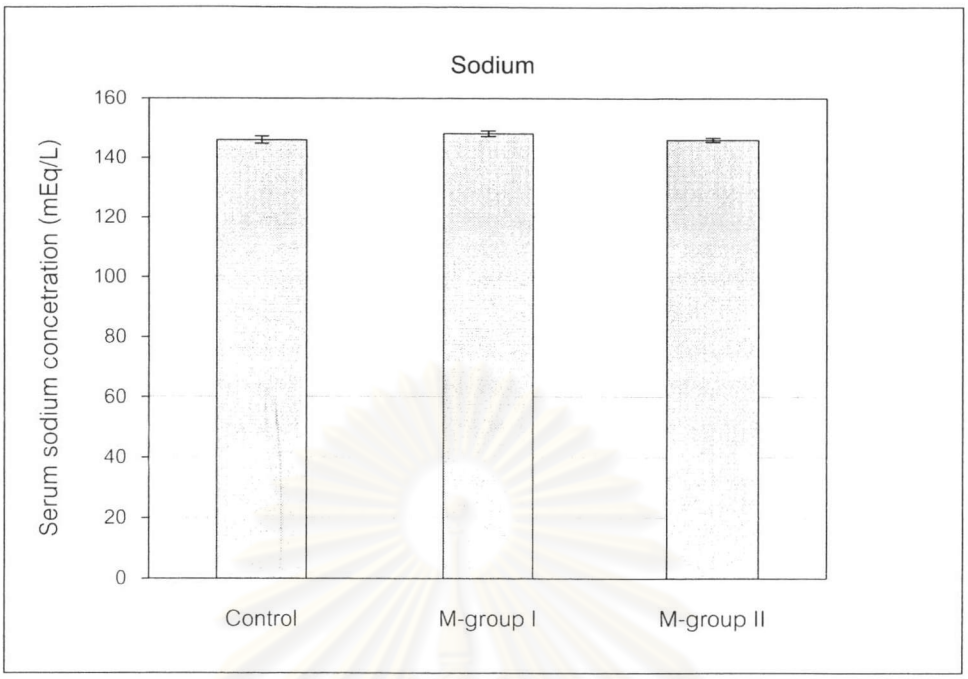

Figure 4.14 Subacute effects of *M. citrifolia* fruit extract on serum sodium

1 ml/kg/day distilled water (Control), 600 and 1200 mg/kg/day *M. citrifolia* (M-group I & M-group II, respectively) were given orally to rats for 30 days. Serum samples were determined for sodium concentrations. The individual bar represented mean of sodium concentrations with an error bar of SEM (n=10). One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.

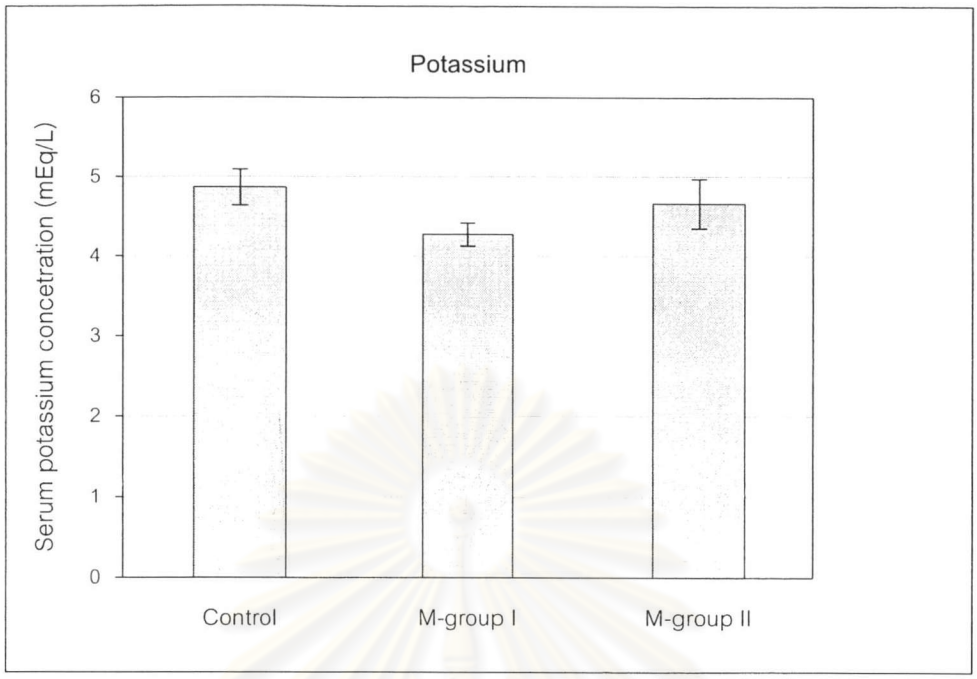

Figure 4.15 Subacute effects of *M. citrifolia* fruit extract on serum potassium

1 ml/kg/day distilled water (Control), 600 and 1200 mg/kg/day *M. citrifolia* (M-group I & M-group II, respectively) were given orally to rats for 30 days. Serum samples were determined for potassium concentrations. The individual bar represented mean of potassium concentrations with an error bar of SEM (n=10). One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.

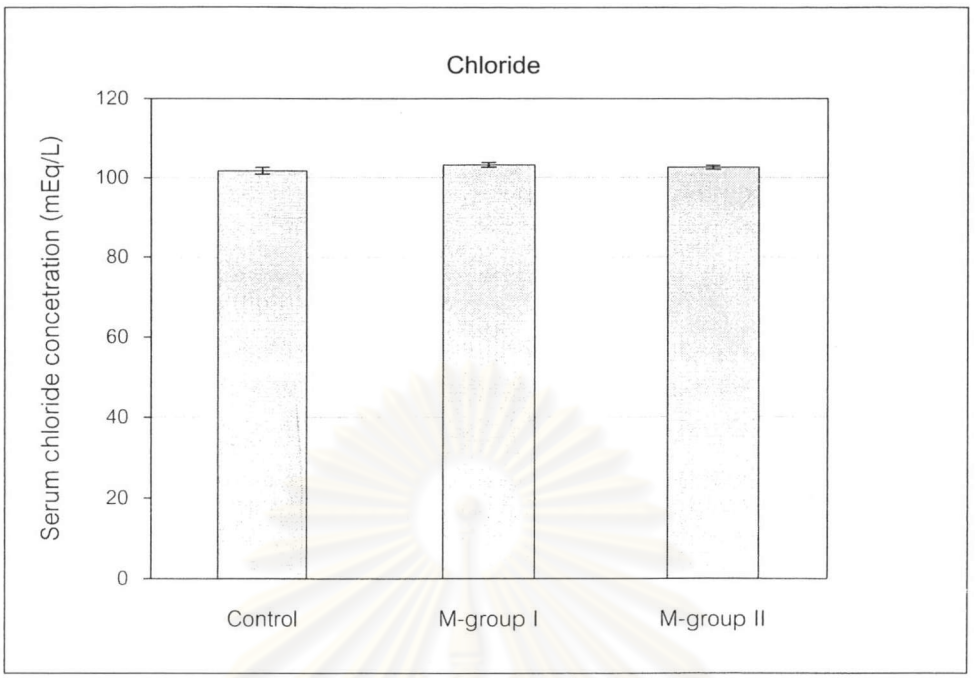

Figure 4.16 Subacute effects of *M. citrifolia* fruit extract on serum chloride

1 ml/kg/day distilled water (Control), 600 and 1200 mg/kg/day *M. citrifolia* (M-group I & M-group II, respectively) were given orally to rats for 30 days. Serum samples were determined for chloride concentrations. The individual bar represented mean of chloride concentrations with an error bar of SEM (n=10). One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.

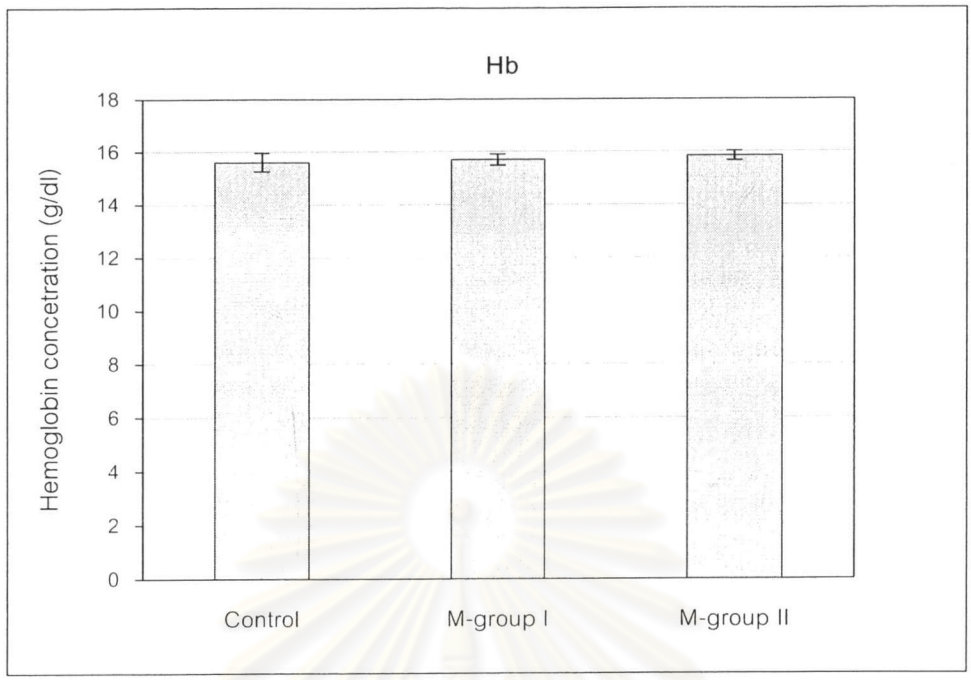

Figure 4.17 Subacute effects of *M. citrifolia* fruit extract on Hb

1 ml/kg/day distilled water (Control), 600 and 1200 mg/kg/day *M. citrifolia* (M-group I & M-group II, respectively) were given orally to rats for 30 days. Whole blood samples were determined for Hb concentrations. The individual bar represented mean of Hb concentrations with an error bar of SEM (n=10). One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.

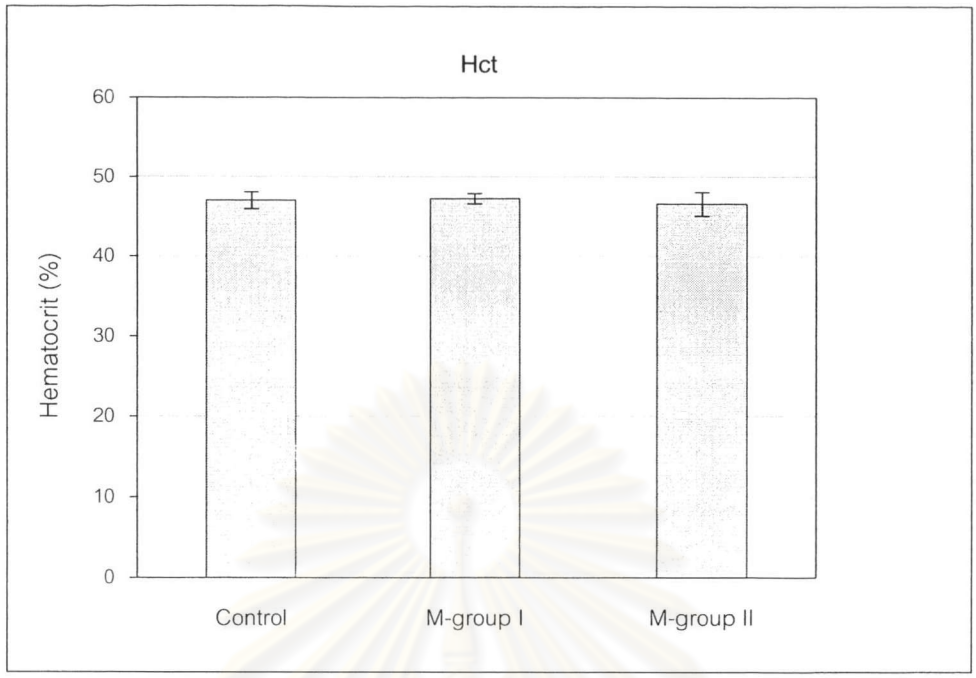

Figure 4.18 Subacute effects of *M. citrifolia* fruit extract on Hct

1 ml/kg/day distilled water (Control), 600 and 1200 mg/kg/day *M. citrifolia* (M-group I & M-group II, respectively) were given orally to rats for 30 days. Whole blood samples were determined for Hct concentrations. The individual bar represented mean of Hct concentrations with an error bar of SEM (n=10). One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.

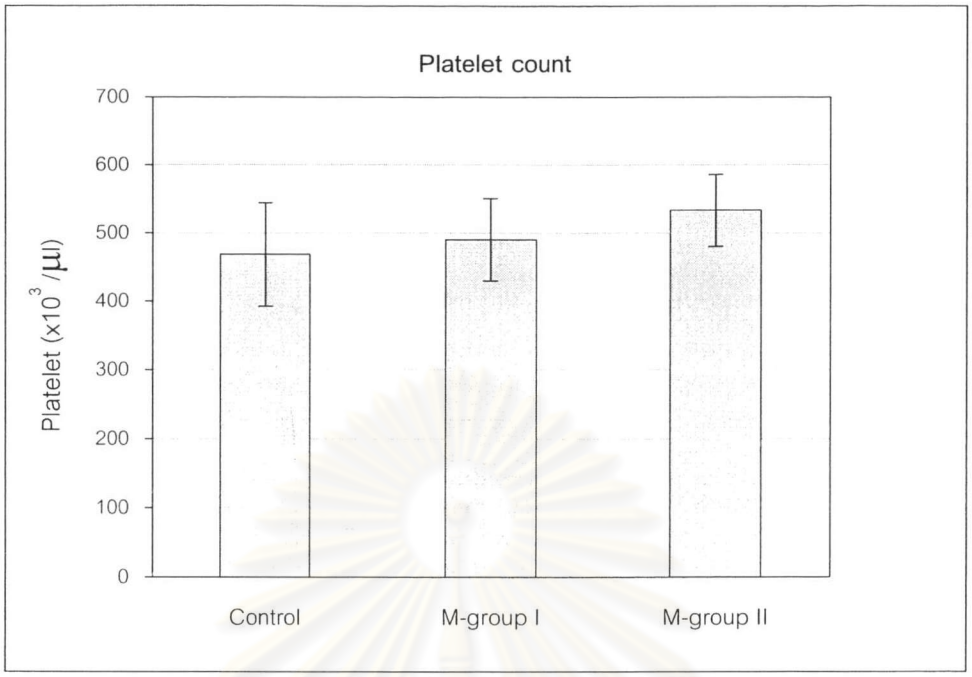

Figure 4.19 Subacute effects of *M. citrifolia* fruit extract on platelet count

1 ml/kg/day distilled water (Control), 600 and 1200 mg/kg/day *M. citrifolia* (M-group I & M-group II, respectively) were given orally to rats for 30 days. Whole blood samples were determined for platelet counts. The individual bar represented mean of platelet counts with an error bar of SEM (n=10). One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.

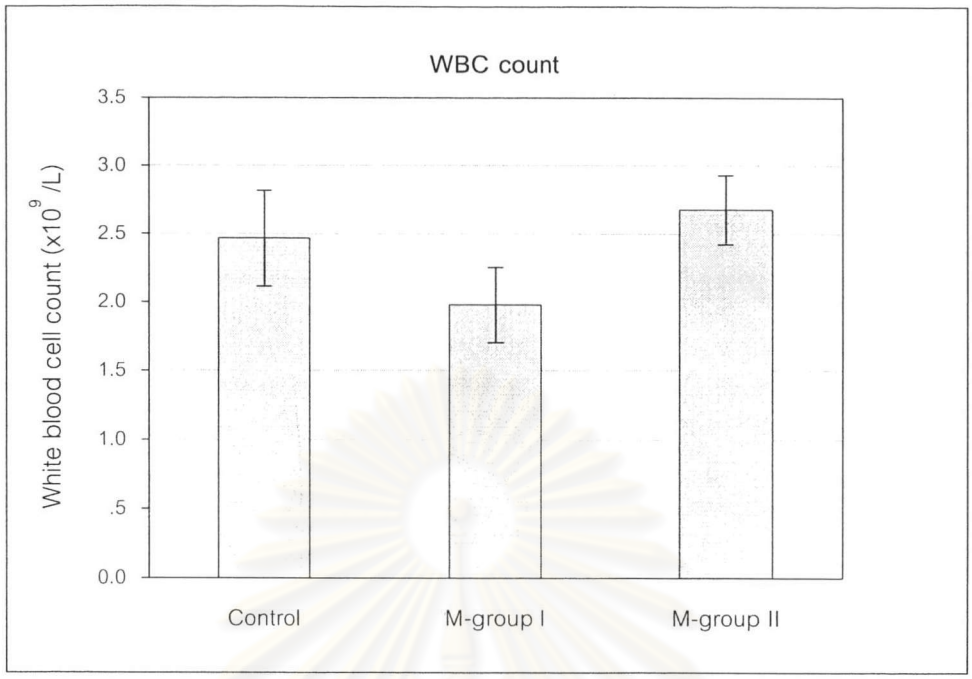

Figure 4.20 Subacute effects of *M. citrifolia* fruit extract on WBC count

1 ml/kg/day distilled water (Control), 600 and 1200 mg/kg/day *M. citrifolia* (M-group I & M-group II, respectively) were given orally to rats for 30 days. Whole blood samples were determined for WBC counts. The individual bar represented mean of WBC counts with an error bar of SEM (n=10). One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.

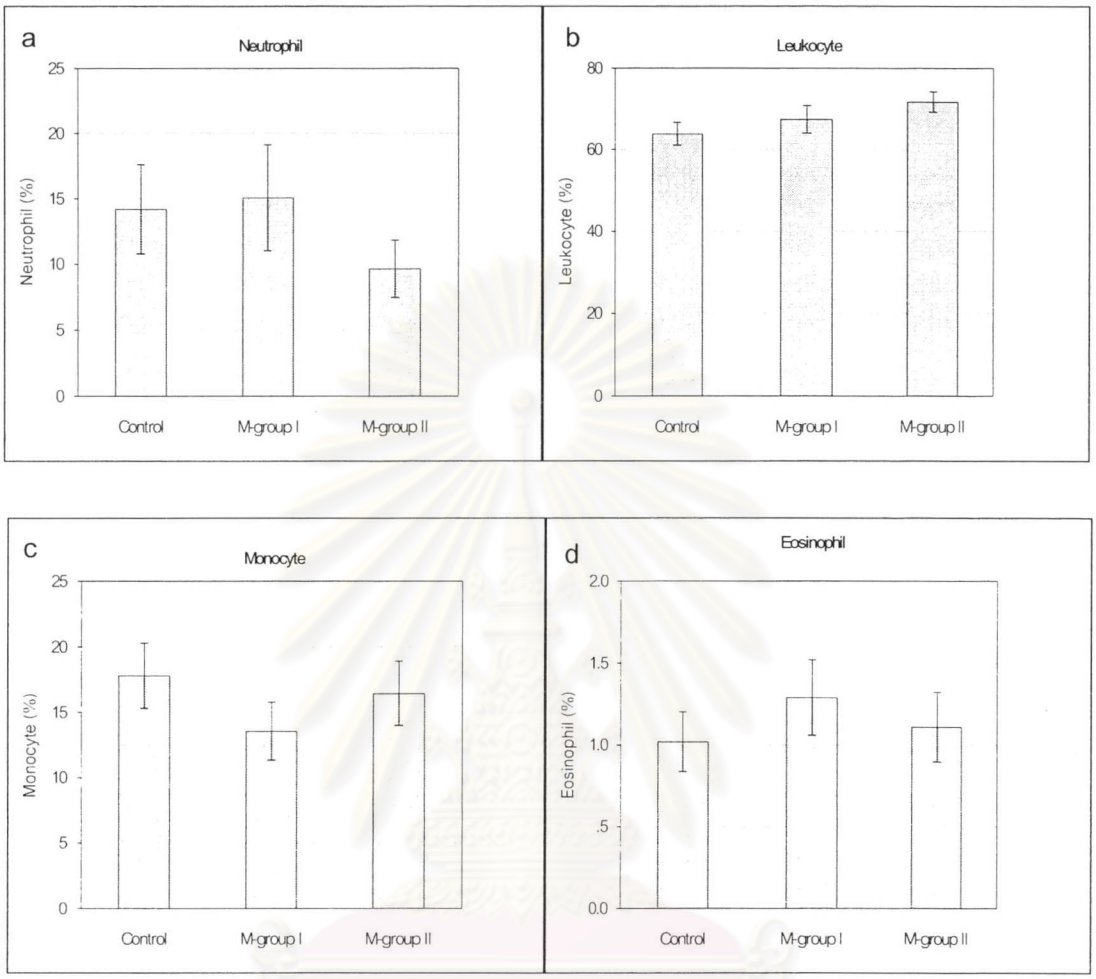

Figure 4.21 Subacute effects of *M. citrifolia* fruit extract on % differential WBCs

1 ml/kg/day distilled water (Control), 600 and 1200 mg/kg/day *M. citrifolia* (M-group I & M-group II, respectively) were given orally to rats for 30 days. Whole blood samples were determined for % differential WBCs. The individual bar represented mean of % differential WBCs that included neutrophil (a), leukocyte (b), monocyte (c) and eosinophil (d) with an error bar of SEM (n=10). One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.

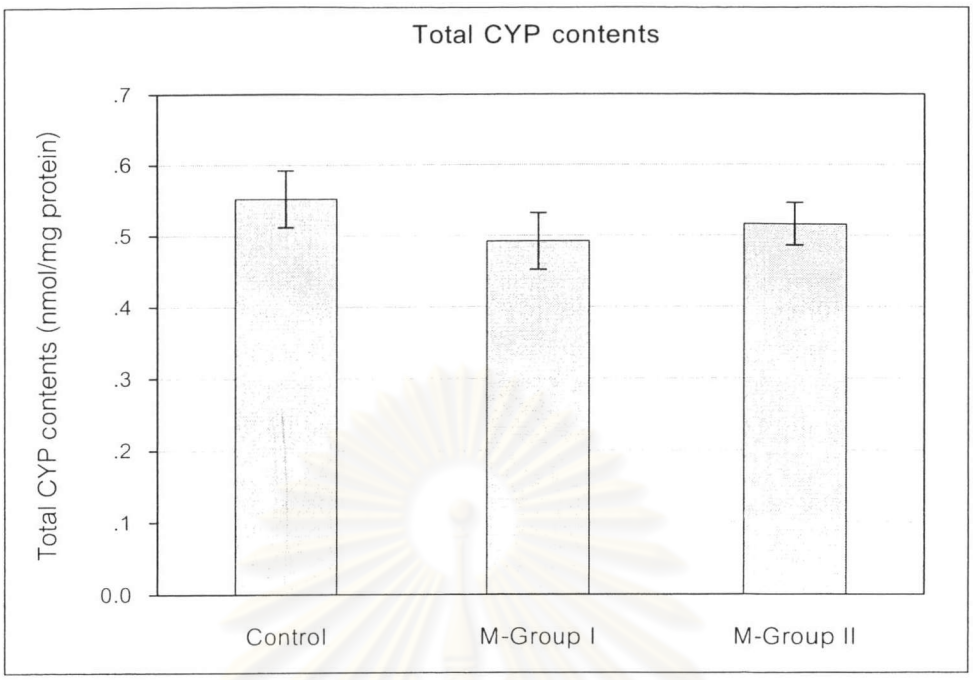

Figure 4.22 Subacute effects of *M. citrifolia* fruit extract on rat hepatic total CYP contents

1 ml/kg/day distilled water (Control), 600 and 1200 mg/kg/day *M. citrifolia* (M-group I & M-group II, respectively) were given orally to rats for 30 days. Liver microsomes were determined for total CYP contents. The individual bar represented mean of total CYP contents with an error bar of SEM (n=10). One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.

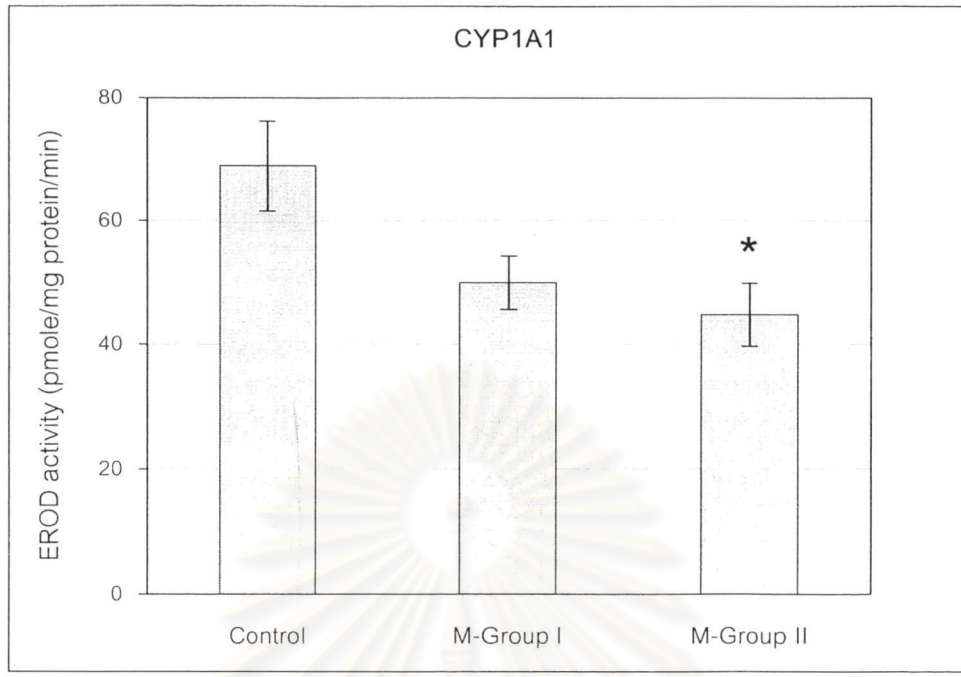

Figure 4.23 Subacute effects of *M. citrifolia* fruit extract on rat hepatic CYP1A1 activity

1 ml/kg/day distilled water (Control), 600 and 1200 mg/kg/day *M. citrifolia* (M-group I & M-group II, respectively) were given orally to rats for 30 days. Liver microsomes were determined for EROD activity. The individual bar represented mean of EROD activity with an error bar of SEM (n=10). One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.

* $p < 0.05$ M-Group II VS control group

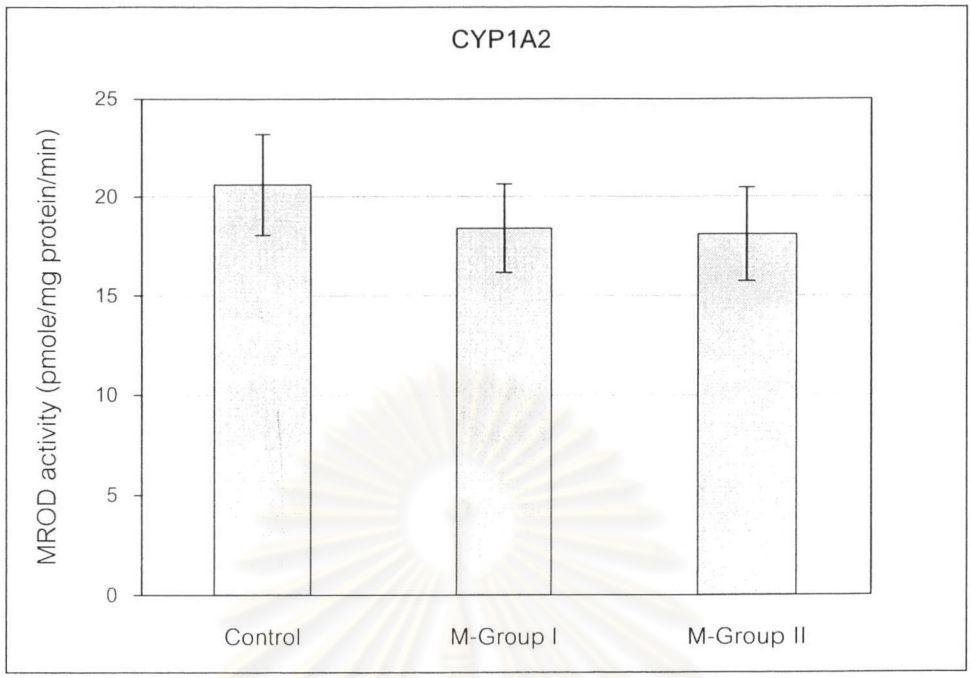

Figure 4.24 Subacute effects of *M. citrifolia* fruit extract on rat hepatic CYP1A2 activity

1 ml/kg/day distilled water (Control), 600 and 1200 mg/kg/day *M. citrifolia* (M-group I & M-group II, respectively) were given orally to rats for 30 days. Liver microsomes were determined for MROD activity. The individual bar represented mean MROD activity with an error bar of SEM (n=10). One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.

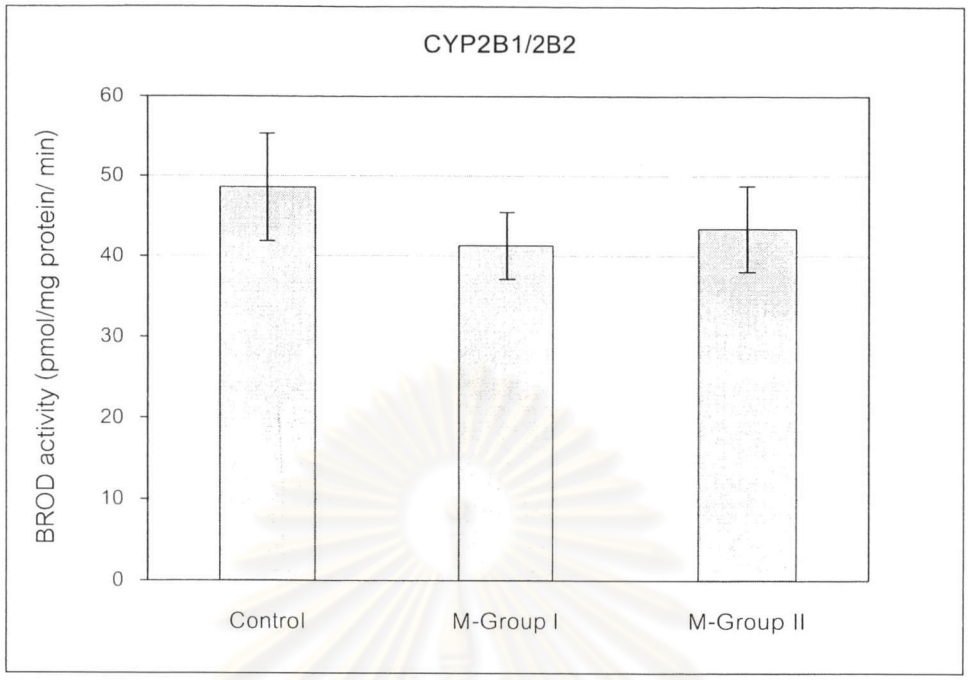

Figure 4.25 Subacute effects of *M. citrifolia* fruit extract on rat hepatic CYP 2B1/2B2 (BROD) activity

1 ml/kg/day distilled water (Control), 600 and 1200 mg/kg/day *M. citrifolia* (M-group I & M-group II, respectively) were given orally to rats for 30 days. Liver microsomes were determined for BROD activity. The individual bar represented mean BROD activity with an error bar of SEM (n=10). One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.

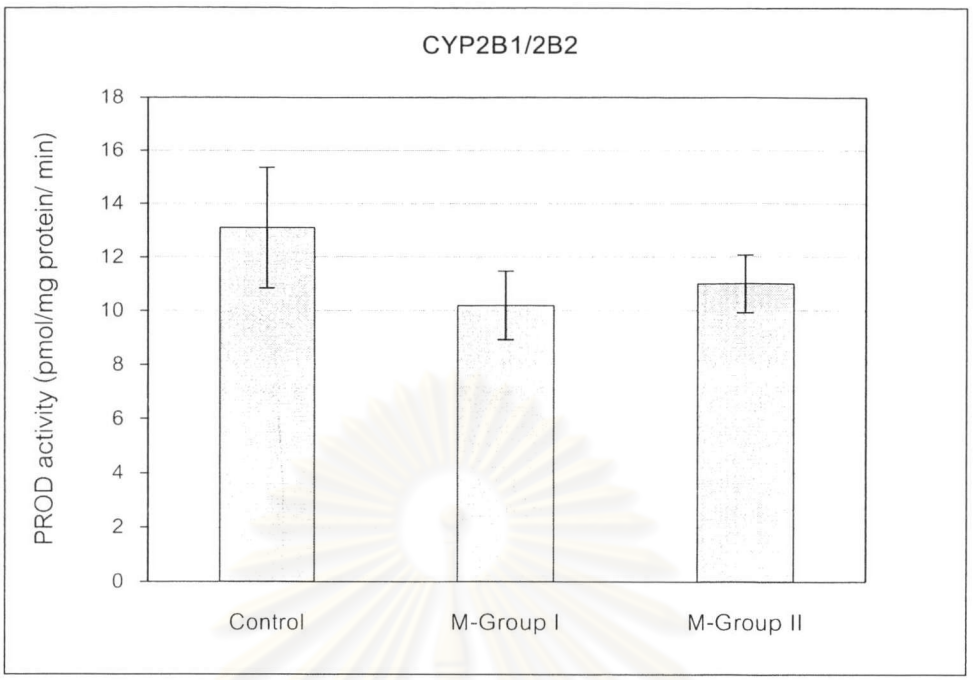

Figure 4.26 Subacute effects of *M. citrifolia* fruit extract on rat hepatic CYP 2B1/2B2 (PROD) activity

1 ml/kg/day distilled water (Control), 600 and 1200 mg/kg/day *M. citrifolia* (M-group I & M-group II, respectively) were given orally to rats for 30 days. Liver microsomes were determined for PROD activity. The individual bar represented mean PROD activity with an error bar of SEM (n=10). One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.

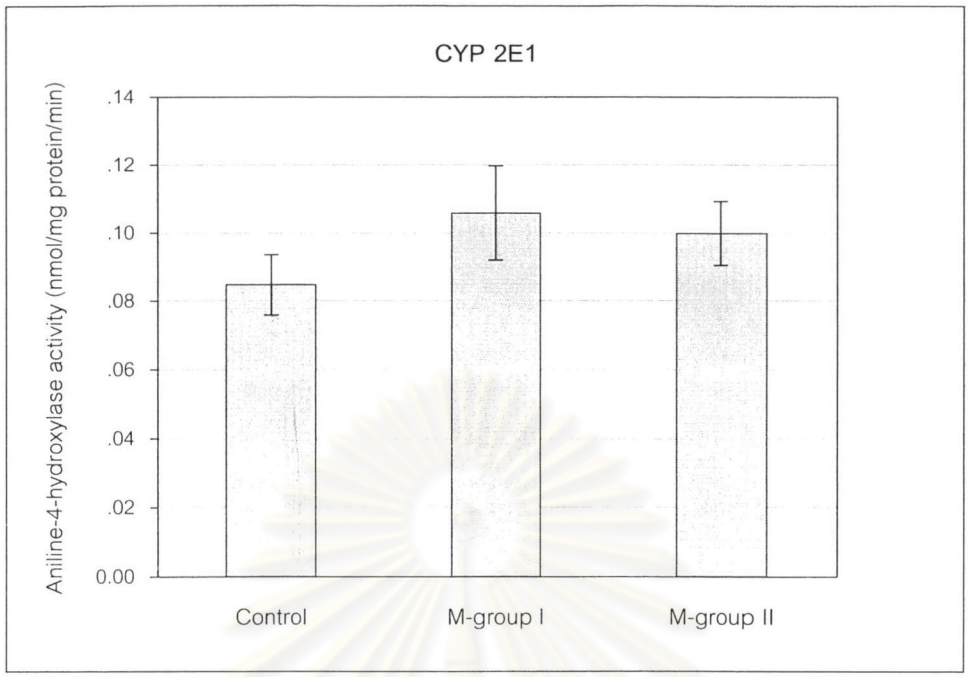

Figure 4.27 Subacute effects of *M. citrifolia* fruit extract on rat hepatic CYP 2E1 activity

1 ml/kg/day distilled water (Control), 600 and 1200 mg/kg/day *M. citrifolia* (M-group I & M-group II, respectively) were given orally to rats for 30 days. Liver microsomes were determined for aniline-4-hydroxylase activity. The individual bar represented mean of aniline-4-hydroxylase activity with an error bar of SEM (n=10). One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$

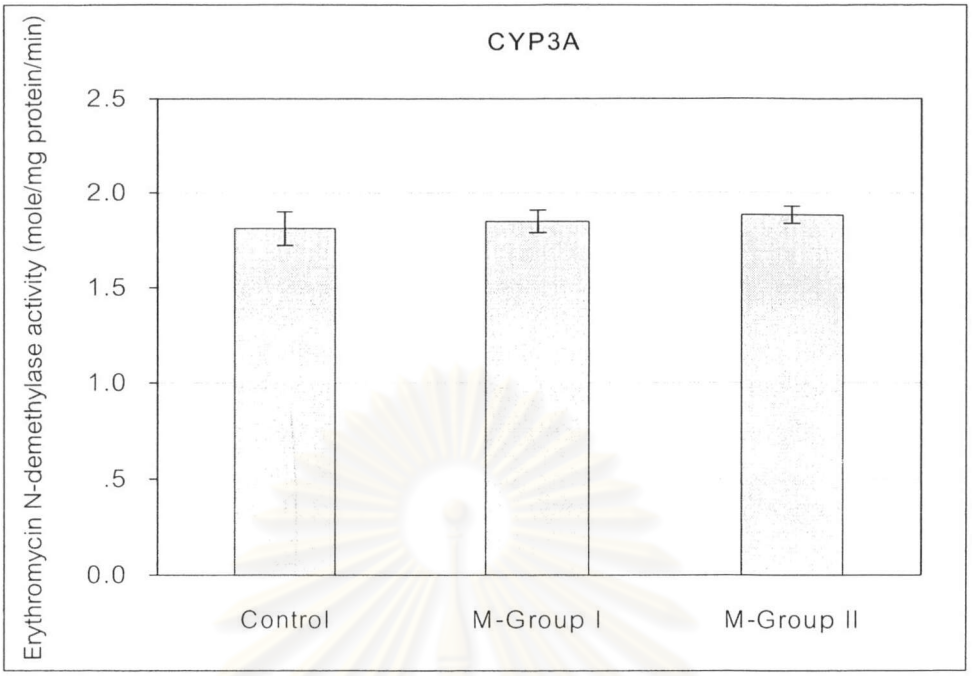

Figure 4.28 Subacute effects of *M. citrifolia* fruit extract on rat hepatic CYP 3A activity 1 ml/kg/day distilled water (Control), 600 and 1200 mg/kg/day *M. citrifolia* (M-group I & M-group II, respectively) were given orally to rats for 30 days. Liver microsomes were determined for erythromycin N-demethylase activity. The individual bar represented mean of erythromycin N-demethylase activity with an error bar of SEM (n=10). One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.