CHAPTER IV

RESULTS

1. Preparation of *O. grandiflorus* aqueous extract

Dry powder of *O. grandiflorus* leaves and tips of the stems was extracted according to the extraction procedure as described in Material and Method to obtain sufficient amount of the extract for using in the study. The extracts obtained from each extraction were combined, mixed homogeneously, and kept in a tightly closed container. The percentage yield of the extract was calculated and was found to be 12.7% w/w.

2. Chemical identification of *O. grandiflorus* aqueous extract

2.1 Preliminary test with color reaction test

Aqueous extract of *O. grandiflorus* was shown to cause a color disappearance of potassium permanganate in the potassium permanganate TS test. Using ferric chloride TS test, the extract was found to change the yellow color of ferric chloride TS to the grayish-green color. These results indicated that *O. grandiflorus* aqueous extract was composed of phenolic compounds.

2.2 Confirmatory test with thin layer chromatographic analysis

Figure 4 showed the TLC chromatograms of *O. grandiflorus* aqueous extract. The specific characteristics of spots on TLC plates conformed those of the reference standards, caffeic acid, ursolic acid and rosmarinic acid.

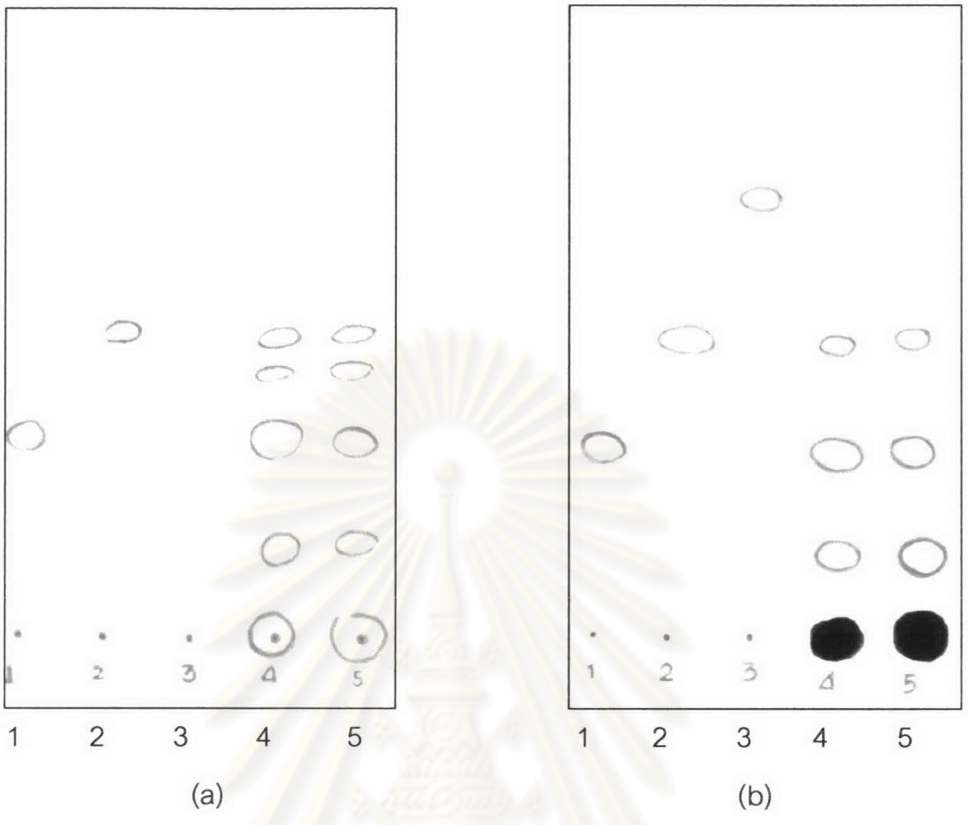


Figure 4 TLC chromatogram of *O. grandiflorus* aqueous extract

Adsorbent	: Silica gel GF_{254} precoated plate
Developing solvent system	: Toluene : ethyl acetate : formic acid (9:9:1)
Detection	: UV 254 nm (a)
	: Vanillin-phosphoric acid reagent, UV 366 nm (b)
Reference standards	: line 1 = rosmarinic acid
	line 2 = caffeic acid
	line 3 = ursolic acid
Sample	: line 4 and 5 = *O. grandiflorus* aqueous extract

2.3 Determination of potassium content in *O. grandiflorus* aqueous extract

Potassium content in *O. grandiflorus* aqueous extract was analyzed and found to be 11.52% w/w.

3. An *ex vivo* study

3.1 Effect of *O. grandiflorus* aqueous extract on body weight, relative liver weight and relative food & water consumptions

O. grandiflorus aqueous extract was given orally to rats at doses of 0.96 and 4.8 g/kg/day for 30 days, whereas the control rats receiving water. Rats of all groups were alive till the end of the experiment. At the beginning of the experiment, an average of body weight of rats in all groups were not different. Eventhough body weights of rats given *O. grandiflorus* at both doses were not significantly different from the control group, body weight gain of rats receiving 4.8 g/kg/day of *O. grandiflorus* was significantly lower then those of the control rats (Figure 5). Liver weight and % relative liver weight were not affected by *O. grandiflorus* administration at both doses used in this study (Table 6).

Relative food and water consumption of rats receiving both doses of *O. grandiflorus* was not significantly different from the control group thoughout the study period (Figure 6-7).

Table 6 Effects of *O. grandiflorus* aqueous extract on body weight, liver weight and %relative liver weight

	Treatment group		
	Control group	*O. grandiflorus* group I 0.96 g/kg/day	*O. grandiflorus* group II 4.8 g/kg/day
Initial body weight[a] (g)	312.39 ± 13.52	324.52 ± 20.64	322.04 ± 14.31
Final body weight[b] (g)	380.31 ± 11.65	370.58 ± 22.26	341.19 ± 13.01
Liver weight[c] (g)	12.38 ± 0.62	12.86 ± 1.14	12.16 ± 0.82
% relative liver weight (g/100 g of body weight)	3.25 ± 0.11	3.42 ± 0.14	3.54 ± 0.16

Data shown were mean ± standard error of the mean (n = 10)

[a] Body weight of rat at the beginning of *O. grandiflorus* administration.

[b] Body weight of rat at the time of sacrification.

[c] Liver weight at the time of sacrification, before preparation of microsomes.

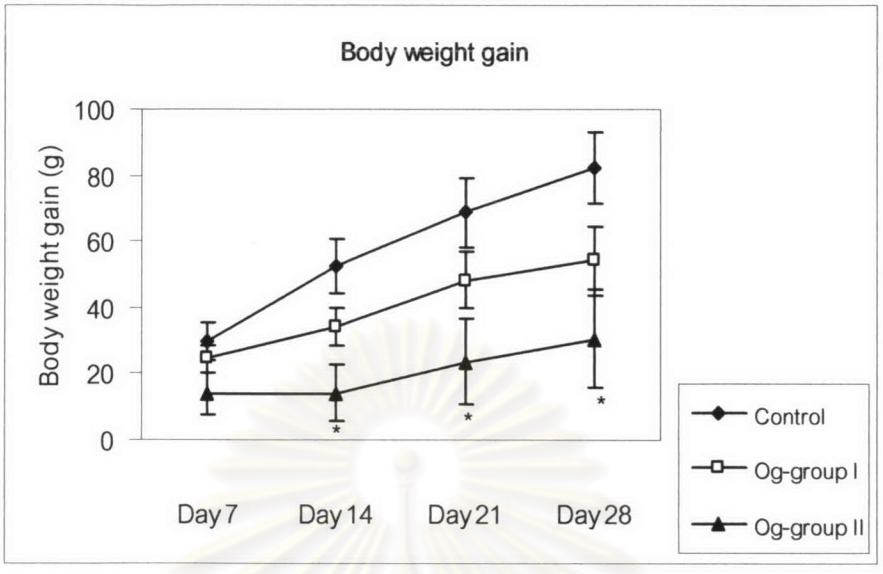


Figure 5 Effect of *O. grandiflorus* aqueous extract on body weight gain of rats.

One milliliter/kg/day of distilled water (Control), 0.96 and 4.8 g/kg/day of *O. grandiflorus* aqueous extract (Og-group I & Og-group II, respectively) (n =10) were given orally to rats for 30 days. The individual mark represented the mean of body weight gain with standard error of the mean. One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.

* $p < 0.05$; *O. grandiflorus* group vs control group

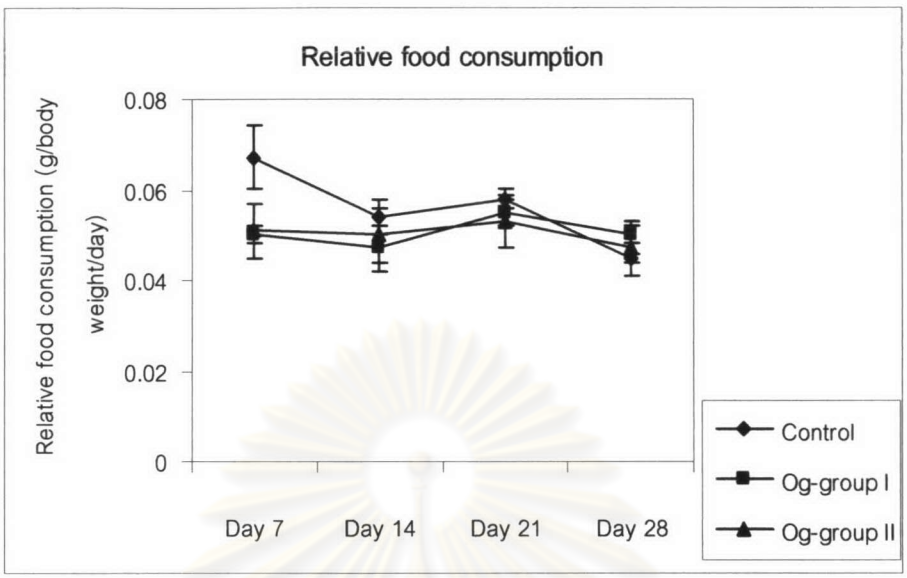


Figure 6 Effect of *O. grandiflorus* aqueous extract on relative food consumption of rats. One milliliter/kg/day distilled water (Control), 0.96 and 4.8 g/kg/day of *O. grandiflorus* (Og-group I & Og-group II, respectively) (n = 10) were given orally to rats for 30 days. Relative food consumption of each rat was recorded. The individual mark represented the mean of relative food consumption with standard error of the mean. One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.

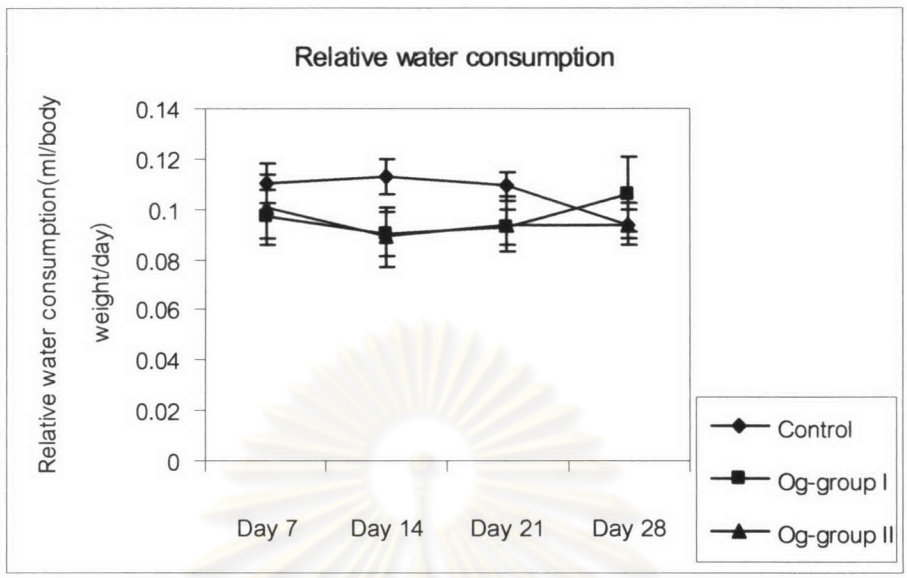


Figure 7 Effect of *O. grandiflorus* aqueous extract on relative water consumption of rats. One milliliter/kg/day distilled water (Control), 0.96 and 4.8 g/kg/day of *O. grandiflorus* (Og-group I & Og-group II, respectively) (n = 10) were given orally to rats for 30 days. Relative water consumption of each rat was recorded. The individual mark represented the mean of relative water consumption with standard error of the mean. One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.

3.2 Effect of *O. grandiflorus* aqueous extract on hematology and clinical blood chemistry

3.2.1 Effect of *O. grandiflorus* aqueous extract on clinical blood chemistry

O. grandiflorus aqueous extract given orally to rats at doses of 0.96 and 4.8 g/kg/day did not cause significant changes on these following clinical blood chemistry in serum: AST, ALT, ALP, total & direct bilirubin, total protein, albumin, globulin, SCr, sodium, chloride, calcium, uric acid, total cholesterol, triglyceride, LDL-C, HDL-C and glucose. Significant increases of BUN (BUN of Og-group II vs control group was 23.60 vs 17.90 mg/dl, $p < 0.05$) and serum potassium (serum potassium of Og-group II vs control group was 5.12 vs 4.43 mEq/L $p < 0.05$) were observed in rats receiving *O. grandiflorus* at 4.8 g/kg/day (Figure 8-21). However, these parameters were within or closed to the normal level of normal rats (Table 64; normal range of BUN and serum potassium in normal rats were 5-29 mg/dl and 5.40-7 mEq/L, respectively).

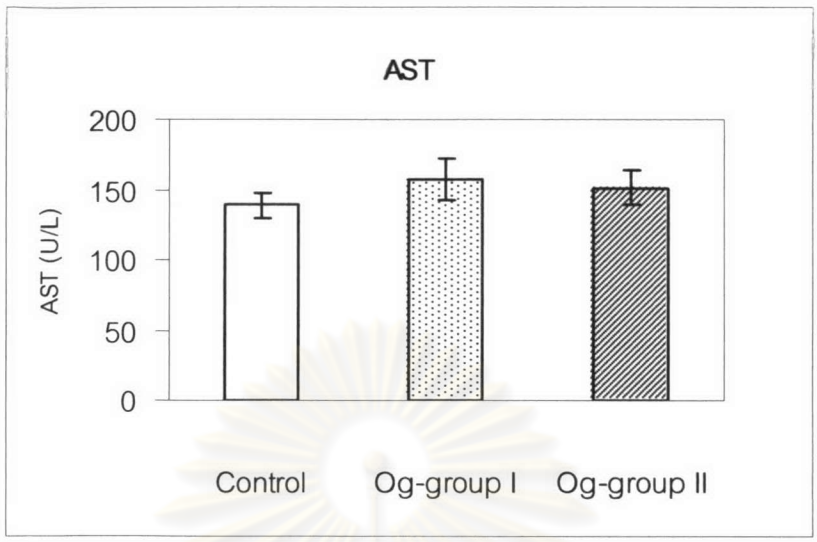


Figure 8 Effect of *O. grandiflorus* aqueous extract on serum AST.

One milliliter/kg/day distilled water (Control), 0.96 and 4.8 g/kg/day of *O. grandiflorus* (Og-group I & Og-group II, respectively) (n = 10) 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 the mean. One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.

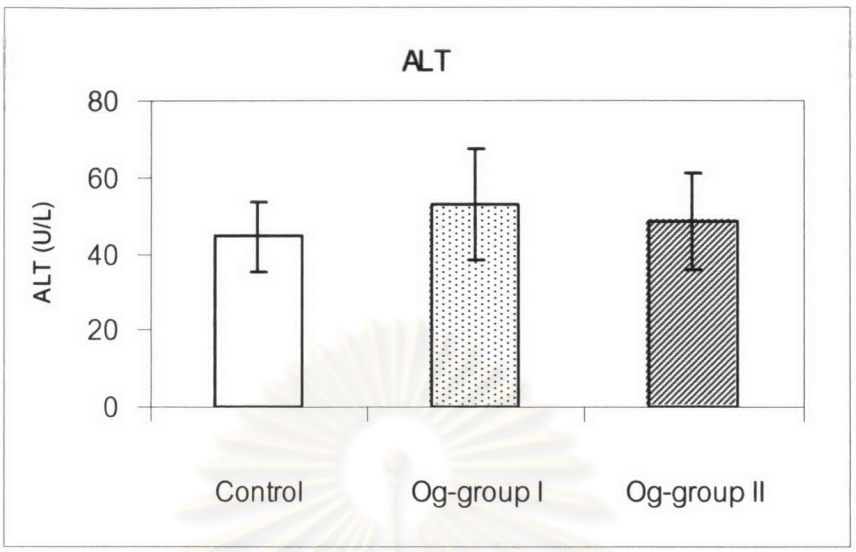


Figure 9 Effect of *O. grandiflorus* aqueous extract on serum ALT.

One milliliter/kg/day distilled water (control), 0.96 and 4.8 g/kg/day of *O. grandiflorus* (Og-group I & Og-group II, respectively) (n = 10) 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 the mean. One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.

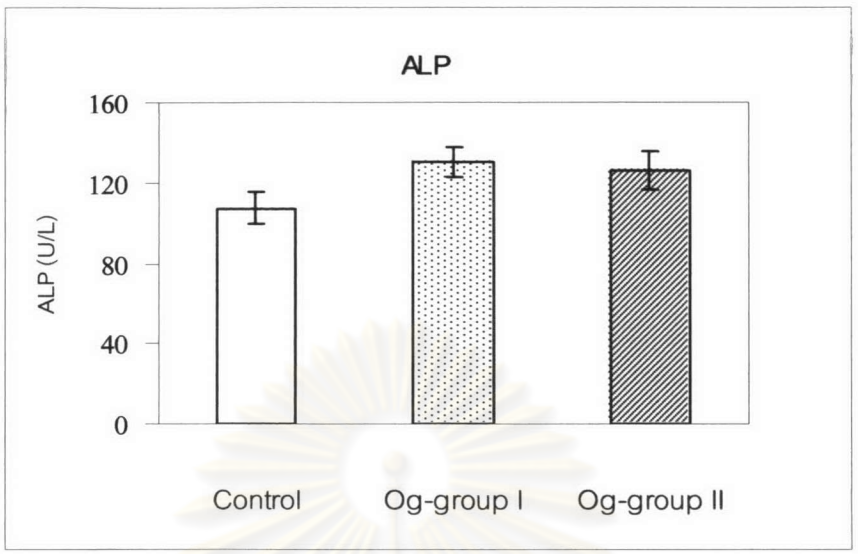
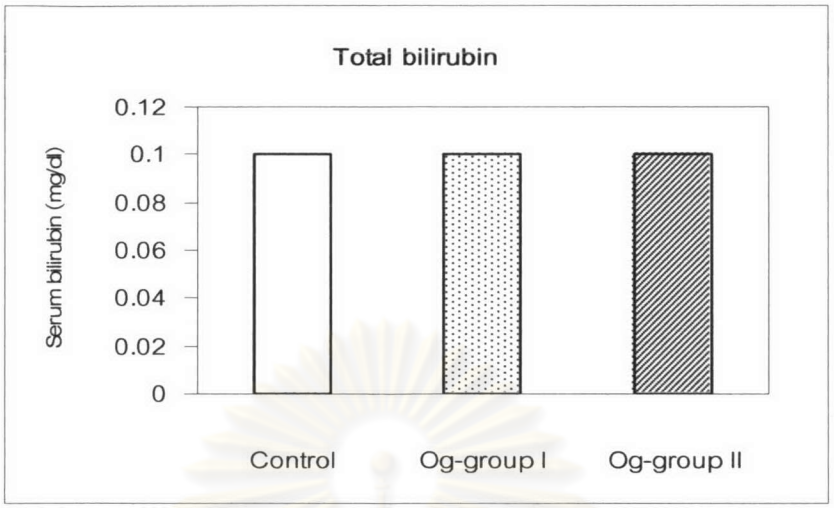
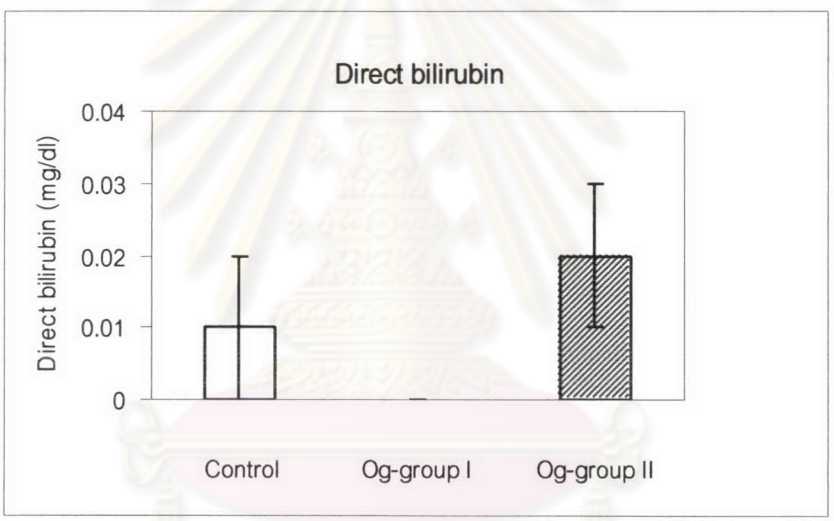


Figure 10 Effect of *O. grandiflorus* aqueous extract on serum ALP.

One milliliter/kg/day distilled water (control), 0.96 and 4.8 g/kg/day of *O. grandiflorus* (Og-group I & Og-group II, respectively) (n = 10) 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 the mean. One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.



(a)



(b)

Figure 11 Effect of *O. grandiflorus* aqueous extract on serum total bilirubin (a) and direct bilirubin (b).

One milliliter/kg/day distilled water (Control), 0.96 and 4.8 g/kg/day of *O. grandiflorus* (Og-group I & Og-group II, respectively) (n = 10) were given orally to rats for 30 days. Serum samples were determined for total bilirubin and direct bilirubin concentrations. The individual bar represented the mean of serum bilirubin with standard error of the mean. One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.

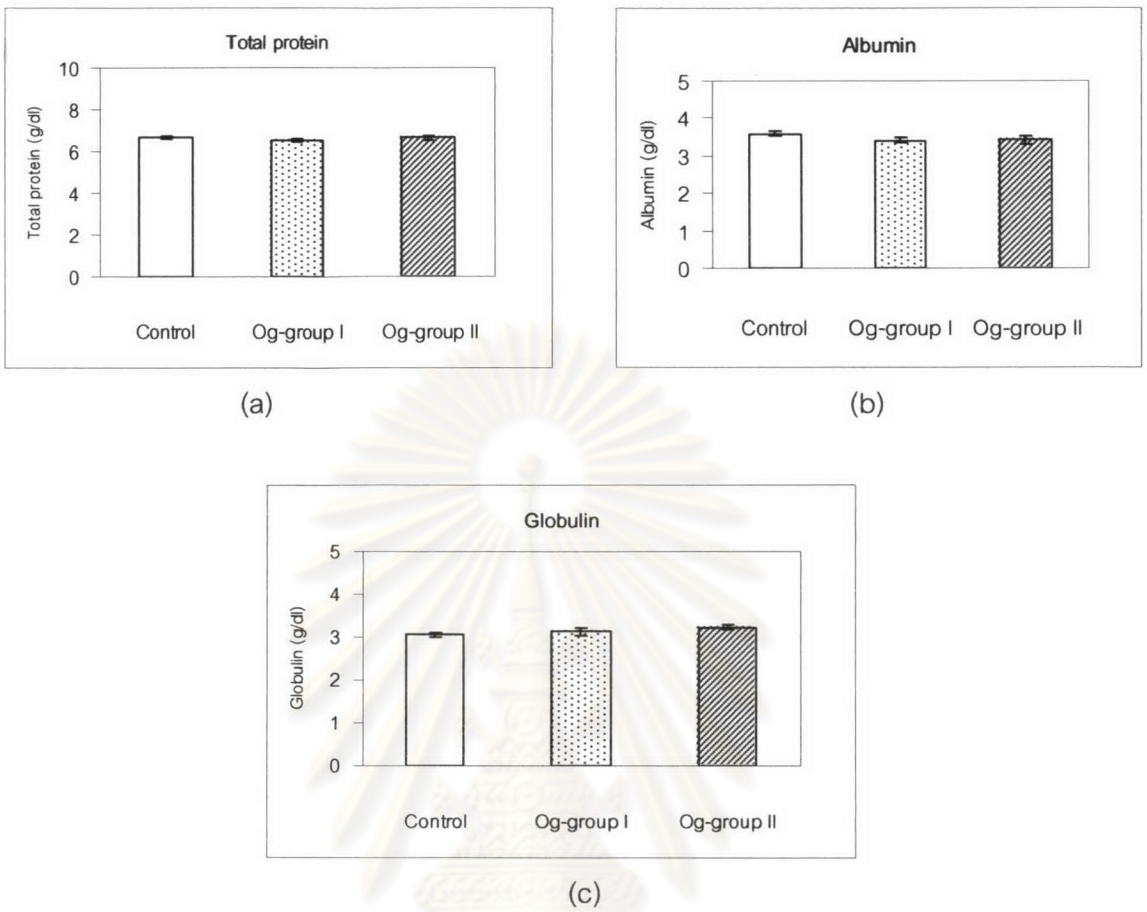
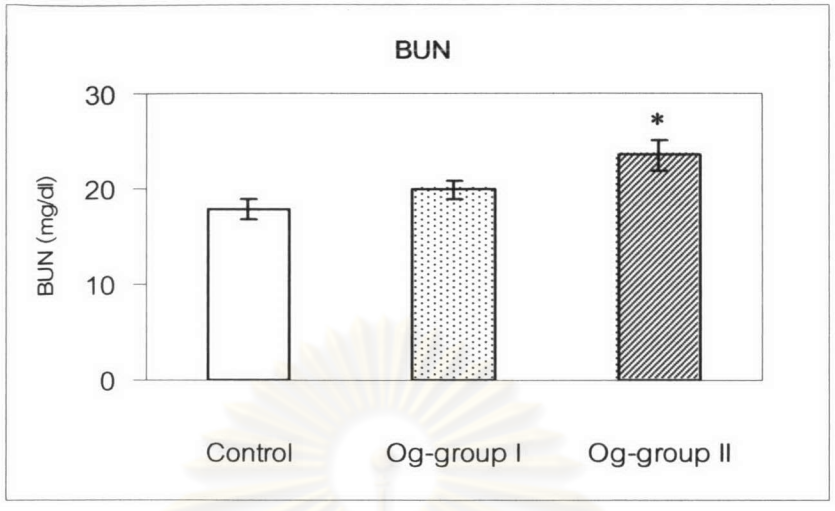
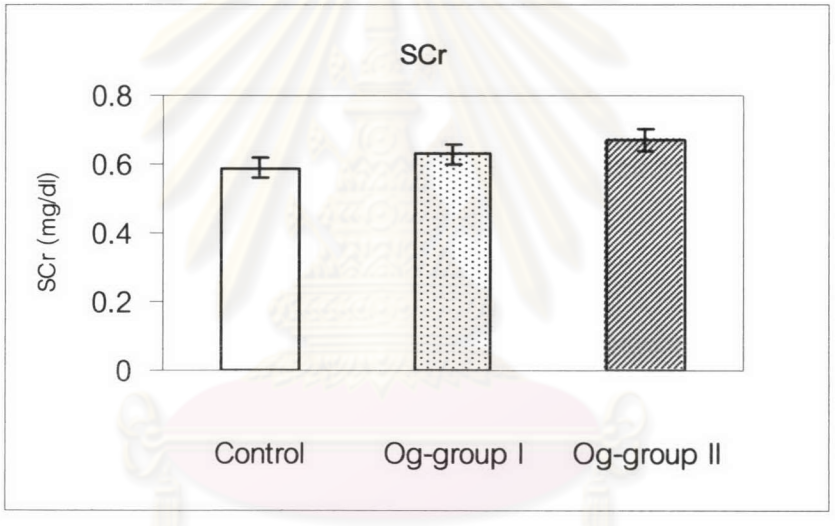


Figure 12 Effect of *O. grandiflorus* aqueous extract on serum total protein (a), albumin (b) and globulin (c).

One milliliter/kg/day distilled water (Control), 0.96 and 4.8 g/kg/day *O. grandiflorus* (Og-group I & Og-group II, respectively) (n = 10) were given orally to rats for 30 days. Serum samples were determined for total protein, albumin and globulin concentrations. The individual bar represented the mean of total protein, albumin and globulin with standard error of the mean. One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.



(a)



(b)

Figure 13 Effect of *O. grandiflorus* aqueous extract on BUN (a) and SCr (b).

One milliliter/kg/day distilled water (Control), 0.96 and 4.8 g/kg/day of *O. grandiflorus* (Og-group I & Og-group II, respectively) (n =10) were given orally to rats for 30 days. Serum samples were determined for BUN and SCr concentrations. The individual bar represented the mean of BUN and SCr with standard error of the mean. One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.

* $p < 0.05$; *O. grandiflorus* group vs control group

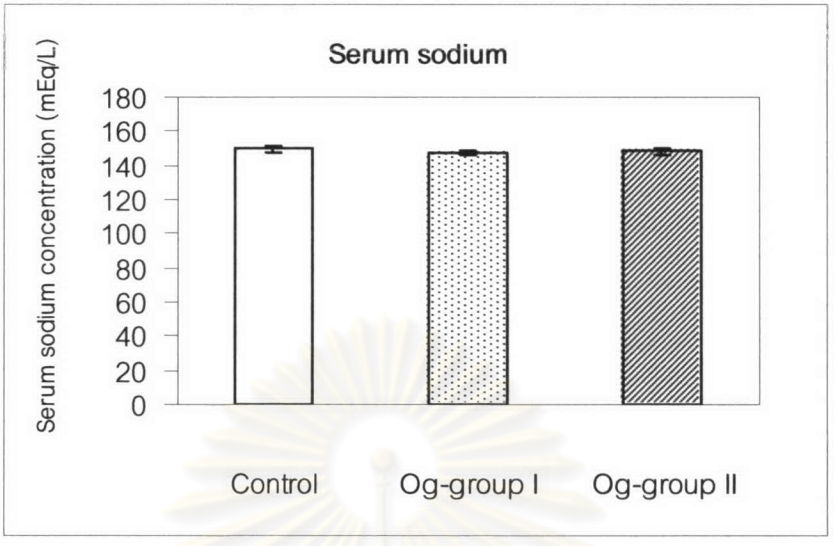


Figure 14 Effect of *O. grandiflorus* aqueous extract on serum sodium concentration.
One milliliter/kg/day distilled water (Control), 0.96 and 4.8 g/kg/day of *O. grandiflorus* (Og-group I & Og-group II, respectively) (n = 10) were given orally to rats for 30 days. Serum samples were determined for sodium concentrations. The individual bar represented the mean of sodium concentrations with standard error of the mean. One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.

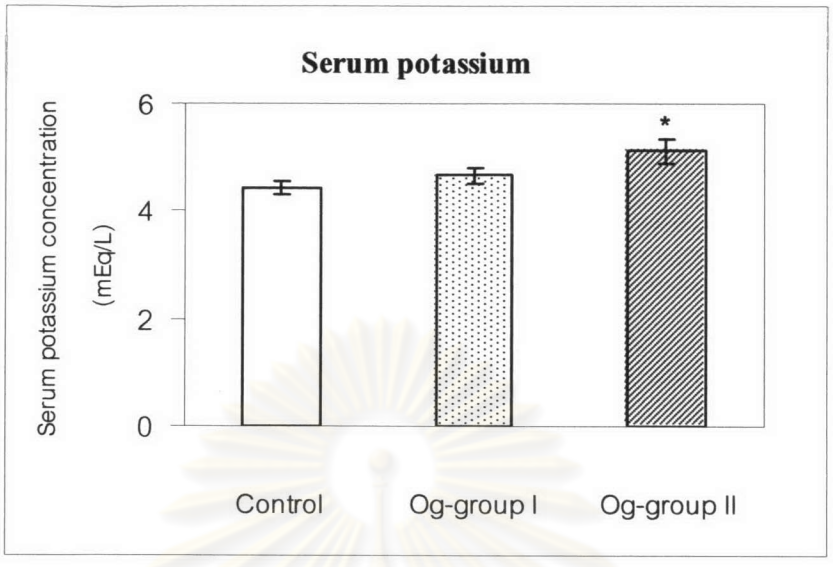


Figure 15 Effect of *O. grandiflorus* aqueous extract on serum potassium concentration. One milliliter/kg/day distilled water (Control), 0.96 and 4.8 g/kg/day of *O. grandiflorus* (Og-group I & Og-group II, respectively) (n = 10) were given orally to rats for 30 days. Serum samples were determined for potassium concentrations. The individual bar represented the mean of potassium concentrations with standard error of the mean. One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.

* $p < 0.05$; *O. grandiflorus* group vs control group

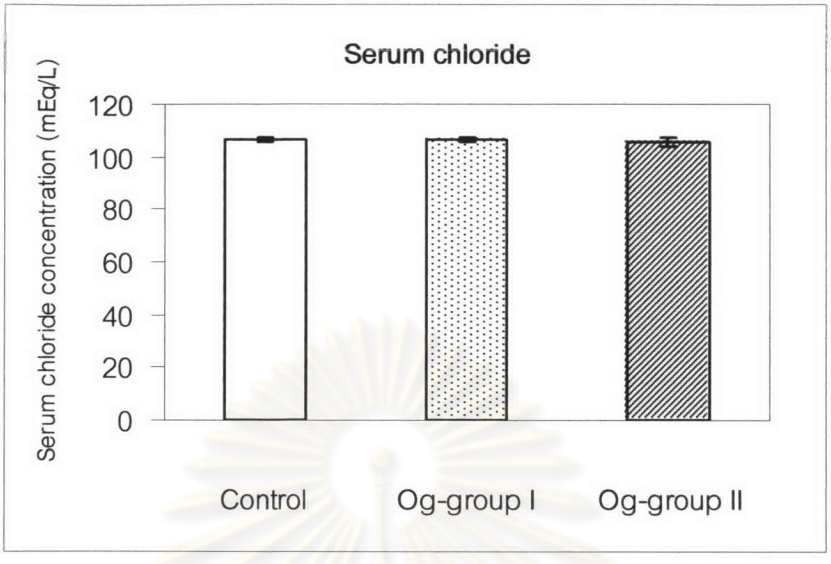


Figure 16 Effect of *O. grandiflorus* aqueous extract on serum chloride concentration. One milliliter/kg/day distilled water (Control), 0.96 and 4.8 g/kg/day of *O. grandiflorus* (Og-group I & Og-group II, respectively) (n = 10) were given orally to rats for 30 days. Serum samples were determined for chloride concentrations. The individual bar represented the mean of chloride concentrations with standard error of the mean. One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.

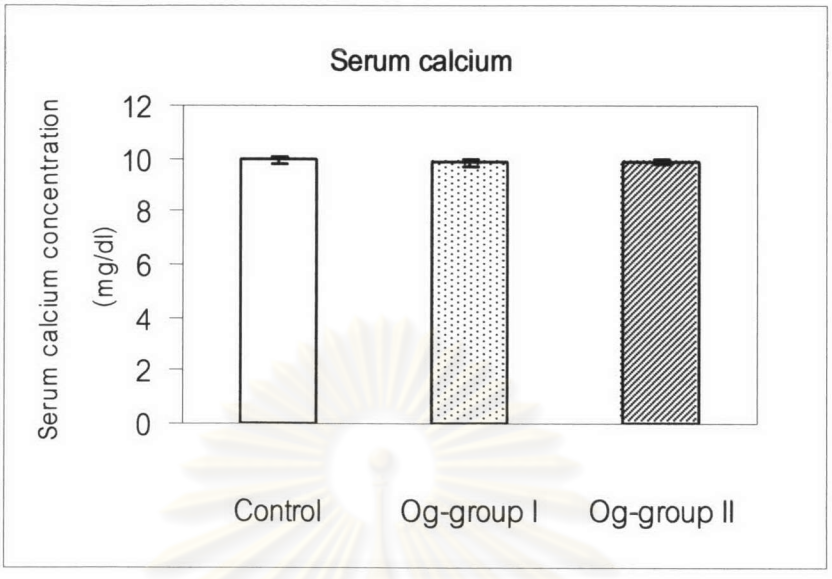


Figure 17 Effect of *O. grandiflorus* aqueous extract on serum calcium concentration. One milliliter/kg/day distilled water (Control), 0.96 and 4.8 g/kg/day of *O. grandiflorus* (Og-group I & Og-group II, respectively) (n = 10) were given orally to rats for 30 days. Serum samples were determined for calcium concentrations. The individual bar represented the mean of calcium concentrations with standard error of the mean. One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.

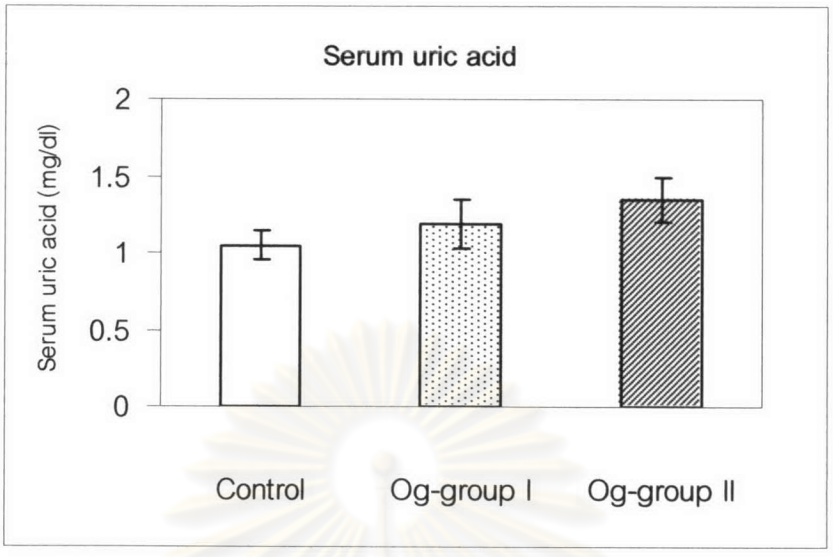
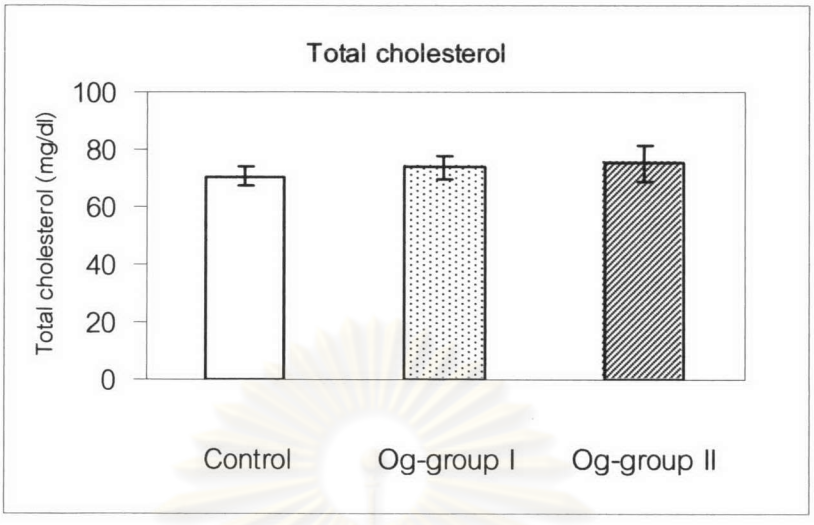


Figure 18 Effect of *O. grandiflorus* aqueous extract on serum uric acid.

One milliliter/kg/day distilled water (Control), 0.96 and 4.8 g/kg/day of *O. grandiflorus* (Og-group I & Og-group II, respectively) (n = 10) 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 the mean. 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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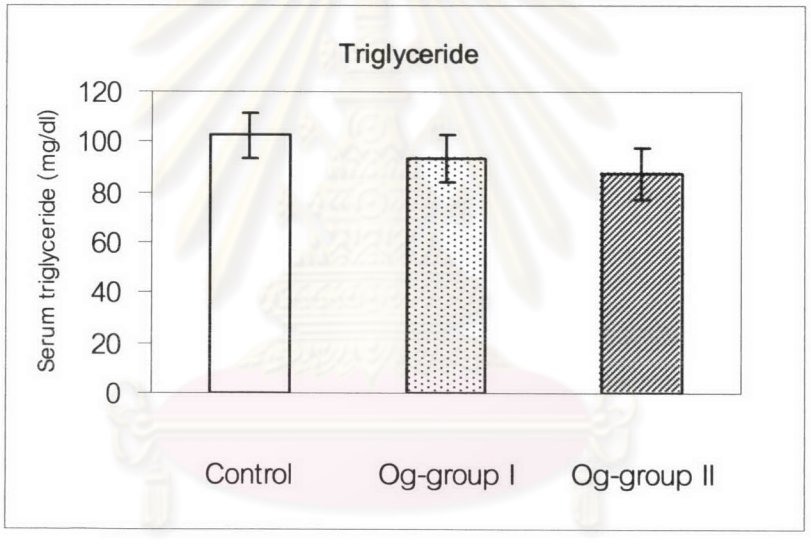
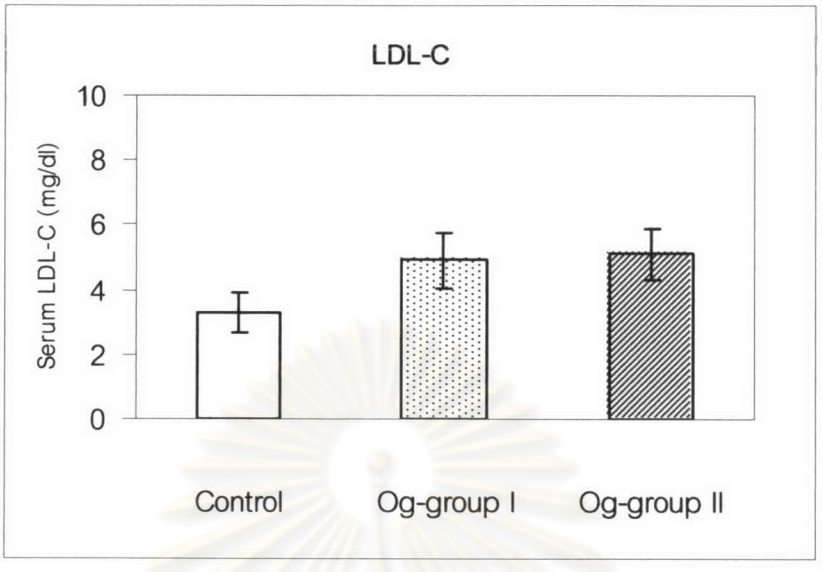


(b)

Figure 19 Effect of *O. grandiflorus* aqueous extract on serum total cholesterol (a) and triglyceride (b).

One milliliter/kg/day distilled water (Control), 0.96 and 4.8 g/kg/day of *O. grandiflorus* (Og-group I & Og-group II, respectively) (n = 10) were given orally to rats for 30 days. Serum samples were determined for total cholesterol and triglyceride concentrations. The individual bar represented the mean of total cholesterol and triglyceride concentrations with standard error of the mean. 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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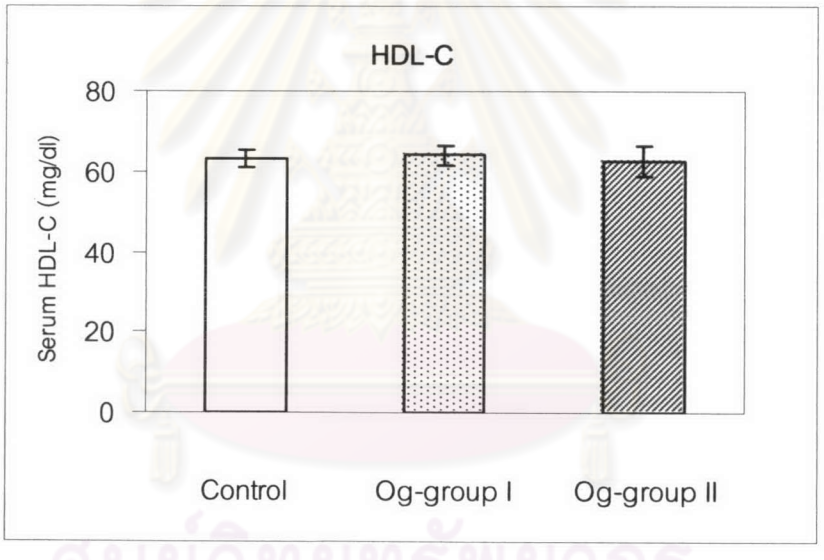


(b)

Figure 20 Effect of *O. grandiflorus* aqueous extract on serum LDL-C (a) and HDL-C (b). One milliliter/kg/day distilled water (Control, n = 10), 0.96 and 4.8 g/kg/day of *O. grandiflorus* (Og-group I, n = 10 & Og-group II, n = 9, respectively) were given orally to rats for 30 days. Serum samples were determined for LDL-C and HDL-C concentrations. The individual bar represented the mean of LDL-C and HDL-C concentrations with standard error of the mean. One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.

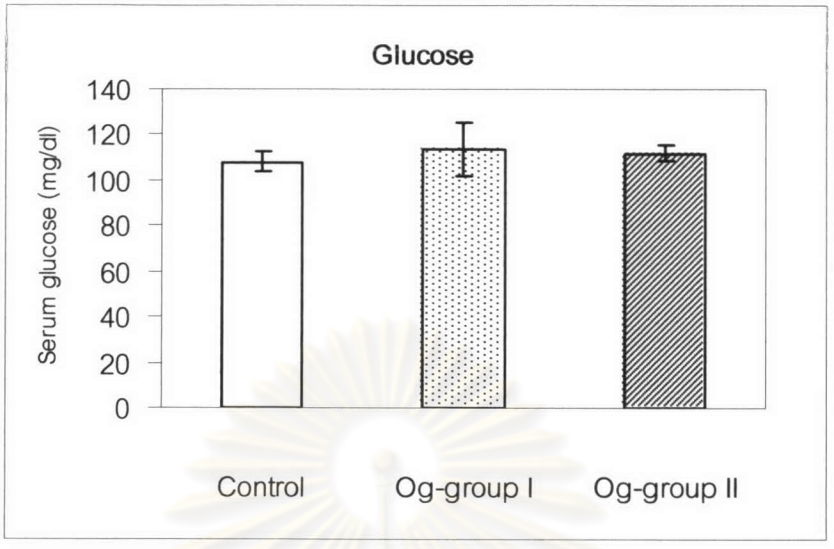
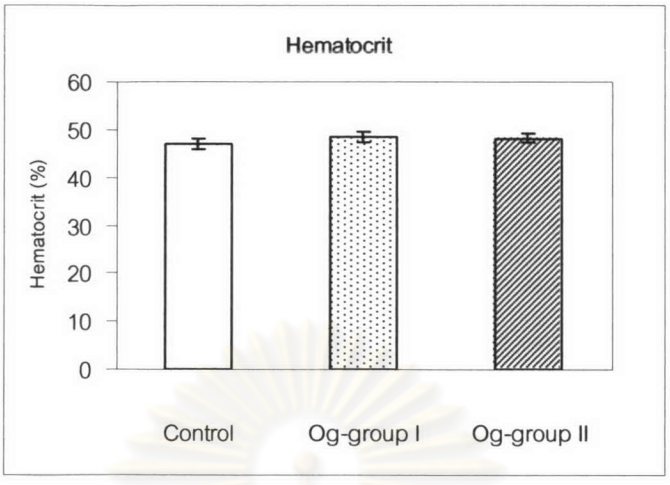


Figure 21 Effect of *O. grandiflorus* aqueous extract on serum glucose.

One milliliter/kg/day distilled water (Control), 0.96 and 4.8 g/kg/day of *O. grandiflorus* (Og-group I & Og-group II, respectively) (n = 10) were given orally to rats for 30 days. Serum samples were determined for glucose concentrations. The individual bar represented the mean of glucose concentrations with standard error of the mean. One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.

3.2.2 Effect of *O. grandiflorus* aqueous extract on hematology

O. grandiflorus aqueous extract at both doses (0.96 and 4.8 g/kg/day) used in this study, did not affect these following hematological parameters: hematocrit, hemoglobin, platelet count, WBC count, RBC count and RBC indices and RBC morphology (Figure 22-25). Significant increase of PMN (PMN of Og-group II vs control group was 40.60 vs 23.60%, $p < 0.05$) but decrease of lymphocyte (lymphocyte of Og-group II vs control group was 55.20 vs 71.50%, $p < 0.05$) were found in rats given *O. grandiflorus* at 4.8 g/kg/day as compared to the control rats. However, these parameters were within or closed to the normal level of normal rats (Table 64, normal range of PMN and lymphocyte in normal rats were 9-34% and 65-84.5%, respectively). Another differential WBC; monocyte and eosinophil were not affected by *O. grandiflorus* administration (Figure 26).



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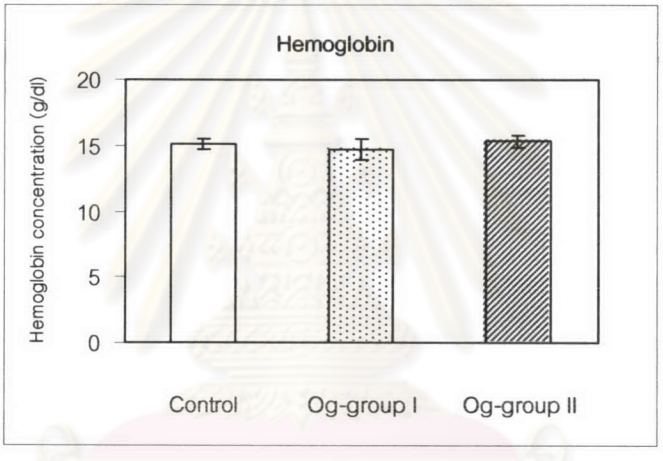
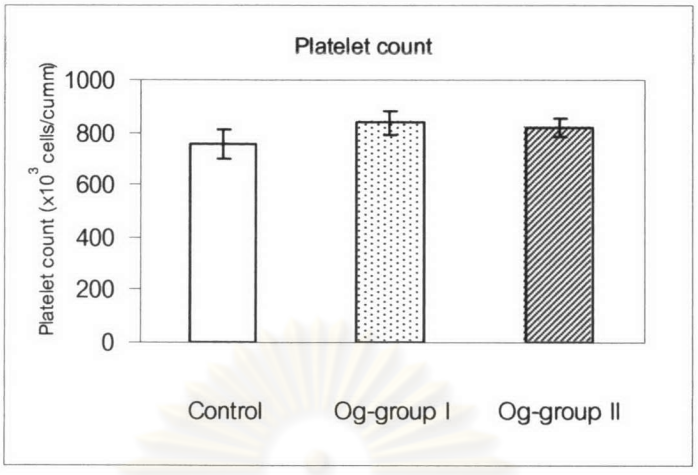


(b)

Figure 22 Effect of *O. grandiflorus* aqueous extract on serum hematocrit (a) and hemoglobin (b).

One milliliter/kg/day distilled water (Control, n = 10), 0.96 and 4.8 g/kg/day of *O. grandiflorus* (Og-group I, n = 9 & Og-group II, n = 10, respectively) were given orally to rats for 30 days. Blood samples were determined for hematocrit and hemoglobin concentrations. The individual bar represented the mean of hematocrit and hemoglobin concentrations with standard error of the mean. 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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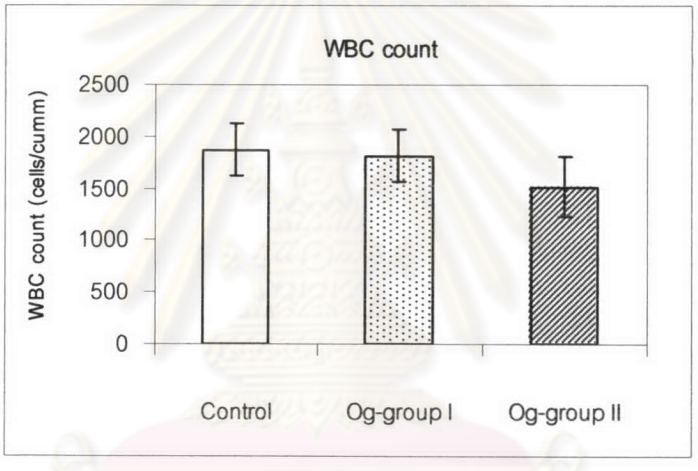

(b)

Figure 23 Effect of *O. grandiflorus* aqueous extract on platelet count (a) and WBC count (b).

One milliliter/kg/day distilled water (Control, n = 10), 0.96 and 4.8 g/kg/day of *O. grandiflorus* (Og-group I, n = 9 & Og-group II, n = 10, respectively) were given orally to rats for 30 days. Blood samples were determined for platelet count and WBC count concentrations. The individual bar represented the mean of platelet count and WBC count concentrations with standard error of the mean. One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.

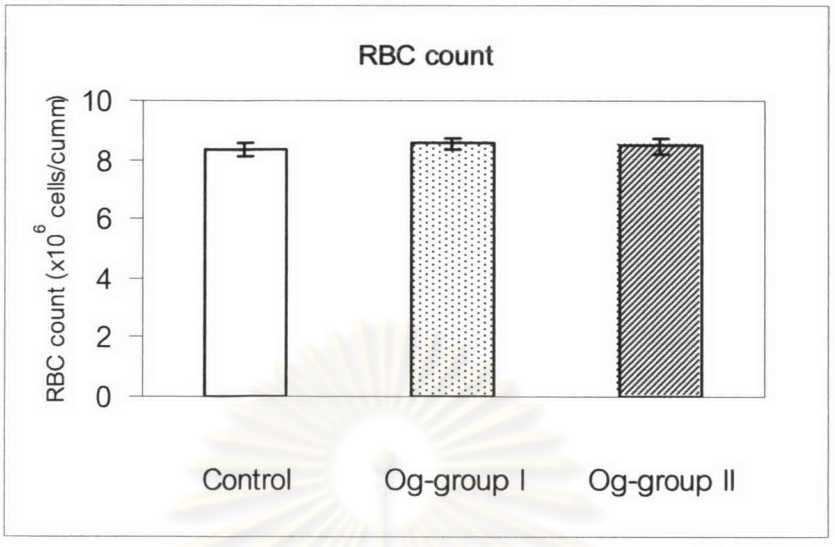

Figure 24 Effect of *O. grandiflorus* aqueous extract on RBC count.

One milliliter/kg/day distilled water (Control, n = 10), 0.96 and 4.8 g/kg/day of *O. grandiflorus* (Og-group I, n = 9 & Og-group II, n = 10, respectively) were given orally to rats for 30 days. Blood samples were determined for RBC count concentrations. The individual bar represented the mean of RBC count concentrations with standard error of the mean. One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.

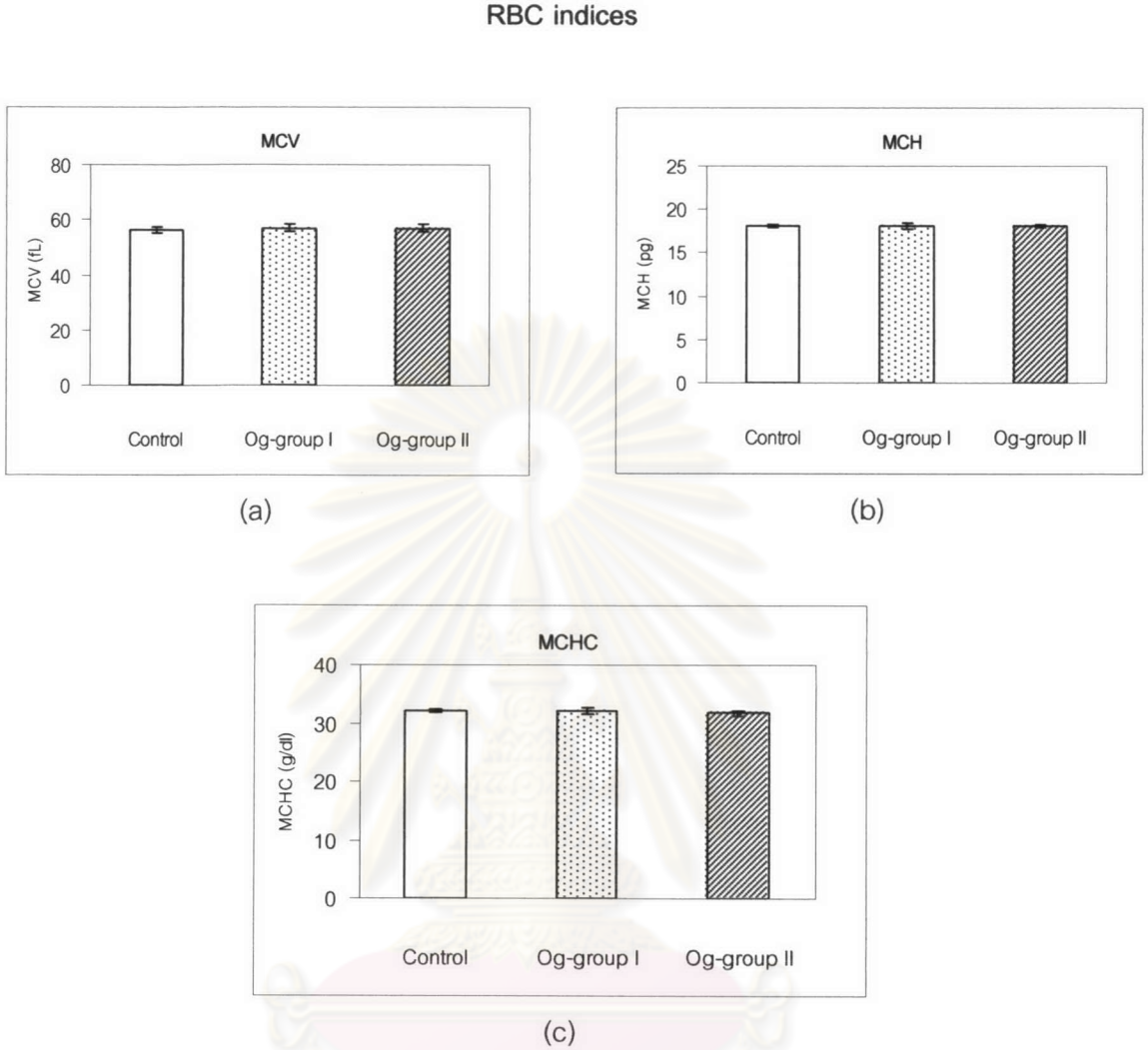

Figure 25 Effect of *O. grandiflorus* aqueous extract on RBC indices.

One milliliter/kg/day distilled water (Control, n = 10), 0.96 and 4.8 g/kg/day of *O. grandiflorus* (Og-group I, n = 9 & Og-group II, n = 10, respectively) were given orally to rats for 30 days. Blood samples were determined for RBC indices. The individual bar represented the mean of RBC indices that includes MCV, MCH and MCHC with standard error of the mean. One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.

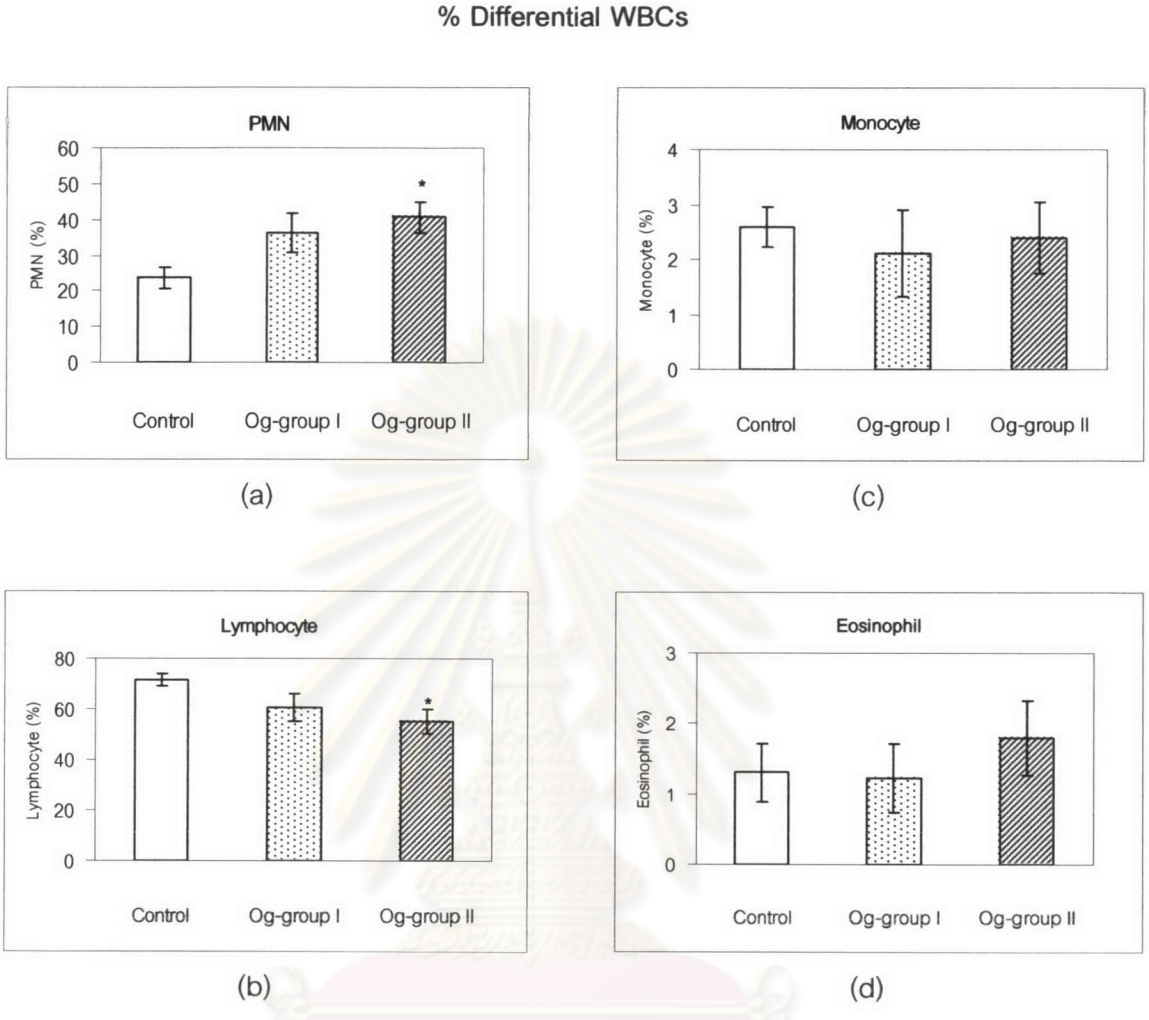

Figure 26 Effect of *O. grandiflorus* aqueous extract on % differential WBCs.

One milliliter/kg/day distilled water (Control, n = 10), 0.96 and 4.8 g/kg/day of *O. grandiflorus* (Og-group I, n = 9 & Og-group II, n = 10, 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 includes PMN, lymphocyte, monocyte and eosinophil with standard error of the mean. One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.

* $p < 0.05$; *O. grandiflorus* group vs control group

3.3 Effect of *O. grandiflorus* aqueous extract on hepatic microsomal CYPs

O. grandiflorus aqueous extract caused a decrease of total CYP contents when giving orally to rats at 4.8 g/kg/day for 30 days (Figure 27). However, no effects on the individual CYP activities such as CYP1A1, 1A2, 2B1/2, 2E1 and 3A were found in both *O. grandiflorus* aqueous extract treated groups (Figure 28-33).

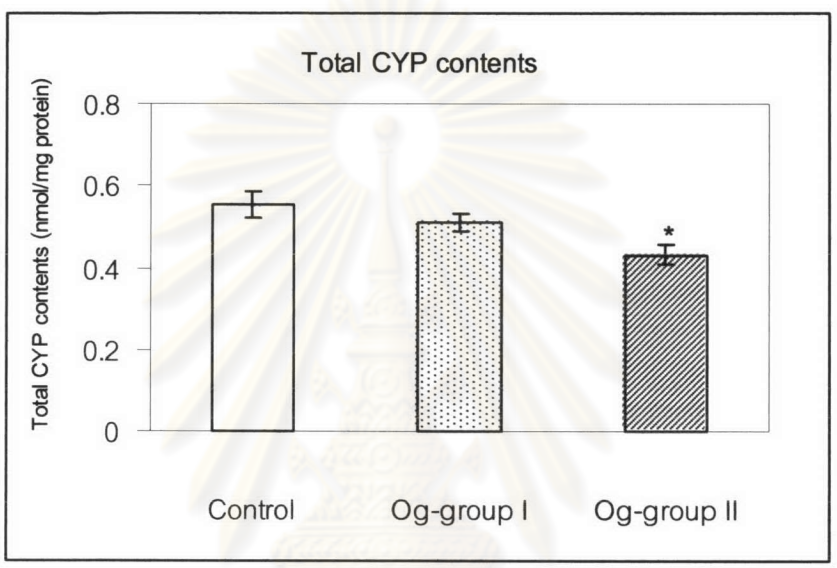

Figure 27 Effect of *O. grandiflorus* aqueous extract on rat hepatic total CYP contents. One milliliter/kg/day distilled water, 0.96 and 4.8 g/kg/day of *O. grandiflorus* (Og-group I & Og-group II, respectively) (n = 10) were given orally to rats for 30 days. Liver microsomes were determined for total CYP contents. The individual bar represented the mean of total CYP contents with standard error of the mean. One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.

* $p < 0.05$; *O. grandiflorus* group vs control group

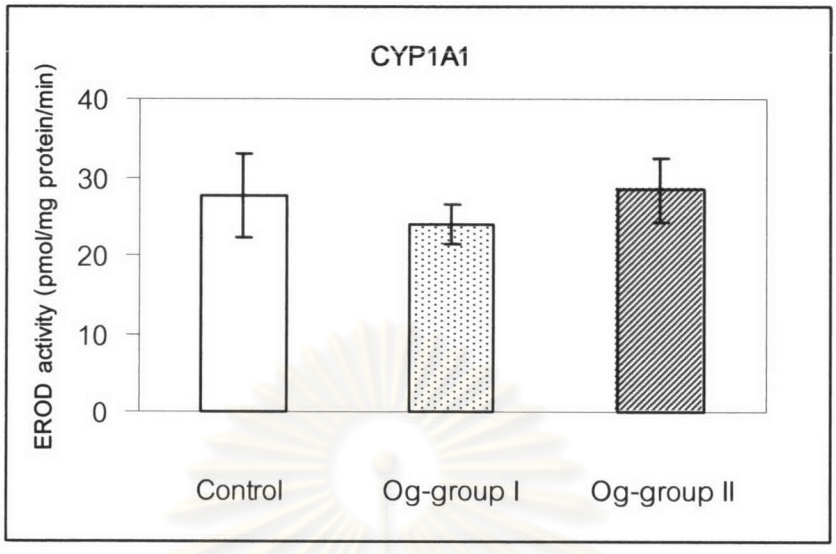

Figure 28 Effect of *O. grandiflorus* aqueous extract on rat hepatic CYP1A1 activity.
One milliliter/kg/day distilled water, 0.96 and 4.8 g/kg/day of *O. grandiflorus* (Og-group I & Og-group II, respectively) (n = 10) were given orally to rats for 30 days. Liver microsomes were determined for EROD activity (CYP1A1 activity). The individual bar represented the mean of EROD activity with standard error of the mean. One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.

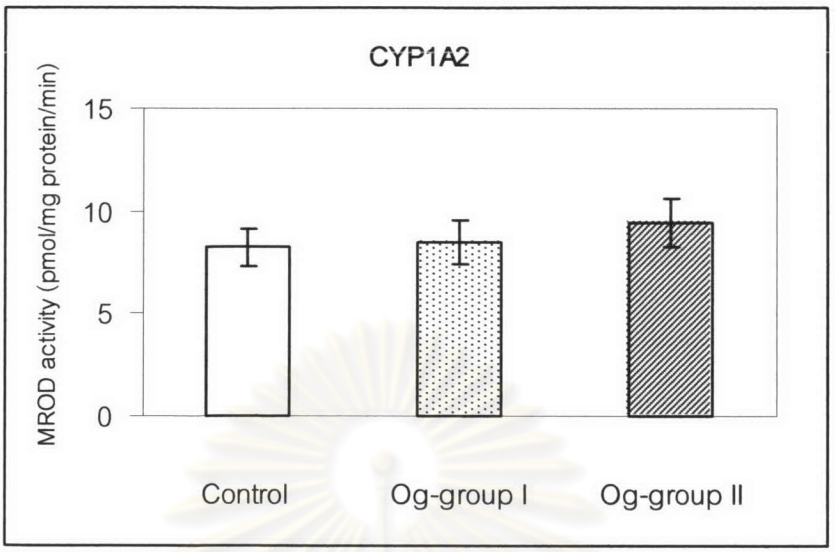

Figure 29 Effect of *O. grandiflorus* aqueous extract on rat hepatic CYP1A2 activity.
One milliliter/kg/day distilled water, 0.96 and 4.8 g/kg/day of *O. grandiflorus* (Og-group I & Og-group II, respectively) (n = 10) were given orally to rats for 30 days. Liver microsomes were determined for MROD activity (CYP1A2 activity). The individual bar represented the mean of MROD activity with standard error of the mean. One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.

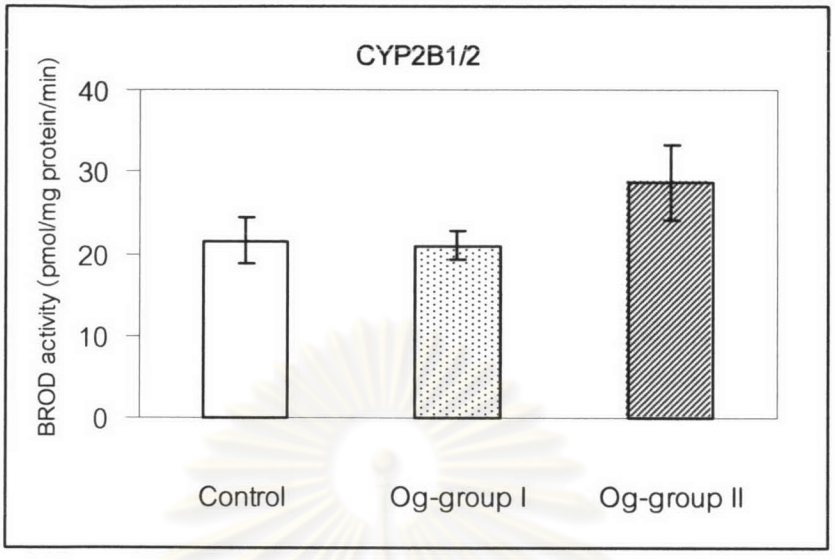

Figure 30 Effect of *O. grandiflorus* aqueous extract on rat hepatic CYP2B1/2 (BROD) activity.

One milliliter/kg/day distilled water, 0.96 and 4.8 g/kg/day of *O. grandiflorus* (Og-group I & Og-group II, respectively) (n = 10) were given orally to rats for 30 days. Liver microsomes were determined for BROD activity (CYP2B1/2 activity). The individual bar represented the mean of BROD activity with standard error of the mean. One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.

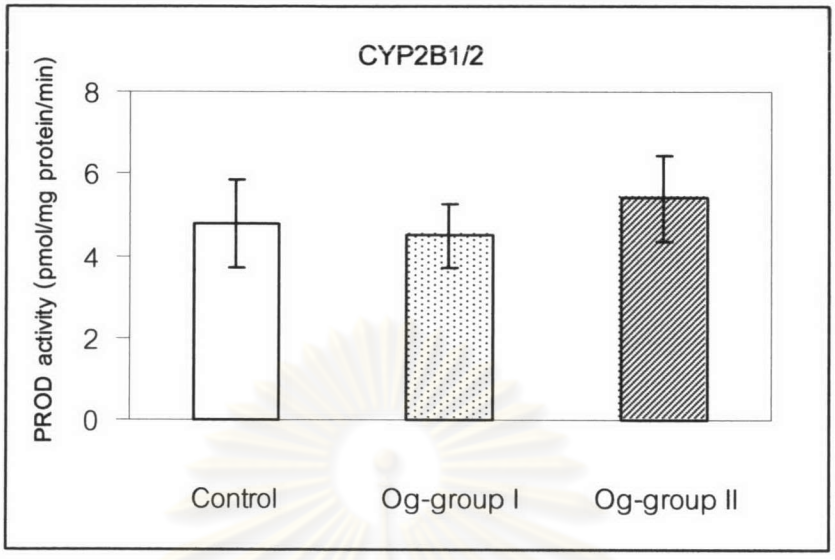

Figure 31 Effect of *O. grandiflorus* aqueous extract on rat hepatic CYP2B1/2 (PROD) activity.

One milliliter/kg/day distilled water, 0.96 and 4.8 g/kg/day of *O. grandiflorus* (Og-group I & Og-group II, respectively) (n = 10) were given orally to rats for 30 days. Liver microsomes were determined for PROD activity (CYP2B1/2 activity). The individual bar represented the mean of PROD activity with standard error of the mean. One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.

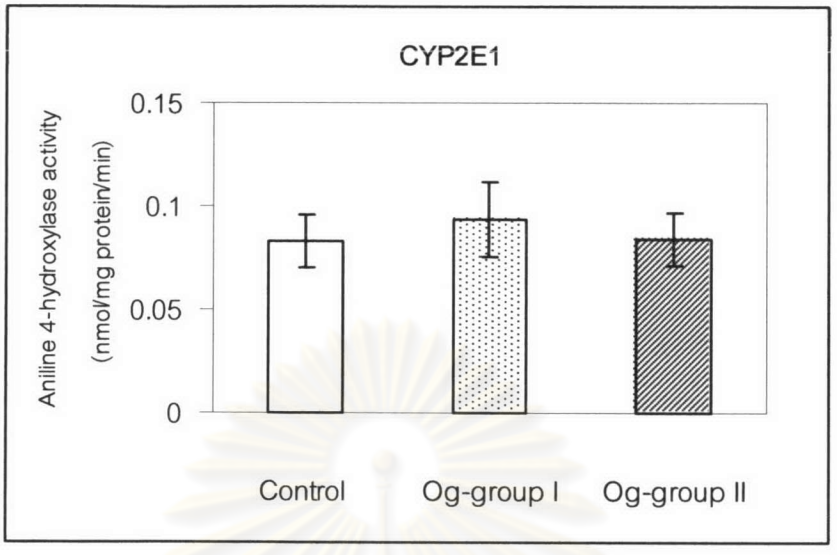

Figure 32 Effect of *O. grandiflorus* aqueous extract on rat hepatic CYP2E1 activity.
One milliliter/kg/day distilled water, 0.96 and 4.8 g/kg/day of *O. grandiflorus* (Og-group I & Og-group II, respectively) (n = 10) were given orally to rats for 30 days. Liver microsomes were determined for aniline-4-hydroxylase activity (CYP2E1 activity). The individual bar represented the mean of aniline-4-hydroxylase activity with standard error of the mean. One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.

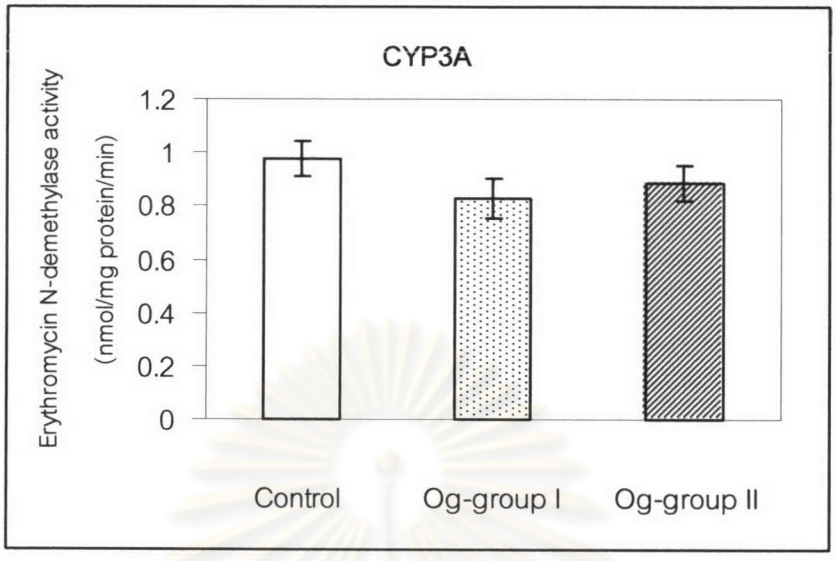

Figure 33 Effect of *O. grandiflorus* aqueous extract on rat hepatic CYP3A activity.

One milliliter/kg/day distilled water, 0.96 and 4.8 g/kg/day of *O. grandiflorus* (Og-group I & Og-group II, respectively) (n = 10) were given orally to rats for 30 days. Liver microsomes were determined for erythromycin *N*-demethylase activity (CYP3A activity). The individual bar represented the mean of erythromycin *N*-demethylase activity with standard error of the mean. One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p < 0.05$.