CHAPTER IV

RESULTS

1. Effect of *C. comosa* extracts on body weight, food and water consumption and liver weight.

Rats were given orally with *C. comosa* hexane extract or ethanolic extract at the doses of 250 and 500 mg/kg/day once daily for 30 consecutive days. At the beginning of the experiment, body weights of all rats were recorded (Table 13). During the administration of *C. comosa*, body weights as well as food and water consumption were recorded every week. At the time of sacrification, rat livers were weighed before the preparation of liver microsomes (Table 13).

Both dosage regimens of *C. comosa* hexane and ethanolic extracts mostly caused significant decreases of body weight gain as compared to the control group. Rats given *C. comosa* at 250 mg/kg/day showed significant decrease of body weight gain only at the 28th day following the extract administration whereas rats in the remaining groups showed significant decrease of body weight gain at the day 14th, 21st and 28th of *C. comosa* administration (Figure 5). However, no difference of food and water consumption were shown in all *C. comosa* treatment groups as compared to the control group (Figure 6).

Significant increases of % relative liver weight were observed in rats given *C. comosa* hexane extract at both doses of 250 and 500 mg/kg/day (Table 13).

Table 13 Effects of *C. comosa* extracts on body weight, liver weight and % relative liver weight

Treatment group	Initial body weight [a] (g)	Final body weight [b] (g)	Liver weight [c] (g)	% relative liver weight (g/100g of body weight)
Control group	322.68 ± 9.39	384.09 ± 11.03	11.593 ± 0.66	3.01 ± 0.14
C.comosa 1	298.78 ± 7.44	320.28 ± 9.55	12.878 ± 0.92	4.03 ± 0.29 *
C.comosa 2	321.47 ± 9.11	331.71 ± 10.39	13.91 ± 0.65	4.19 ± 0.12 *
C.comosa 3	310.83 ± 7.29	348.56 ± 7.39	11.241 ± 0.41	3.22 ± 0.09
C.comosa 4	318.79 ± 7.70	350.98 ± 9.21	12.05 ± 0.48	3.38 ± 0.11

Data shown were mean ± SEM (n = 9-10)

[a] Body weight of rats at the beginning of *C. comosa* extracts administration

[b] Body weight of rats at the time of sacrification

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

* $p<0.05$; *C. comosa* group vs control group

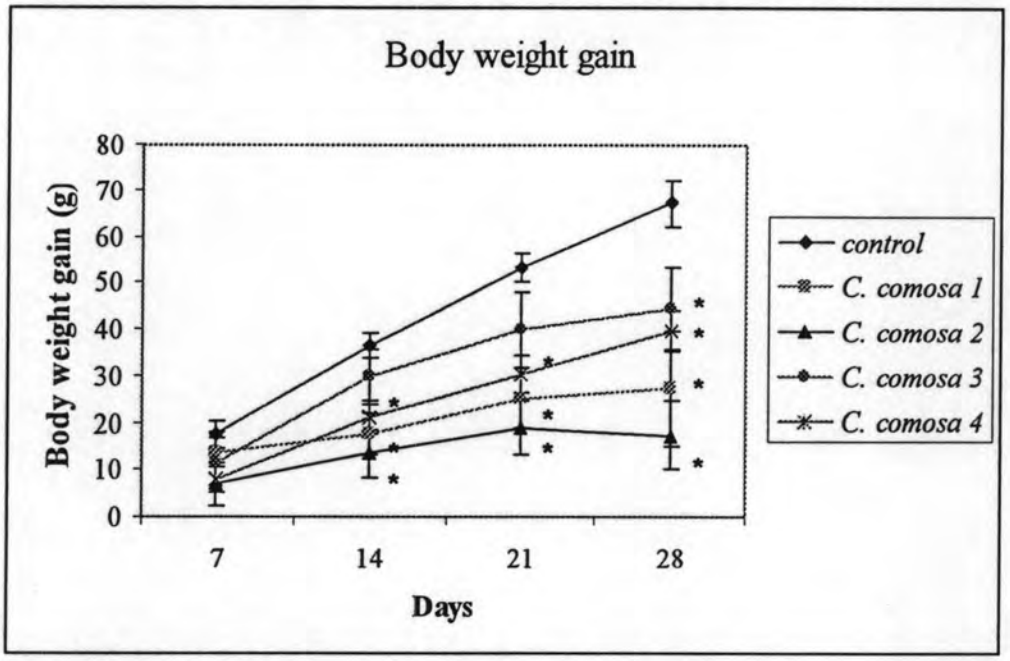
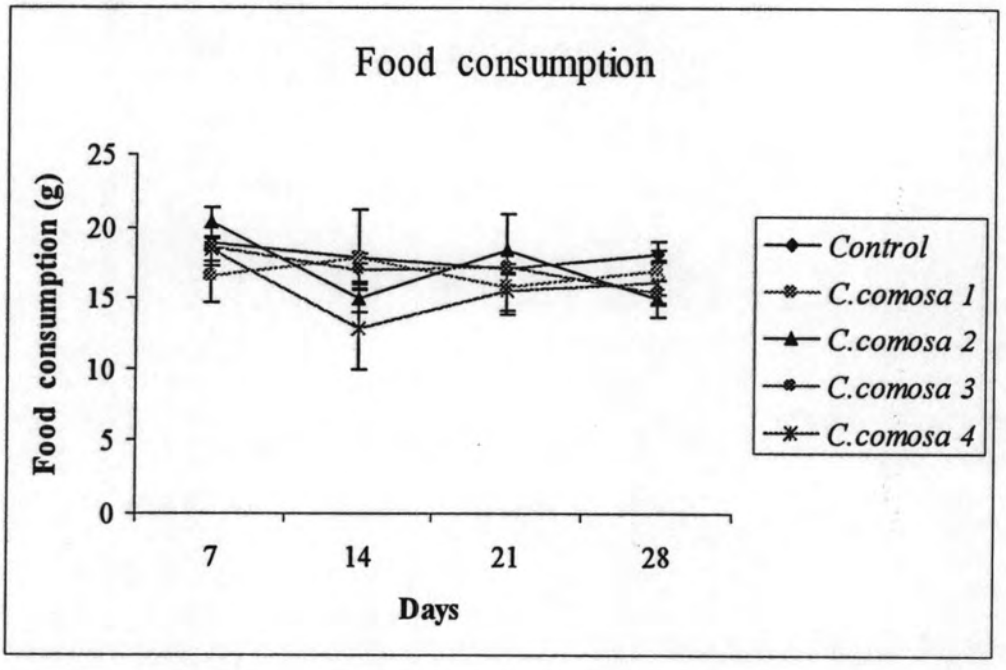


Figure 5 Effect of *C. comosa* extracts on body weight gain

Data shown were mean ± SEM (n=9-10)

* $p<0.05$; *C. comosa* group vs control group

A)



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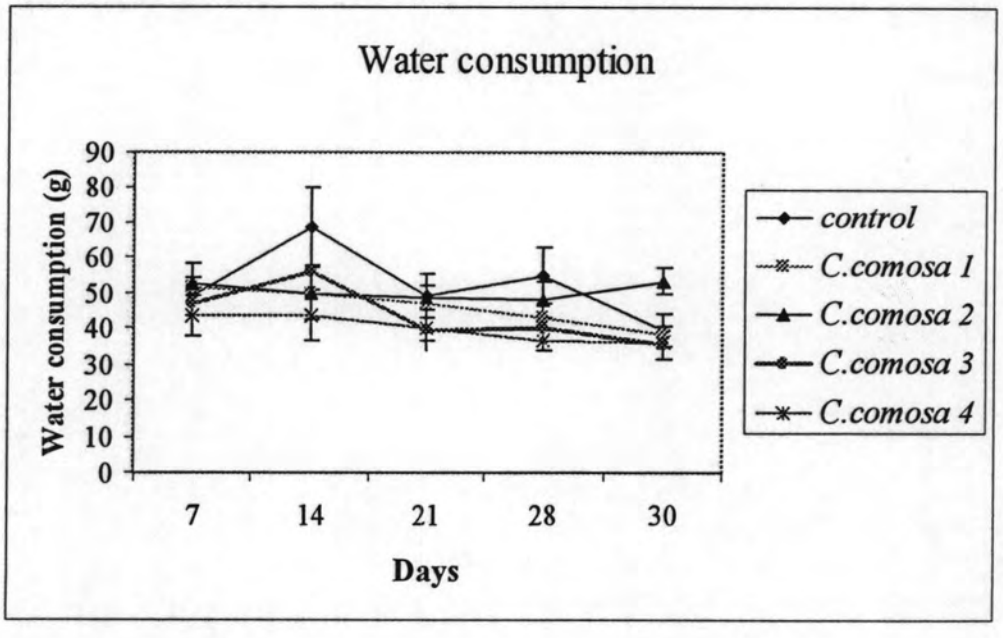


Figure 6 Effect of *C. comosa* extracts on food (A) and water (B) consumption
Data shown were mean ± SEM (n = 9-10)

2. Effects of *C. comosa* extracts on clinical blood chemistry and hematology

Both doses of *C. comosa* hexane extract and ethanolic extract did not cause significant effects on these following clinical blood chemistry parameters: sugar, BUN, creatinine, LDL-C, SGOT, SGPT, ALP, albumin, total bilirubin and direct bilirubin. Total cholesterol and HDL-C level were significantly decreased in rats treated with *C. comosa* hexane extract at the dose of 500 mg/kg/day. Serum triglyceride were increased when *C. comosa* hexane extract was given to rats at the dose of 250 mg/kg/day but the level was decreased when the extract was given to rats at 500 mg/kg/day. Both doses of *C. comosa* ethanolic extracts caused a significant decrease of serum triglyceride. Significant increase of total protein was observed only when *C. comosa* hexane extract was given at 500 mg/kg/day (Table 14).

No effects of *C. comosa* hexane extract and ethanolic extract on the hematological parameters were observed. These parameters included hematocrit, hemoglobin, RBC count, RBC indices (% mean corpuscular volume, % MCV; % mean corpuscular hemoglobin, % MCH; % mean corpuscular hemoglobin concentration; % MCHC) RBC morphology, platelet count, WBC count and % differential WBC (Table 14).

Table 14 Effects of *C. comosa* extracts on clinical blood chemistry and hematology

Clinical blood chemistry	Control group	*C. comosa* 1	*C. comosa* 2	*C. comosa* 3	*C. comosa* 4
Sugar (mg/dl)	133.89 ± 11.67	120.75 ± 7.94	122.33 ± 11.47	124.44 ± 11.57	109.50 ± 10.58
BUN (mg/dl)	22.22 ± 1.79	27.57 ± 3.08	21.00 ± 1.06	26.00 ± 2.01	26.71 ± 3.30
Creatinine (mg/dl)	0.622 ± 0.04	0.600 ± 0.04	0.700 ± 0.04	0.644 ± 0.03	0.614 ± 0.04
Total cholesterol (mg/dl)	75.33 ± 2.79	62.29 ± 4.70	38.00 ± 4.11*↓	65.56 ± 4.12	70.86 ± 7.18
Triglyceride (mg/dl)	115.44 ± 10.95	145.57 ± 26.37*↑	77.33 ± 8.26*↓	56.11 ± 4.64*↓	59.86 ± 9.65*↓
HDL-C (mg/dl)	65.44 ± 2.46	50.14 ± 5.65	32.00 ± 3.61*↓	60.44 ± 3.83	63.86 ± 6.00
LDL-C (mg/dl)	3.11 ± 0.48	2.00 ± 0.49	1.67 ± 0.33	4.44 ± 0.99	4.43 ± 1.07
AST (U/L)	145.56 ± 11.43	132.29 ± 22.68	147.00 ± 24.43	138.56 ± 7.13	147.57 ± 14.35
ALT (U/L)	33.33 ± 0.94	42.00 ± 10.56	34.00 ± 4.42	38.33 ± 3.44	40.86 ± 6.64
ALP (U/L)	114.56 ± 13.24	133.29 ± 10.12	117.50 ± 14.82	119.56 ± 8.38	132.71 ± 16.10
Total protein (g/dl)	6.40 ± 0.05	6.80 ± 0.169	6.93 ± 0.06*↑	6.467± 0.11	6.529 ± 0.182
Albumin (g/dl)	3.54 ± 0.06	3.843 ± 0.127	3.783 ± 0.149	3.578 ± 0.106	3.543 ± 0.132

Table 14 (con't) Effects of *C. comosa* extracts on clinical blood chemistry and hematology

Clinical blood chemistry	Control group	*C. comosa* 1	*C. comosa* 2	*C. comosa* 3	*C. comosa* 4
Total bilirubin (mg/dl)	0.12 ± 0.11	0.02 ± 0.02	0.18 ± 0.02	0.13 ± 0.05	0.03 ± 0.02
Direct bilirubin (mg/dl)	0.01 ± 0.01	0.01 ± 0.01	0.12 ± 0.04	0.11 ± 0.04	0.02 ± 0.01
Hematology	Control group	*C. comosa* 1	*C. comosa* 2	*C. comosa* 3	*C. comosa* 4
WBC count (cell/cumm)	2868.89±411.54	2638.75±326.21	2653.33±302.60	2651.11±242.49	2613.75±418.95
RBC count (10^6 cell/cumm)	7.661 ± 0.12	8.074 ± 0.15	7.657 ± 0.13	7.9589 ± 0.14	7.7963 ± 0.13
Hemoglobin (g/dl)	15.17 ± 0.25	15.06 ± 0.17	14.35 ± 0.29	15.22 ± 0.18	15.00 ± 0.19
Hematocrit (%)t	48.00 ± 0.73	49.00 ± 0.87	47.17 ± 6.81	48.33 ± 0.65	46.75 ± 0.67
% MCV (fL)	62.44 ± 0.94	61.175 ± 0.94	62.00 ± 1.29	60.78 ± 0.81	60.08 ± 1.12
% MCH (pg)	19.811 ± 0.29	18.825 ± 0.28	18.75 ± 0.30	19.178 ± 0.25	19.275 ± 0.35
% MCHC (g/dl)	31.833 ± 0.79	30.80 ± 0.43	30.567 ± 0.31	31.578 ± 0.27	32.10 ± 0.47
Platelet count (10^3 cell/cumm)	551.78 ± 56.23	670.50 ± 58.10	622.83 ± 100.24	570.33 ± 68.43	577.75 ± 84.96
PMN (%)	16.33 ± 2.49	17.63 ± 3.21	20.33 ± 4.24	22.44 ± 2.71	25.75 ± 4.85

Table 14 (con't) Effects of *C. comosa* extracts on clinical blood chemistry and hematology

Hematology	Control group	*C. comosa* 1	*C. comosa* 2	*C. comosa* 3	*C. comosa* 4
Lymphocyte (%)	75.22 ± 3.13	77.38 ± 3.90	74.00 ± 4.96	71.44 ± 3.21	69.63 ± 5.38
Monocyte (%)	5.44 ± 0.75	2.50 ± 0.53	4.17 ± 0.98	4.22 ± 0.60	3.50 ± 0.63
Eosinophil (%)	2.67 ± 0.71	2.50 ± 0.57	1.50 ± 0.34	1.89 ± 0.26	1.29 ± 0.18
Basophil (%)	0.75± 0.7	0.00	0.00	0.00	0.00
Platelet Increase Adequate	 67 % 33 %	 87.5 % 12.5 %	 67 % 33 %	 67 % 33 %	 62.5 % 37.5 %
RBC morphology Micro 1+ Micro 2+ Micro 3+	 44.4 % 44.4 % 11.2 %	 50 % 50 %	 50 % 50 %	 44.4 % 44.4 % 11.2 %	 50 % 37.5 % 12.5 %

Rats were administered orally with 1 ml/kg/day of corn oil (Control), 250 and 500 mg/kg/day of the *C. comosa* hexane extract (*C. comosa* 1 and *C. comosa* 2, respectively), 250 and 500 mg/kg/day of the *C. comosa* ethanolic extract (*C. comosa* 3 and *C. comosa* 4, respectively) for 30 days. Data shown were mean ± SEM. Significance was determined using an One-way ANOVA follow by the Student-Newman-Keuls test in which $p < 0.05$ was required for a statistically significant difference.

* $p < 0.05$; *C. comosa* vs control group.

3. Effect of *C. comosa* extracts on rat hepatic CYP

3.1. Effect of *C. comosa* extracts on rat hepatic total CYP content

C. comosa hexane extract caused significant increase of hepatic total CYP content while *C. comosa* ethanolic extract increased total CYP content only at the dose of 250 mg/kg/day (Figure 7).

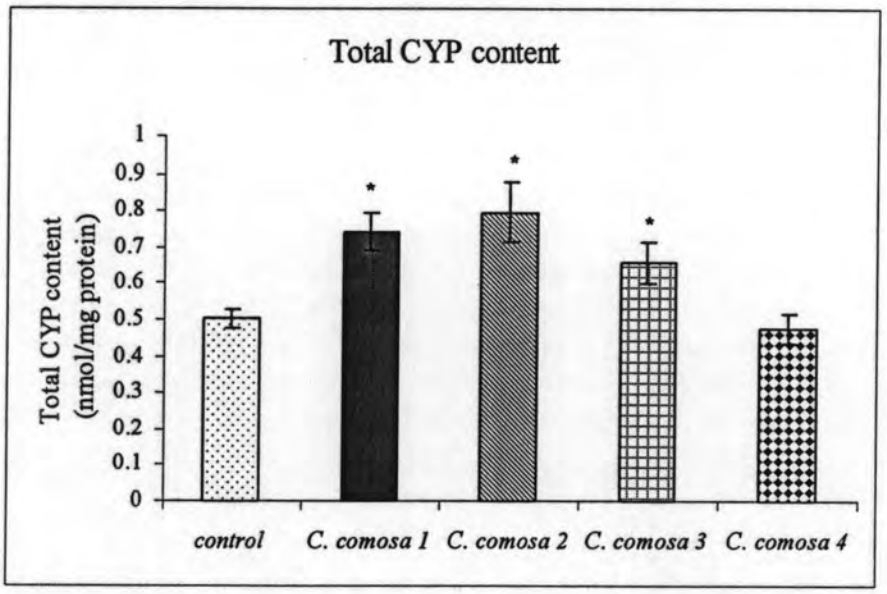


Figure 7 Effect of *C. comosa* hexane extract and ethanolic extract on total CYP contents. Rats were administered orally with 1 ml/kg/day of corn oil (Control), 250 and 500 mg/kg/day of *C. comosa* hexane extract (*C. comosa* 1 and *C. comosa* 2, respectively), 250 and 500 mg/kg/day of *C. comosa* ethanolic extract (*C. comosa* 3 and *C. comosa* 4, respectively) for 30 days. The individual bar graph represented mean of total CYP content with a standard error of the mean (n = 9-10). Significance was determined using One-way ANOVA follow by the Student-Newman-Keuls test in which $p < 0.05$ was required for a statistically significant difference.

* $p < 0.05$; *C. comosa* group vs control group.

3.2. Effect of *C. comosa* extracts on rat hepatic CYP activities

CYP1A1 activity which represented by the rate reaction of ethoxyresorufin O-dealkylation (EROD) was significantly increased in hepatic microsomes of rats treated with both doses of *C. comosa* hexane extract (250 and 500 mg/kg/day) and *C. comosa* ethanolic extract only at the dose of 250 mg/kg/day compared to the control group. The lower dose (250 mg/kg/day) of both hexane and ethanolic extracts caused a significant higher increase of CYP 1A1 activity than the higher dose (500 mg/kg/day) (Figure 8).

Both *C. comosa* hexane extract and ethanolic extract at the doses of 250 and 500 mg/kg/day did not caused any significant effects on the rate reaction of methoxyresorufin O-dealkylation (MROD) which represented the activity of CYP1A2 (Figure 9).

Both *C. comosa* hexane extract and ethanolic extract at the doses of 250 and 500 mg/kg/day increased the rate reaction of pentoxyresolufin O-dealkylation (PROD) which represented the activities of CYP2B1/2B2 as compared to the control group. No significant difference of the rate reaction of PROD was observed between the group received the lower dose of *C. comosa* (250 mg/kg/day) and the group received the higher dose (500 mg/kg/day). As comparing the groups receiving the same dose of the *C. comosa* extracts, the rate reaction of PROD in the hexane extract treatment groups was significantly higher than that of the ethanolic extract treatment groups (Figure 10A).

Both doses of *C. comosa* hexane extract caused significant increases of CYP2B1/2B2 activities which represented by the rate reaction of benzyloxyresorufin O-dealkylation (BROD) with a dose dependent manner. Likewise, both doses of *C. comosa* ethanolic extract caused an increase of the rate reaction of BROD but the increment was lower than the corresponding dosage of *C. comasa* hexane extract group (Figure 10B).

Both *C. comosa* hexane extract and ethanolic extract did not affect the rate of aniline 4-hydroxylation, which represented the activity of CYP2E1 (Figure 11) as well as the rate of erythromycin N-demethylation which represented the activity of CYP3A (Figure 12)

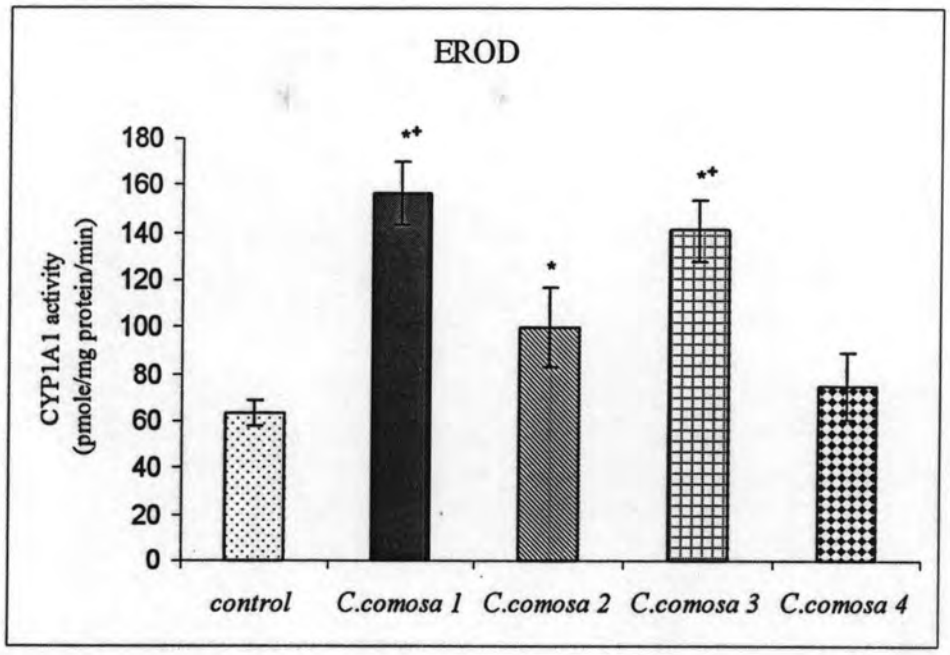


Figure 8 Effect of *C. comosa* hexane extract and ethanolic extract on rat hepatic CYP1A1 activity. Rats were administered orally with 1 ml/kg/day of corn oil (Control), 250 and 500 mg/kg/day of the *C. comosa* hexane extract (*C. comosa* 1 and *C. comosa* 2), 250 and 500 mg/kg/day of the *C. comosa* ethanolic extract (*C. comosa* 3 and *C. comosa* 4) for 30 days. The individual bar graph represented mean of EROD activity with a standard error of the mean (n = 9-10). Significance was determined using One-way ANOVA follow by the Student-Newman-Keuls test in which $p < 0.05$ was required for a statistically significant difference.

* $p < 0.05$; *C. comosa* group vs control group.

$^{+}$ $p < 0.05$; C. comosa 500 mg/kg/day vs *C. comosa* 250 mg/kg/day.

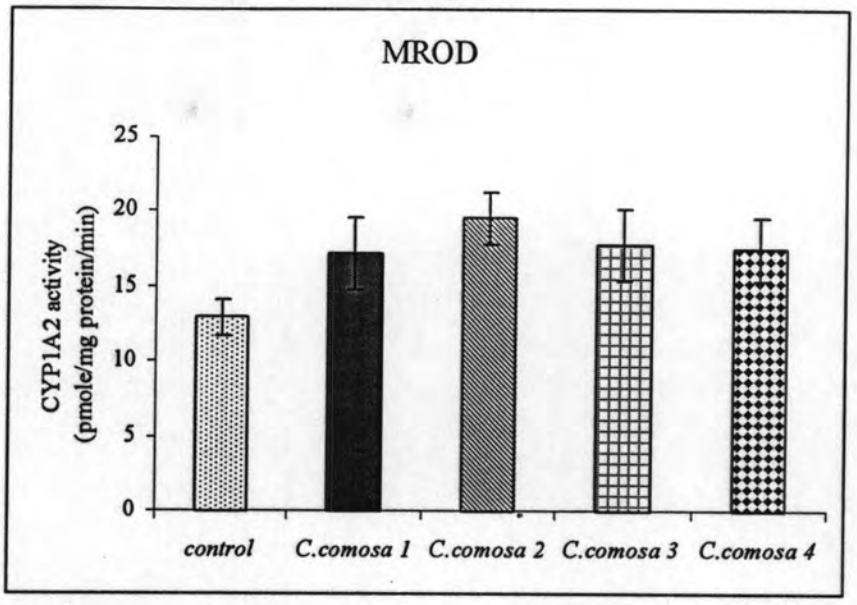
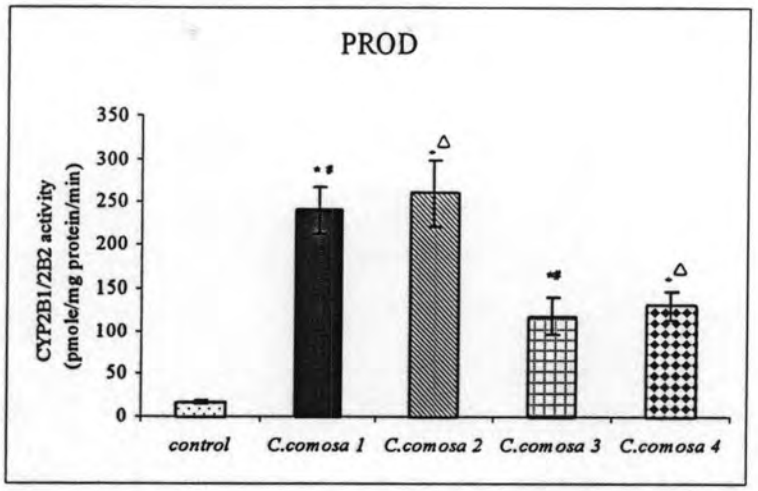


Figure 9 Effect of *C. comosa* hexane extract and ethanolic extract on rat hepatic CYP1A2 activity. Rats were administered orally with 1 ml/kg/day of corn oil (Control), 250 and 500 mg/kg/day of the *C. comosa* hexane extract (*C. comosa* 1 and *C. comosa* 2), 250 and 500 mg/kg/day of the *C. comosa* ethanolic extract (*C. comosa* 3 and *C. comosa* 4) for 30 days. The individual bar graph represented mean of MROD activity with a standard error of the mean (n = 9-10). Significance was determined using One-way ANOVA followed by the Student-Newman-Keuls test in which $p < 0.05$ was required for a statistically significant difference.

A)



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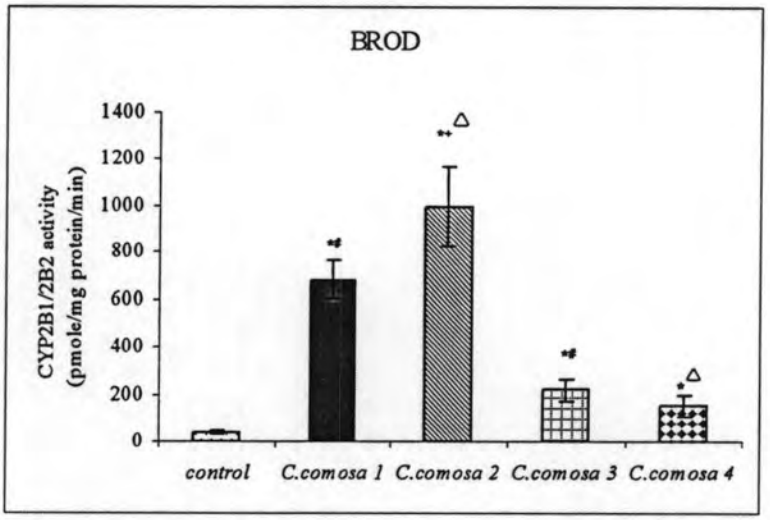

Figure 10 Effect of *C. comosa* hexane extract and ethanolic extract on rat hepatic CYP2B1/2B2 activity. Rats were administered orally with 1 ml/kg/day of corn oil (Control), 250 and 500 mg/kg/day of the *C. comosa* hexane extract (*C. comosa* 1 and *C. comosa* 2), 250 and 500 mg/kg/day of the *C. comosa* ethanolic extract (*C. comosa* 3 and *C. comosa* 4) for 30 days. The individual bar graph represented mean of PROD (A) or BROD (B) activity with a standard error of the mean (n = 9-10). Significance was determined using One-way ANOVA followed by the Student-Newman-Keuls test in which $p < 0.05$ was required for a statistically significant difference.

* $p < 0.05$; *C. comosa* group vs control group.

$^{+}$ $p < 0.05$; *C. comosa* hexane extract 500 mg/kg/day vs *C. comosa* hexane extract 250 mg/kg/day.

$^{\#}$ $p < 0.05$; *C. comosa* hexane extract 250 mg/kg/day vs *C. comosa* ethanolic extract 250 mg/kg/day.

$^{\Delta}$ $p < 0.05$; *C. comosa* hexane extract 500 mg/kg/day vs *C. comosa* ethanolic extract 500 mg/kg/day.

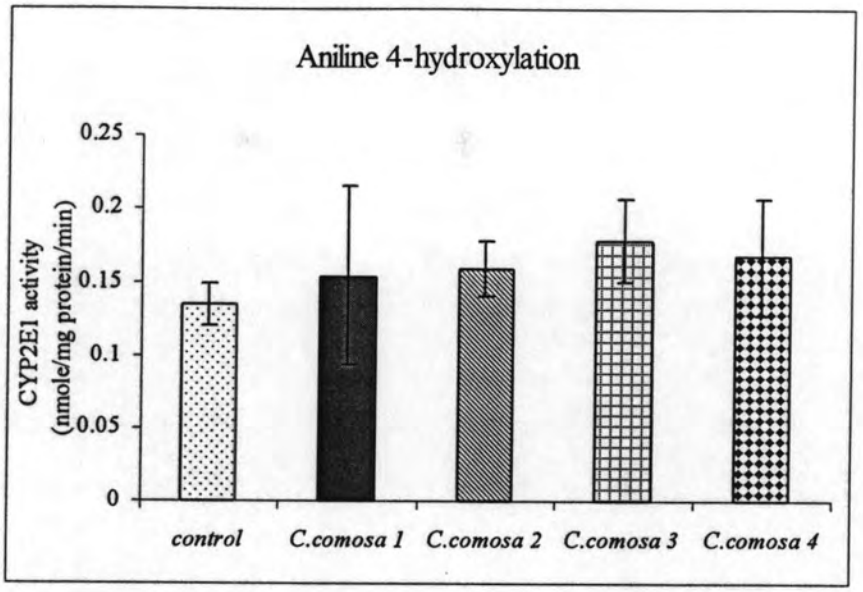

Figure 11 Effect of *C. comosa* hexane extract and ethanolic extract on rat hepatic CYP2E1 activity. Rats were administered orally with 1 ml/kg/day of corn oil (Control), 250 and 500 mg/kg/day of the *C. comosa* hexane extract (*C. comosa* 1 and *C. comosa* 2), 250 and 500 mg/kg/day of the *C. comosa* ethanolic extract (*C. comosa* 3 and *C. comosa* 4) for 30 days. The individual bar graph represented mean of aniline 4-hydroxylase activity with a standard error of the mean (n=9-10).

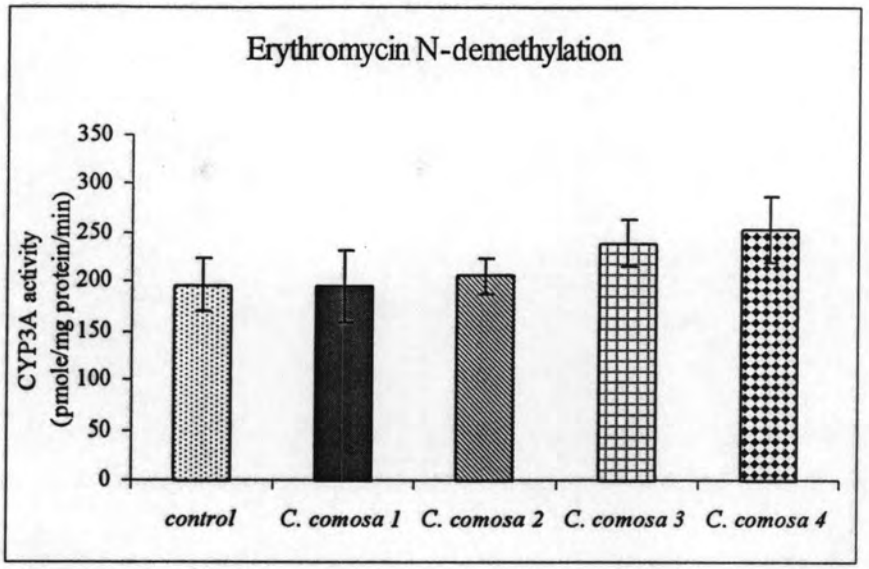

Figure 12 Effects of the *C. comosa* hexane extract and ethanolic extract on rat hepatic CYP3A activity. Rats were administered orally with 1 ml/kg/day of corn oil (Control), 250 and 500 mg/kg/day of the *C. comosa* hexane extract (*C. comosa* 1 and *C. comosa* 2), 250 and 500 mg/kg/day of the *C. comosa* ethanolic extract (*C. comosa* 3 and *C. comosa* 4) for 30 days. The individual bar graph represented mean of erythromycin N-demethylase activity with a standard error of the mean (n = 9-10).