CHAPTER IV

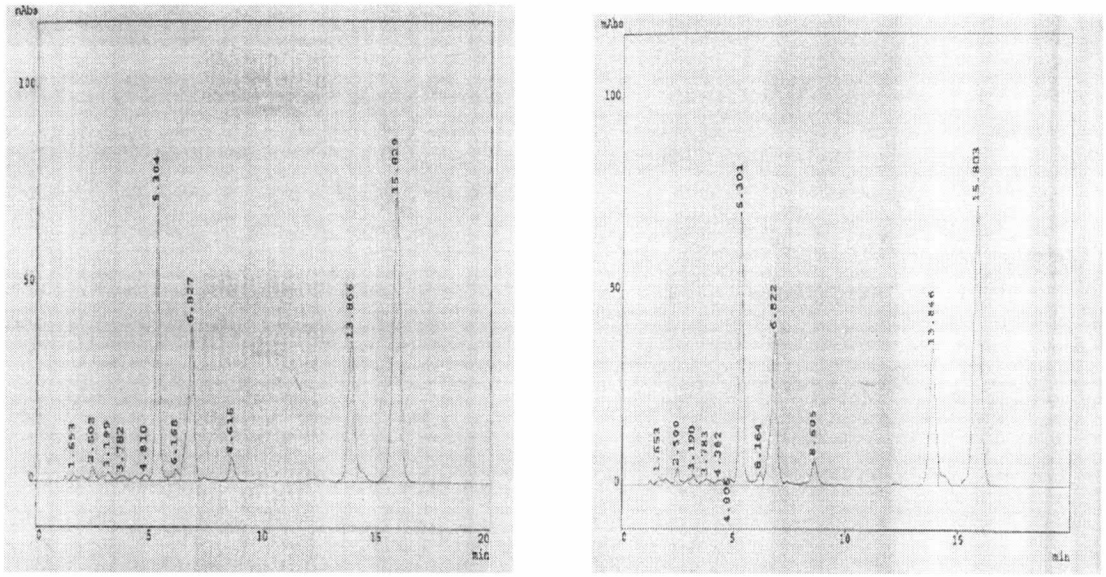
RESULTS

1. Preparation of *C. comosa* ethanolic extract

Nine kilograms of dried fine powder of *C. comosa* rhizomes were used in this study. Following the extraction and solvent evaporating process, 1310 g of the ethanolic extract of *C. comosa* were obtained. Thus, yield of *C. comosa* ethanolic extract was 14.5 % w/w.

2. Chemical identification test

C. comosa ethanolic extract was identified by HPLC on the basis of UV-spectra and retention time in comparision with reference standard, 1,7 diphenyl-4,6-heptadiene-3-ol (Figure 2, 3 and 14). Total 1,7 diphenyl-4,6-heptadiene-3-ol in *C. comosa* ethanolic extract used in the study was 23.6 % w/w.



(a) (b)

Figure 2 HPLC chromatograms of *C. comosa* ethanolic extract.

Twenty microlitres of solution of *C. comosa* ethanolic extract in methanol were injected into the HPLC system as mentioned in Material and Method. The injection was repeated 2 times and the chromatograms were shown (a, b) with the presence of 1,7 diphenyl-4,6-heptadiene-3-ol at the retention time of 13.8 min.

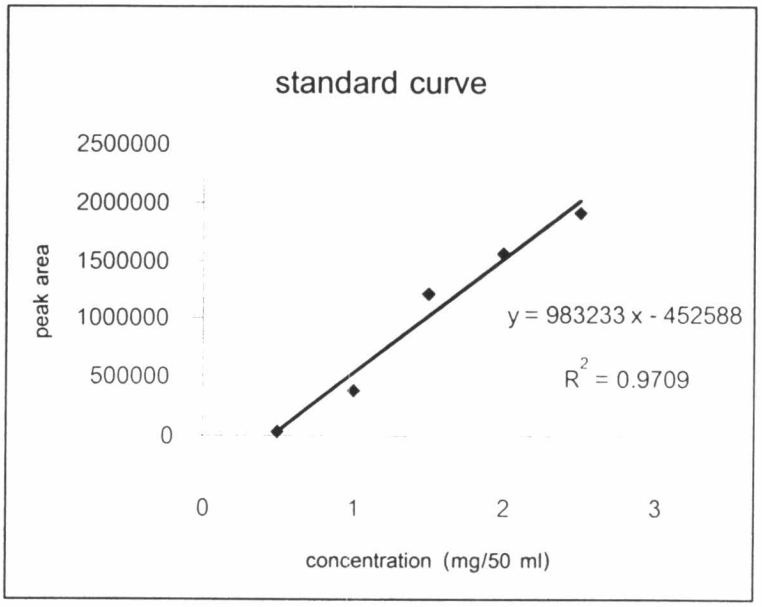


Figure 3 Standard curve of 1,7 diphenyl-4,6-heptadiene-3-ol

Twenty microlitres of various concentration of the reference standard, 1,7 diphenyl-4,6-heptadiene-3-ol were injected into the HPLC system as mentioned in Material and Method. The injection was repeated 2 times for each concentration of the standard and the chromatograms were shown in Figure 14. The standard curve was constructed between average peak area under the chromatogram of 13.8 min retention time against the corresponding concentrations of 1,7 diphenyl-4,6-heptadiene-3-ol.

3. An *ex vivo* study

3.1 Effects of *C. comosa* on body weight, food & water consumption, liver weight and relative liver weight

C. comosa ethanolic extract at the doses of 100, 250 and 500 mg/kg/day given orally once daily for 30 days, did not cause any effects on body weight (Table 5), and body weight gain (Figure 4). Significant increases of liver weight and %relative liver weight were observed in rats given the extract at dosages of 250 and 500 mg/kg/day as compared to those of the control group (Table 5). Likewise, no changes of food and water intake were observed in the animals of all three *C. comosa* treatment groups as

compared to the control group (Figure 5 and Figure 6). All rats were alive till the end of the experiment and exhibited no apparent signs of toxicity.

Table 5 Effect of *C. comosa* ethanolic extract on body weight, liver weight and %relative liver weight

	Treatment group			
	Control group	*C. comosa* group I	*C. comosa* group II	*C. comosa* group III
Initial body weight[a] (g)	233.01±6.38	234.31±3.73	234.93±6.36	232.54±4.09
Final body weight[b] (g)	237.68±4.18	233.78±4.01	234.79±5.66	234.20±6.21
Liver weight[c] (g)	7.50±0.36	8.59±0.52	10.67±0.73*	11.58±0.54*
%relative liver weight (g/100 g of body weight)	3.15±0.12	3.67±0.19	4.52±0.24*	4.95±0.24*

Data shown were mean ± SEM (n=9-10)

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

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

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

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

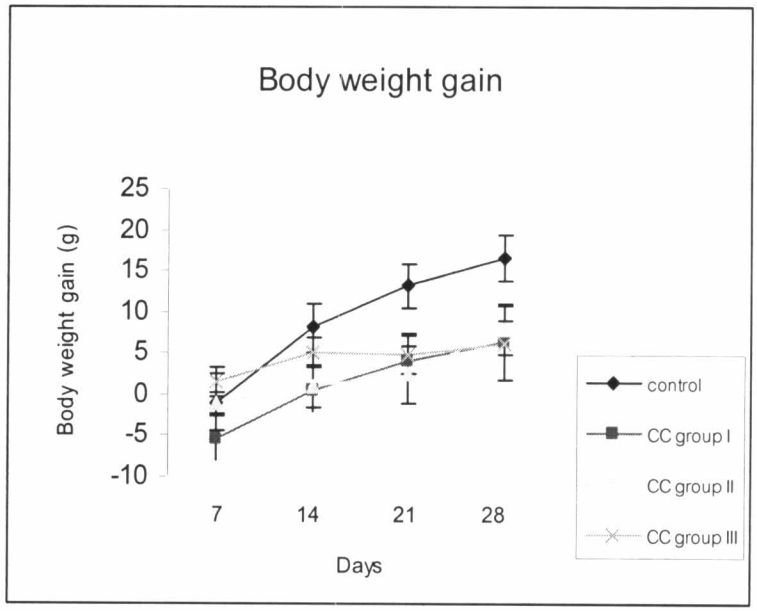


Figure 4 Effect of *C. comosa* ethanolic extract on body weight gain

Data shown were mean ± SEM (n=9-10)

Figure 5 Effect of *C. comosa* ethanolic extract on food consumption

Data shown were mean ± SEM (n=9-10)

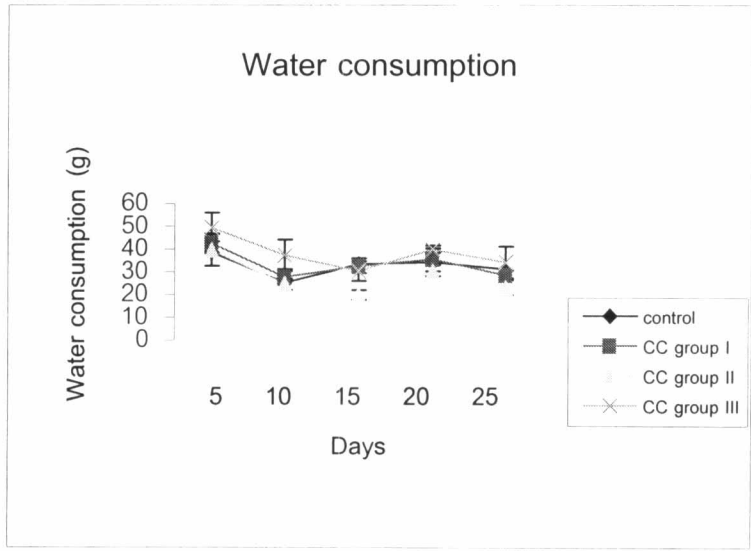


Figure 6 Effect of *C. comosa* ethanolic extract on water consumption

Data shown were mean ± SEM (n=9-10)

3.2 Effects of *C. comosa* ethanolic extract on clinical blood chemistry and hematology

Subacute exposure (30 days) of *C. comosa* ethanolic extract at the doses of 100, 250 and 500 mg/kg/day caused no effects on these following clinical blood chemistry in serum: AST, ALT, total bilirubin, direct bilirubin, total protein, albumin, globulin, BUN, SCr, glucose, total cholesterol, triglyceride, HDL-C, LDL-C, sodium, calcium and chloride (Table 6). Serum ALP and potassium levels were significantly increased in rats receiving the 500 mg/kg/day dosage of *C. comosa* ethanolic extract as compared to the control group (Table 6). However, the levels of both parameters were still within normal range (Table E14). Estradiol concentrations of rats receiving the extract at the doses of 250 and 500 mg/kg/day were significantly higher than those of the control. The increase of estradiol concentrations in rats treated with *C. comosa* ethanolic extract was shown to be dose-related (Table 6).

Likewise, no effects of *C. comosa* ethanolic extract were observed on several hematological parameters. Those 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 6).

Table 6 Effects of the *C. comosa* ethanolic extract on clinical blood chemistry and hematology

Clinical blood chemistry	Control group	*C. comosa* group I	*C. comosa* group II	*C. comosa* group III
AST (U/L)	187.78±15.38	186.62±21.03	194.0±20.03	209.0±16.88
ALT (U/L)	46.11±2.46	53.0±7.66	54.29±7.08	58.71±6.83
ALP (U/L)	67.11±6.26	92.62±8.15	88.71±11.31	156.29±29.67*
Total bilirubin (mg/dl)	0.1±0	0.1±0	0.13±0.02	0.21±0.06
Direct bilirubin (mg/dl)	0.02±0.01	0.0±0.0	0.06±0.03	0.13±0.06
Total protein (g/dl)	6.9±0.1	7.0±0.1	7.23±0.11	6.81±0.26
Albumin (g/dl)	3.73±0.1	3.9±0.09	4.2±0.06	3.57±0.26
Globulin (g/dl)	3.17±0.07	3.1±0.1	3.03±0.08	3.24±0.07
BUN (mg/dl)	22.9±1.23	30.5±4.83	31.43±2.62	30.37±4.33
SCr (mg/dl)	0.67±0.03	0.8±0.08	0.83±0.06	0.78±0.05
Glucose (mg/dl)	92.0±6.83	88.87±4.34	87.71±7.86	82.5±3.37
Total cholesterol (mg/dl)	55.9±3.96	51.0±2.28	52.0±2.24	66.57±8.41
TG (mg/dl)	58.4±4.08	65.12±3.01	71.0±8.32	67.37±10.63
HDL-C (mg/dl)	50.33±4.17	38.75±4.94	40.14±6.39	52.5±5.35
LDL-C (mg/dl)	2.44±0.44	2.12±0.29	2.57±0.3	4.0±0.60
Sodium (mEq/L)	148.44±1.57	154.37±3.78	150.29±2.71	150.29±4.09
Potassium (mEq/L)	4.37±0.14	4.57±0.27	4.64±0.16	5.4±0.28*
Calcium (mg/dl)	10.1±0.12	10.32±0.19	10.06±0.20	10.39±0.16
Chlorine (mEq/L)	110.44±0.96	112.87±3.00	107.71±2.67	109.86±2.94
Estradiol (pmol/L)	115.71±15.52	347.62±44.55	628.83±41.92*	1324.0±253.76*

Table 6 (con't) Effects of the *C. comosa* ethanolic extract on clinical blood chemistry and hematology

Hematology	Control group	*C. comosa* group I	*C. comosa* group II	*C. comosa* group III
Hematocrit (%)	45.2±0.96	44.72±1.62	41.57±3.70	43.33±0.67
Hemoglobin (g/dl)	14.51±0.12	14.06±0.52	13.31±1.16	13.85±0.39
RBC count (10^6 cell/cumm)	7.64±0.12	7.85±0.30	7.35±0.64	7.52±0.07
MCV (fL)	59.23±1.52	53.3±1.74	56.1±0.68	56.93±1.38
MCH (pg)	19.1±0.29	17.9±0.22	18.09±0.13	18.15±0.44
MCHC (g/dl)	32.2±0.59	31.39±0.62	32.3±0.44	31.92±0.39
RBC morphology	Normal	Normal	Normal	Normal
Platelet count (10^3 cell/cumm)	830.3±39.24	935.12±58.96	915.29±85.80	940.83±42.58
WBC count (cell/cumm)	1179.2±282.94	1131.75±143.28	976.29±134.74	1718.5±589.23
PMN (%)	21.5±1.98	31.12±8.1	22.29±3.32	22.33±3.86
Lymphocyte (%)	74.1±2.07	65.62±8.34	74.43±3.40	71.17±3.75
Monocyte (%)	3.2±0.47	2.5±0.38	2.71±0.36	3.67±0.8
Eosinophil (%)	1.1±0.35	0.75±0.41	0.57±0.43	0.5±0.22

Rats were administered orally with 1 ml/kg/day of corn oil (Control group), 100, 250 and 500 mg/kg/day of *C. comosa* ethanolic extract (*C. comosa* group I, *C. comosa* group II & *C. comosa* group III, respectively) for 30 days.

Data shown were mean ± SEM. One way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p<0.05$.

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

3.3 Effects of *C. comosa* ethanolic extract on hepatic microsomal CYPs

3.3.1 Effect of *C. comosa* on hepatic microsomal total CYP contents

C. comosa at the doses of 100, 250 and 500 mg/kg/day used in this study did not cause any significant changes of hepatic microsomal total CYP contents (Figure 7).

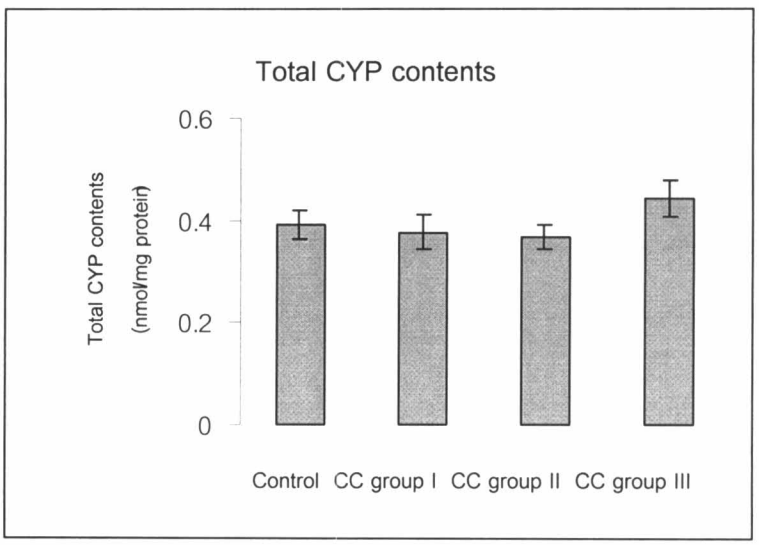


Figure 7 Effects of *C. comosa* ethanolic extract on hepatic total CYP contents. Rats were administered orally with 1 ml/kg/day of corn oil (Control group), 100, 250 and 500 mg/kg/day of the *C. comosa* ethanolic extract (CC group I, CC group II & CC group III, respectively) for 30 days. Liver microsomes were determined for total CYP contents. The individual bar graph represented mean of total CYP contents with an error bar of standard error of the mean. One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p<0.05$.

3.3.2 Effect of *C. comosa* on hepatic CYP activity

C. comosa ethanolic extract did not show any significant effects on the rate of both ethoxyresorufin O-dealkylation (EROD) and methoxyresorufin O-dealkylation (MROD) which represented the activities of CYP1A1 and CYP1A2, respectively (Figure 8 & Figure 9).

Rate of both benzyloxy- and pentoxyresorufin O-dealkylation (BROD and PROD, respectively), which represented the activities of CYP2B1&2B2, were significantly increased by *C. comosa* administration at the dosages of 250 and 500 mg/kg/day. The induction of *C. comosa* ethanolic extract on CYP2B1&2B2 was shown to be dose-related (Figure 10a & Figure 11a) with the correlation coefficient (r^2) between doses of *C. comosa* and the correspondly activities of CYP 2B1/2B2 of 0.9293 (for BROD) and 0.8867 (for PROD) (Figure 10b & Figure 11b).

C. comosa ethanolic extract did not affect the rate of aniline 4-hydroxylation, which represented the activity of CYP 2E1 (Figure 12) as well as the rate of erythromycin N-demethylation which represented the activity of CYP 3A (Figure 13).

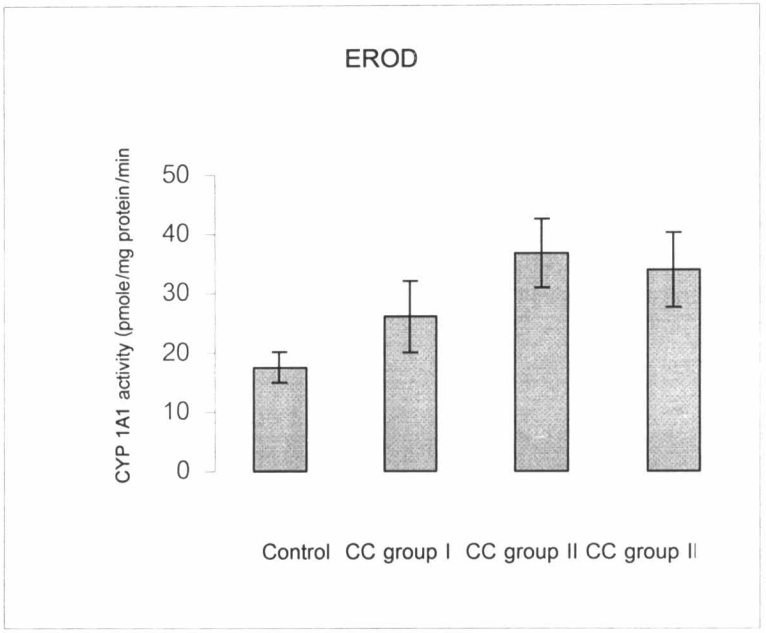


Figure 8 Effects of the *C. comosa* ethanolic extract on hepatic CYP1A1 activity. Rats were administered orally with 1 ml/kg/day of corn oil (Control group), 100, 250 and 500 mg/kg/day of the *C. comosa* ethanolic extract (CC group I, CC group II & CC group III, respectively) for 30 days. Liver microsomes were prepared and determined for EROD activity. The individual bar graph represented mean of EROD activity with an error bar of standard error of the mean. One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p<0.05$.

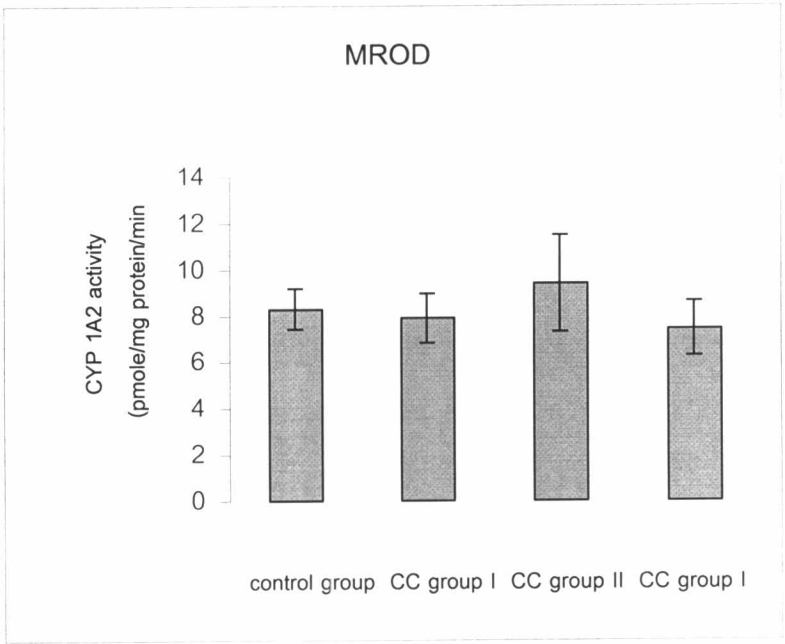
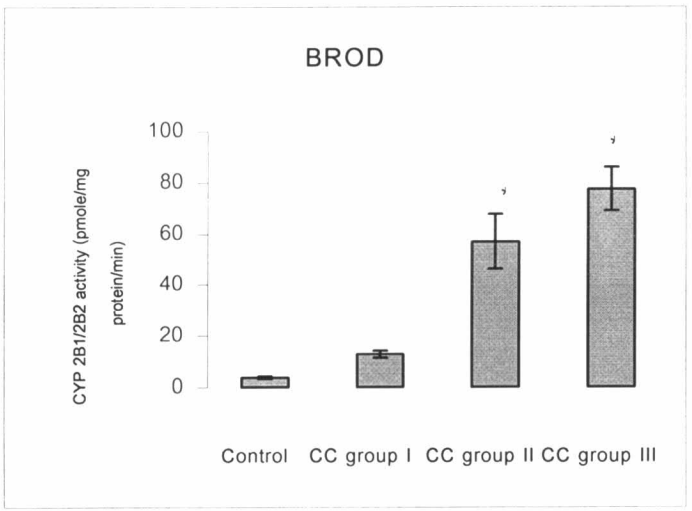
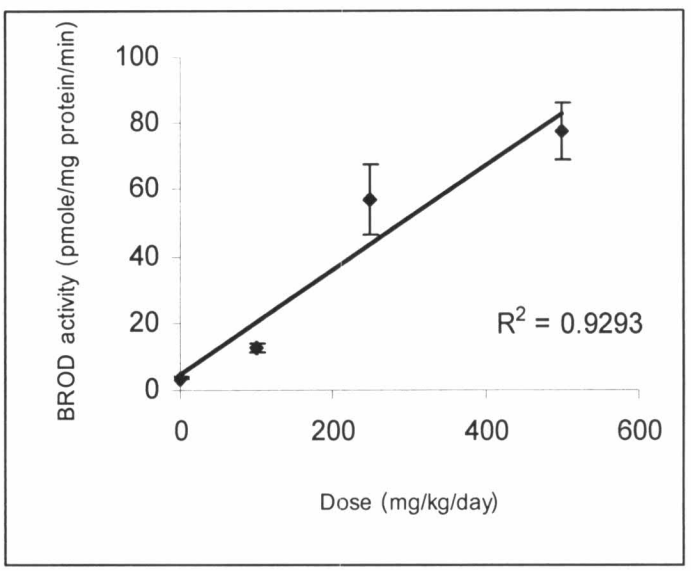


Figure 9 Effects of the *C. comosa* ethanolic extract on hepatic CYP1A2 activity. Rats were administered orally with 1 ml/kg/day of corn oil (Control group), 100, 250 and 500 mg/kg/day of the *C. comosa* ethanolic extract (CC group I, CC group II & CC group III, respectively) for 30 days. Liver microsomes were determined for MROD activities. The individual bar graph represented mean of MROD activity with an error bar of standard error of the mean. One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p<0.05$.



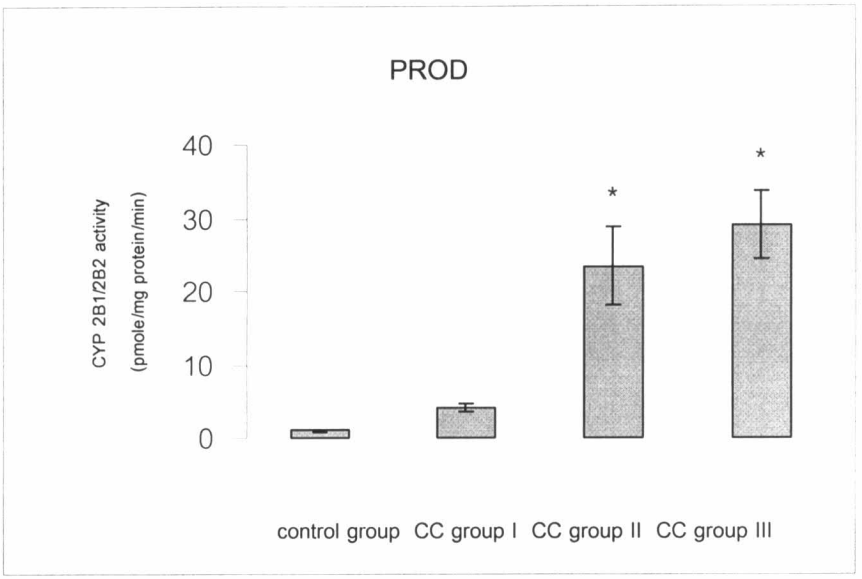
(a)



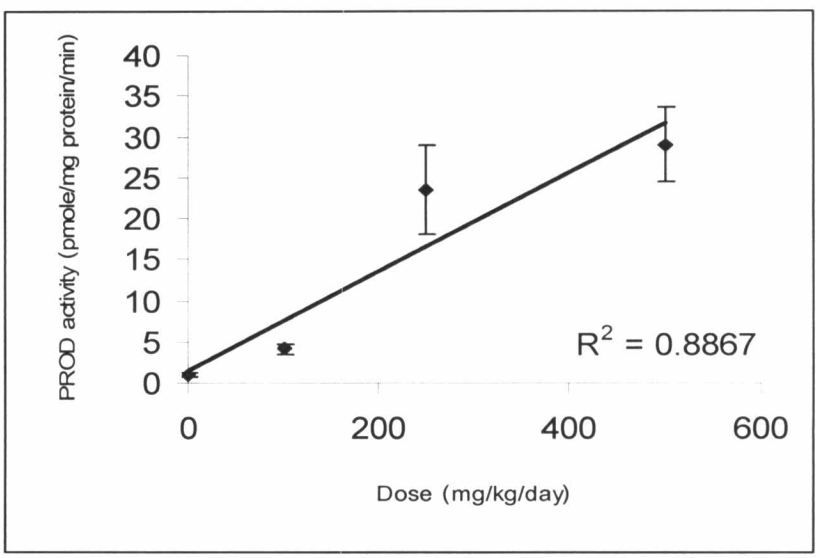
(b)

Figure 10 Effects of the *C. comosa* ethanolic extract on hepatic CYP 2B1/2B2 activity. Rats were administered orally with 1 ml/kg/day of corn oil (Control group), 100, 250 and 500 mg/kg/day of the *C. comosa* ethanolic extract (CC group I, CC group II & CC group III, respectively) for 30 days. Liver microsomes were determined for BROD activity. The individual bar graph represented mean of BROD activity with an error bar of standard error of the mean. One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons (a). The correlation coefficient between BROD activity and doses of C. comosa administration was shown to be 0.9293 (b).

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



(a)



(b)

Figure 11 Effects of the *C. comosa* ethanolic extract on hepatic CYP 2B1/2B2 activity. Rats were administered orally with 1 ml/kg/day of corn oil (Control group), 100, 250 and 500 mg/kg/day of the *C. comosa* ethanolic extract (CC group I, CC group II & CC group III, respectively) for 30 days. Liver microsomes were determined for PROD activity. The individual bar graph represented mean of PROD activity with an error bar of standard error of the mean. One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons (a). The correlation coefficient between PROD activity and doses of *C. comosa* administration was shown to be 0.8867 (b).

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

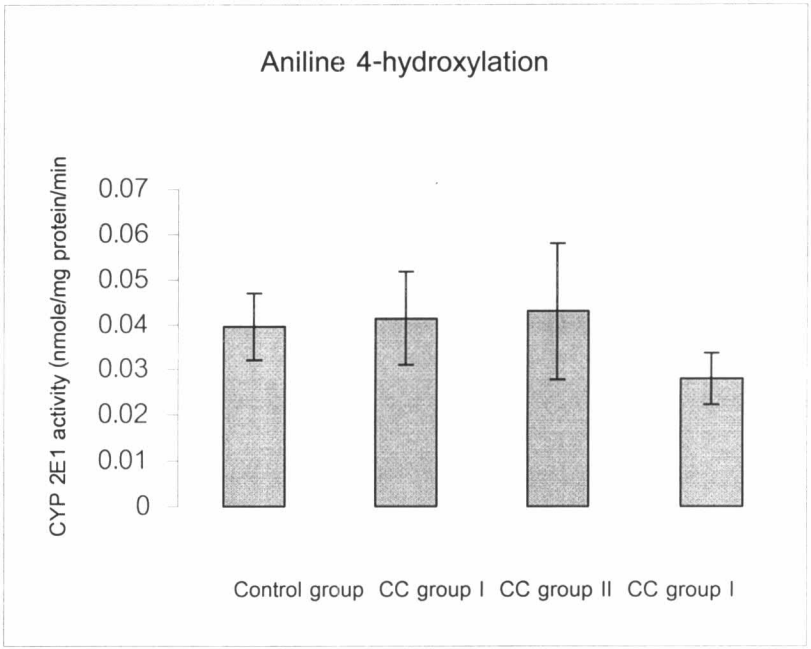


Figure 12 Effects of the *C. comosa* ethanolic extract on hepatic CYP 2E1 activity. Rats were administered orally with 1 ml/kg/day of corn oil (Control group), 100, 250 and 500 mg/kg/day of the *C. comosa* ethanolic extract (CC group I, CC group II & CC group III, respectively) for 30 days. Liver microsomes were determined for aniline 4-hydroxylase activity. The individual bar graph represented mean of aniline 4-hydroxylase activity with an error bar of standard error of the mean. One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p<0.05$

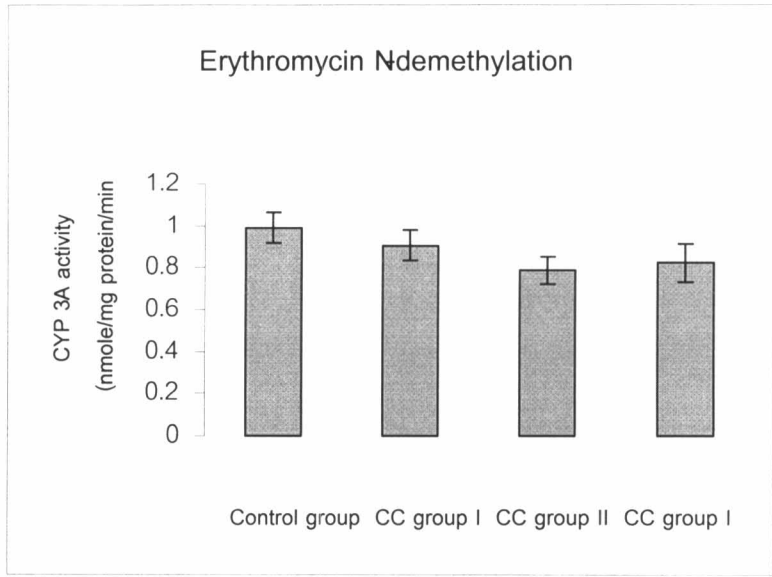


Figure 13 Effects of the *C. comosa* ethanolic extract on hepatic CYP 3A activity. Rats were administered orally with 1 ml/kg/day of corn oil (Control group), 100, 250 and 500 mg/kg/day of the *C. comosa* ethanolic extract (CC group I, CC group II & CC group III, respectively) for 30 days. Liver microsomes were determined for erythromycin N-demethylase activity. The individual bar graph represented mean of erythromycin N-demethylase activity with an error bar of standard error of the mean. One-way ANOVA and Student-Newman-Keuls test were used for statistical comparisons at a significant level of $p<0.05$