CHAPTER IV

RESULTS

1. An *in vivo* study

1.1 Effect of the standardized extract of *C. asiatica* on rat hepatic total CYP content.

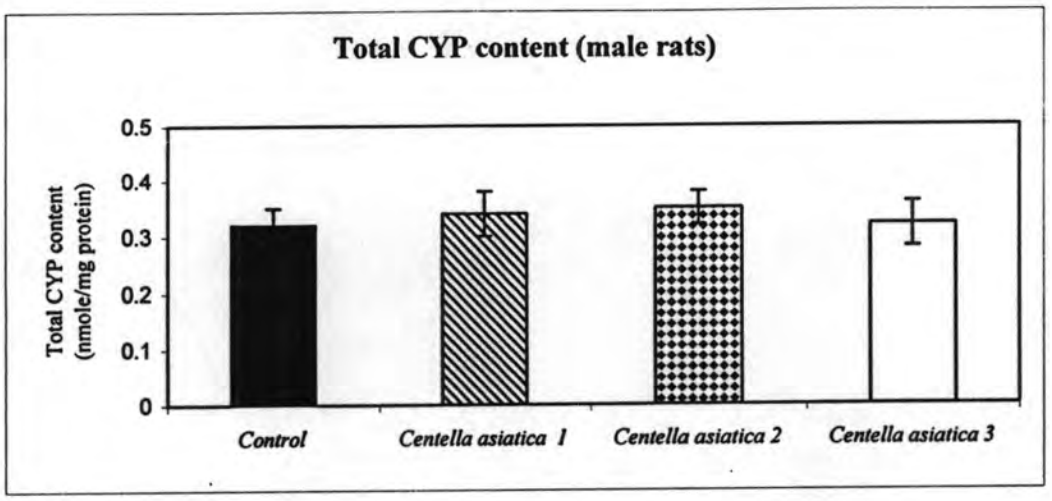
The standardized extract of *C. asiatica* given at 10, 100 and 1000 mg/kg/day for 90 days did not affect total CYP content in both male (Figure 5A) and female rats (Figure 5B).

1.2 Effect of the standardized extract of *C. asiatica* on rat hepatic CYP activities.

Effects of the standardized extract of *C. asiatica* on CYP1A1, CYP1A2, were determined using the rate reaction of ethoxyresorufin O-delkylation (EROD) and methoxyresorufin O-dealkylation (MROD), respectively. Rate reaction of benzyloxyresorufin O-dealkylation (BROD) and penthoxyresorufin O-dealkylation (PROD) were used for determining effects of the standardized extract of *C. asiatica* on CYP2B1/2B2 whereas aniline 4-hydroxilation and erythromycin N-demethylation were used for determining of CYP 2E1 and 3A, respectively.

The results showed that the standardized extract of *C. asiatca* did not cause any effects on the activities CYP1A1 (Figure6), 1A2 (Figure 7), 2B1/ 2B2 (Figure 8 & 9), 2E1 (Figure 10), and 3A (Figure 11) in both male and female rats.

A)



B)

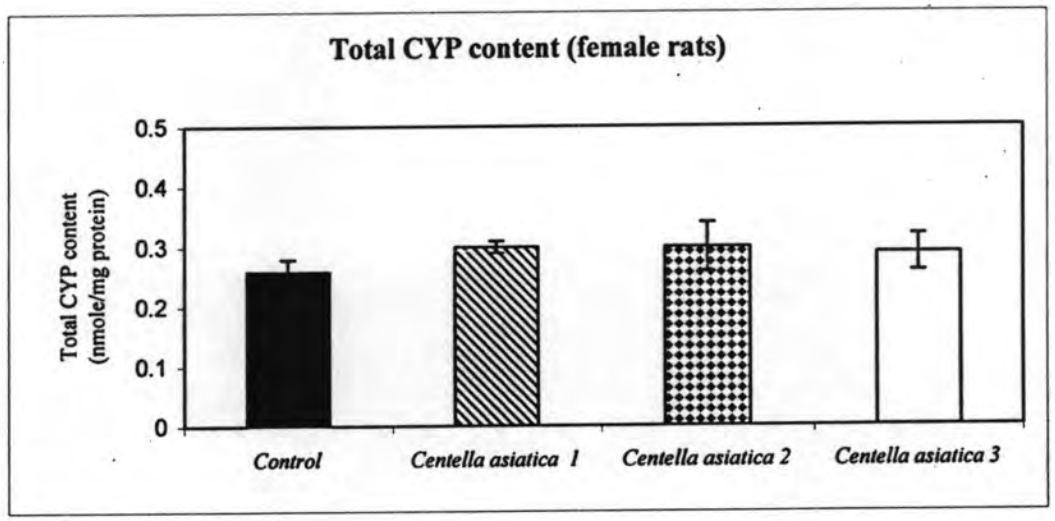
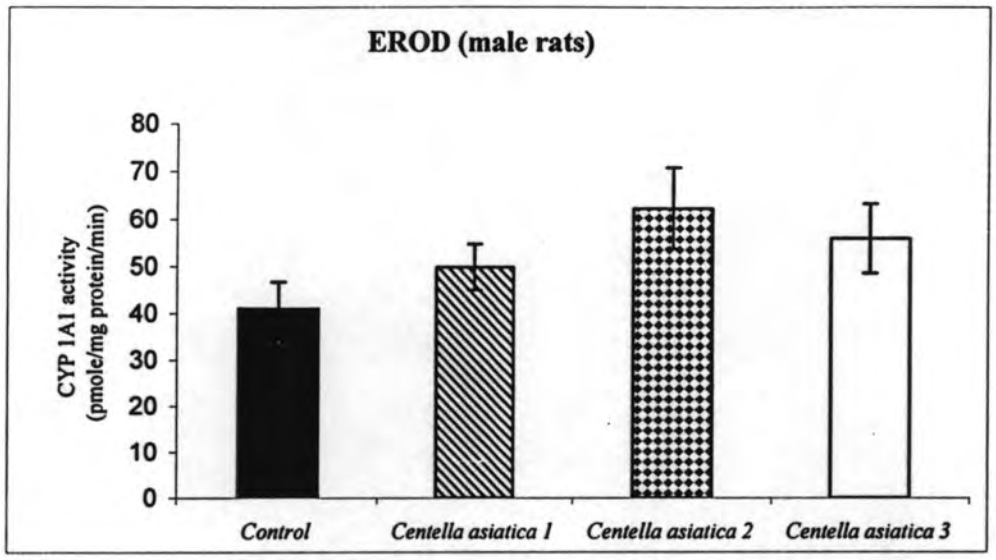


Figure 5 Effect of the standardized extract of *C. asiatica* on hepatic total CYP content of male rats (A) and female rats (B). Rats were administered orally with 10 ml/kg/day of distilled water (control), 10 mg/kg/day, 100 mg/kg/day and 1000 mg/kg/day of the standardized extract of *C. asiatica (Centella asiatica* 1, *Centella asiatica* 2, *Centella asiatica* 3, respectively). The individual bar graph represented mean of total CYP content with a standard error of the mean (n=10). Difference 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.

A)



B)

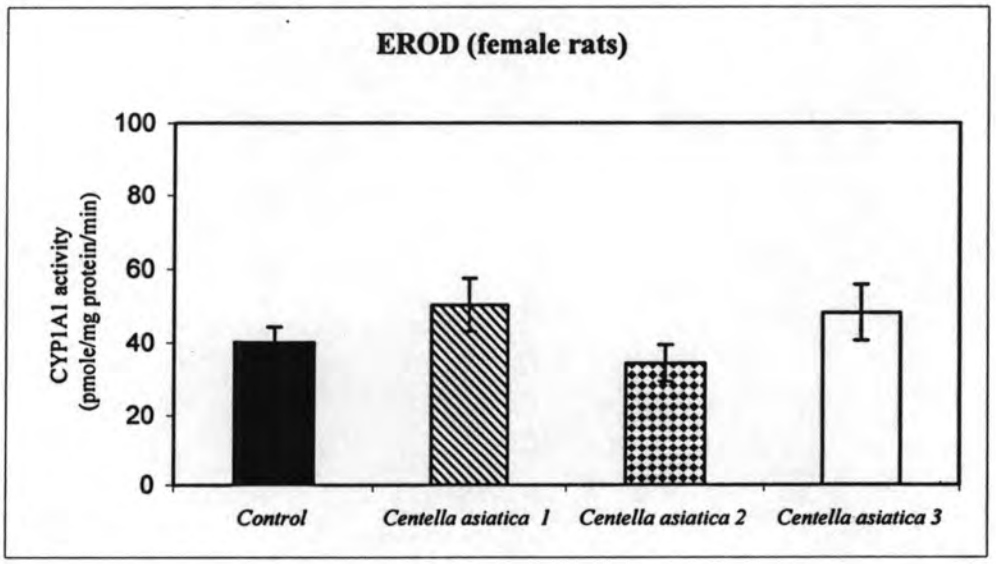
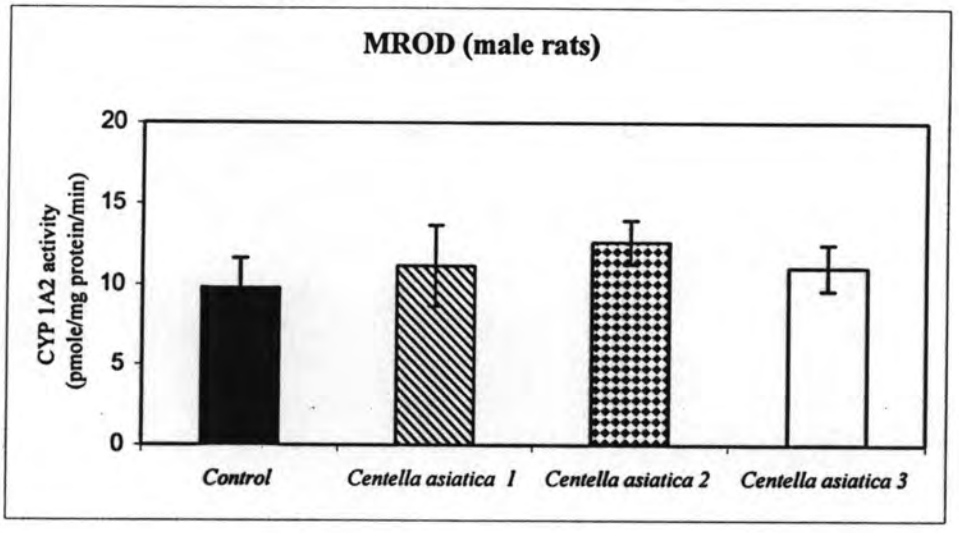


Figure 6 Effect of the standardized extract of *C. asiatica* on hepatic CYP1A1 activity of male rats (A) and female rats (B). Rats were administered orally with 10 ml/kg/day of distilled water (control), 10 mg/kg/day, 100 mg/kg/day and 1000 mg/kg/day of the standardized extract of *C. asiatica (Centella asiatica* 1,*Centella asiatica* 2, *Centella asiatica* 3, respectively). The individual bar graph represented mean of CYP1A1 activity with a standard error of the mean (n=10). Difference 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.

A)



B)

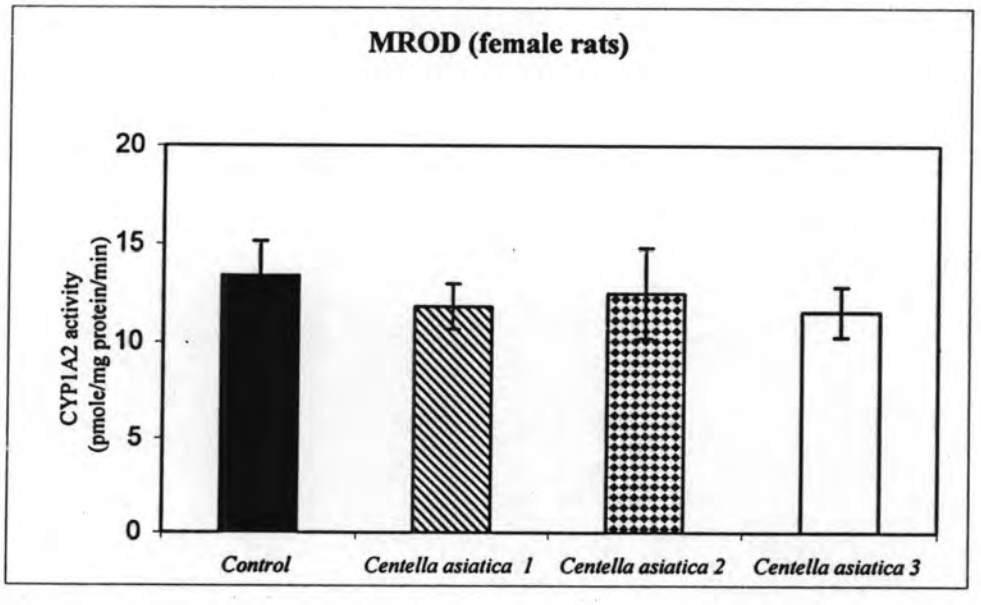
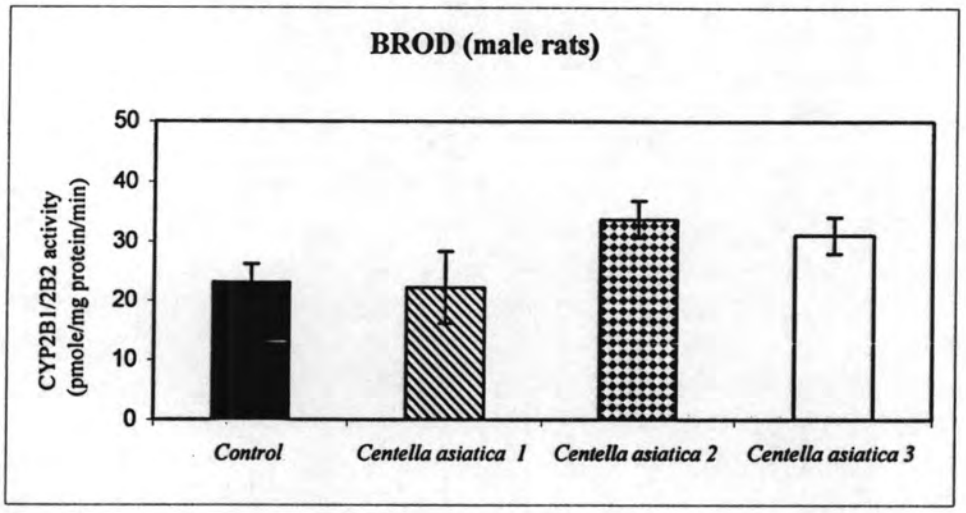


Figure 7 Effect of the standardized extract of *C. asiatica* on hepatic CYP1A2 activity of male rats (A) and female rats (B). Rats were administered orally with 10 ml/kg/day of distilled water (control), 10 mg/kg/day, 100 mg/kg/day and 1000 mg/kg/day of the standardized extract of *C. asiatica (Centella asiatica* 1, *Centella asiatica* 2, *Centella asiatica* 3, respectively). The individual bar graph represented mean of CYP1A2 activity with a standard error of the mean (n=10). Difference 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.

A)



B)

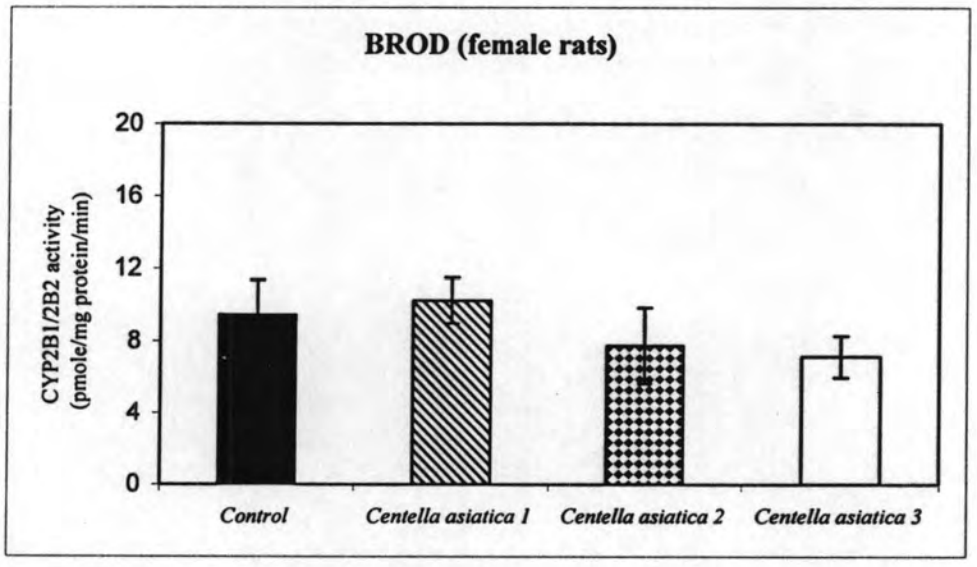
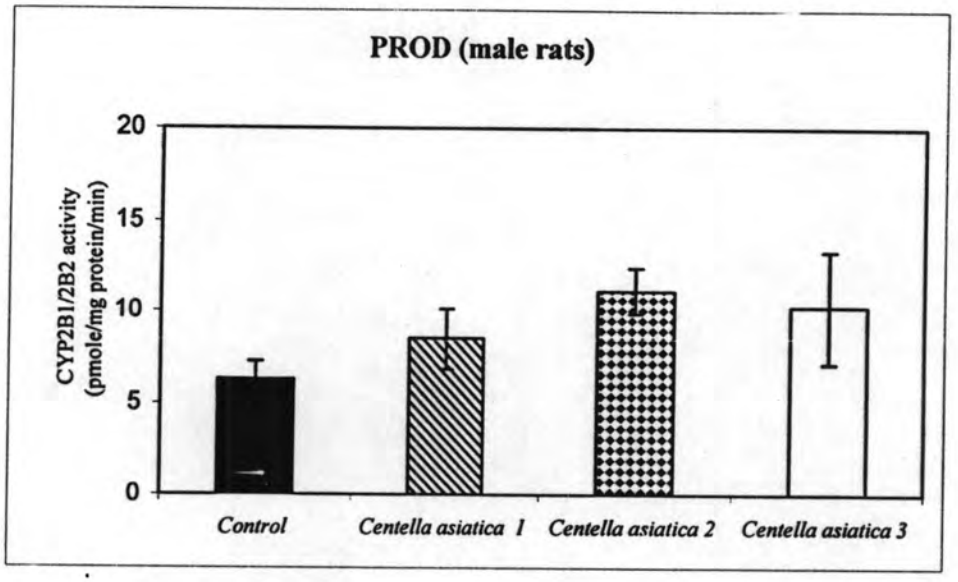


Figure 8 Effect of the standardized extract of *C. asiatica* on hepatic CYP2B1/2B2 activity of male rats (A) and female rats (B). Rats were administration orally with 10 ml/kg/day of distilled water (control), 10 mg/kg/day, 100 mg/kg/day and 1000 mg/kg/day of the standardized extract of *C. asiatica (Centella asiatica* 1, *Centella asiatica* 2, *Centella asiatica* 3, respectively). The individual bar graph represented mean of CYP2B1/2B2 activity with a standard error of the mean (n=10). Difference 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.

A)



B)

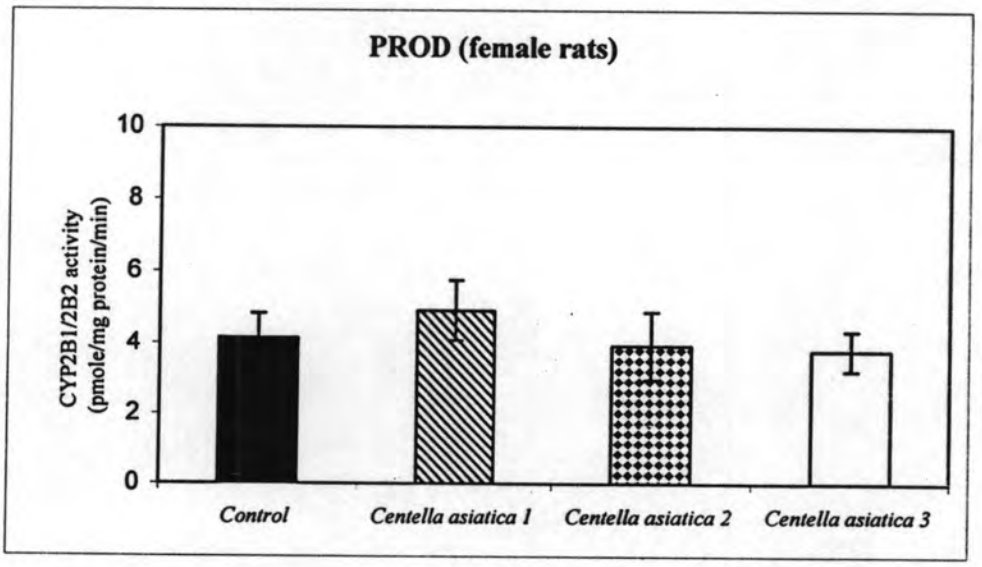
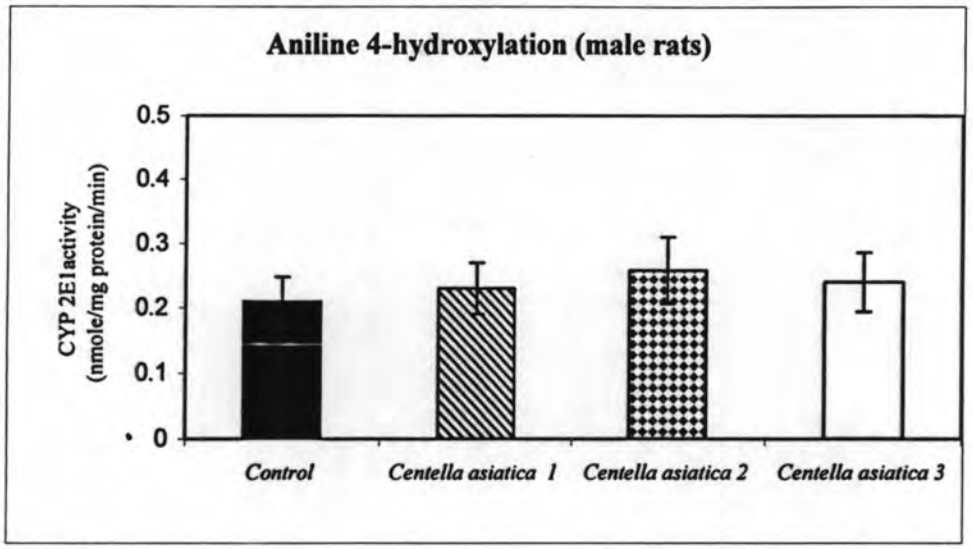


Figure 9 Effect of the standardized extract of *C. asiatica* on hepatic CYP2B1/2B2 activity of male rats (A) and female rats (B). Rats were administered orally with 10 ml/kg/day of distilled water (control), 10 mg/kg/day, 100 mg/kg/day and 1000 mg/kg/day of the standardized extract of *C. asiatica (Centella asiatica* 1, *Centella asiatica* 2, *Centella asiatica* 3, respectively). The individual bar graph represented mean of CYP2B1/2B2 activity with a standard error of the mean (n=10). Difference 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.

A)



B)

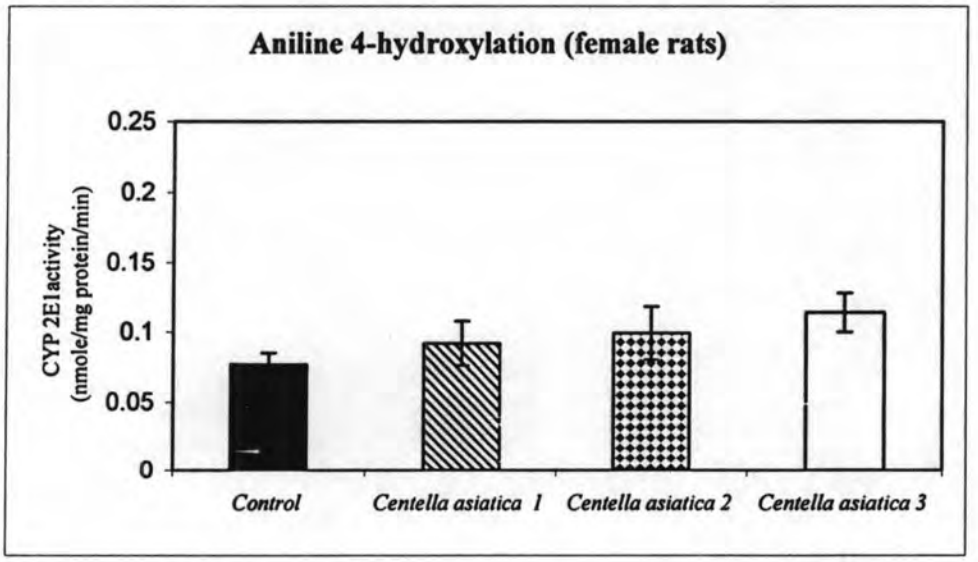
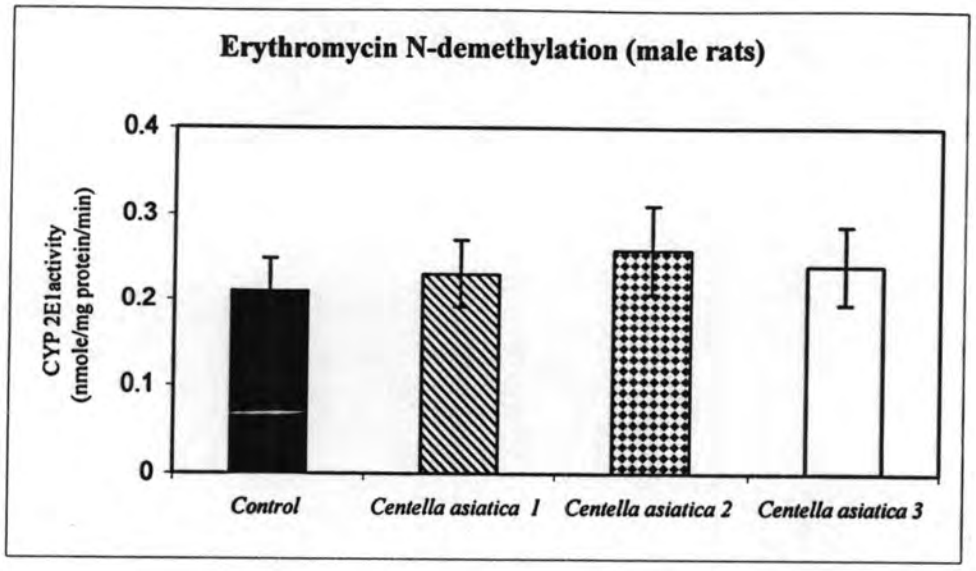


Figure 10 Effect of the standardized extract of *C. asiatica* on hepatic CYP2E1 activity of male rats (A) and female rats (B). Rats were administered orally with 10 ml/kg/day of distilled water (control), 10 mg/kg/day, 100 mg/kg/day and 1000 mg/kg/day of the standardized extract of *C. asiatica (Centella asiatica* 1, *Centella asiatica* 2, *Centella asiatica* 3, respectively). The individual bar graph represented mean of CYP2E1activity with a standard error of the mean (n=10). Difference 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.

A)



B)

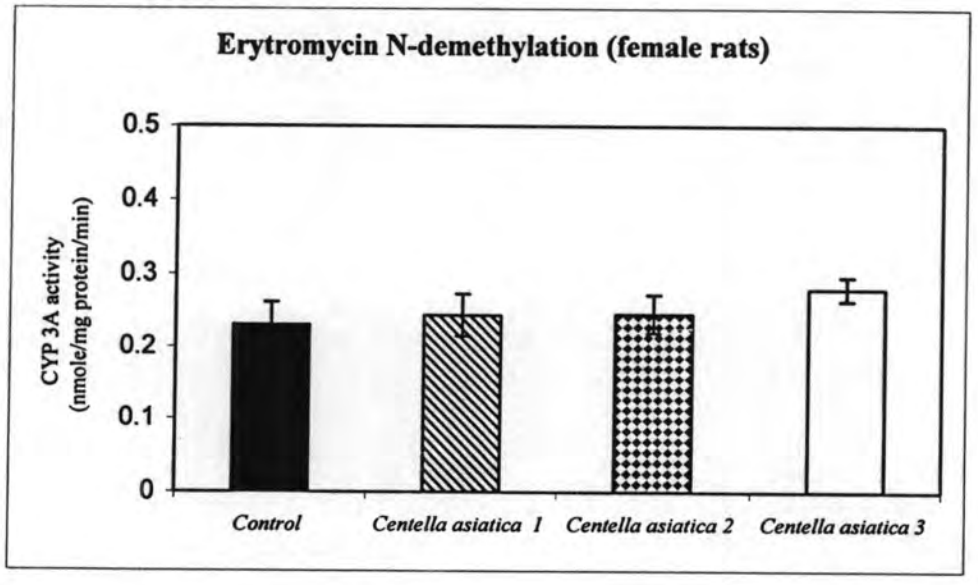


Figure 11 Effect of the standardized extract of *C. asiatica* on hepatic CYP3A activity of male rats (A) and female rats (B). Rats were administered orally with 10 ml/kg/day of distilled water (control), 10 mg/kg/day, 100 mg/kg/day and 1000 mg/kg/day of the standardized extract of *C. asiatica (Centella asiatica* 1, *Centella asiatica* 2, *Centella asiatica* 3, respectively). The individual bar graph represented mean of CYP3A activity with a standard error of the mean (n=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.

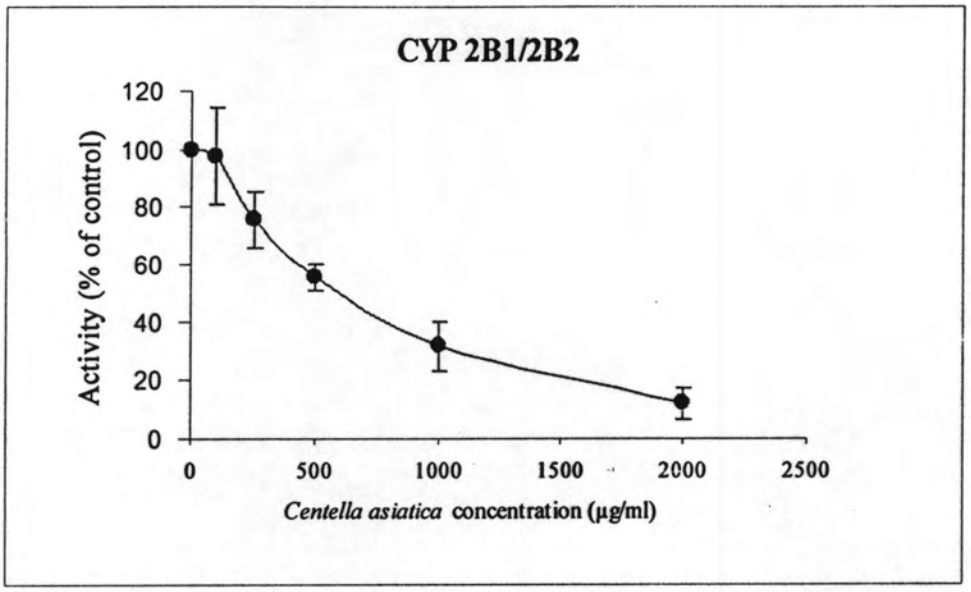
2. An *in vitro* study

Inhibitory effects of the standardized extract of *C. asiatica* on activities of various CYP isforms were studied by *in vitro* co-incubation of the standardized extract of *C. asiatica* simultaneously with the selective substrates for the particular CYP isoforms.

The results showed that the standardized extract of *C. asiatica* possessed an inhibitory effect on CYP2B1/2B2 in a concentration dependent manner with a median inhibitory concentration (IC_{50}) of 523 μg/ml as determined by BROD reaction (Figure 12A) and 563 μg/ml as determine by PROD reaction (Figure 12B).

Very slightly decrease or no effect of the extract was shown on the activity of CYP1A2 resulting in a high IC_{50} of more than 1000 μg/ml (Figure 13). Likewise, no inhibitory effects of the standardized of *C. asiatica* were shown on the activities of CYP1A1 (Table 8), 2E1 (Table 9) and 3A (Table 10).

A)



B)

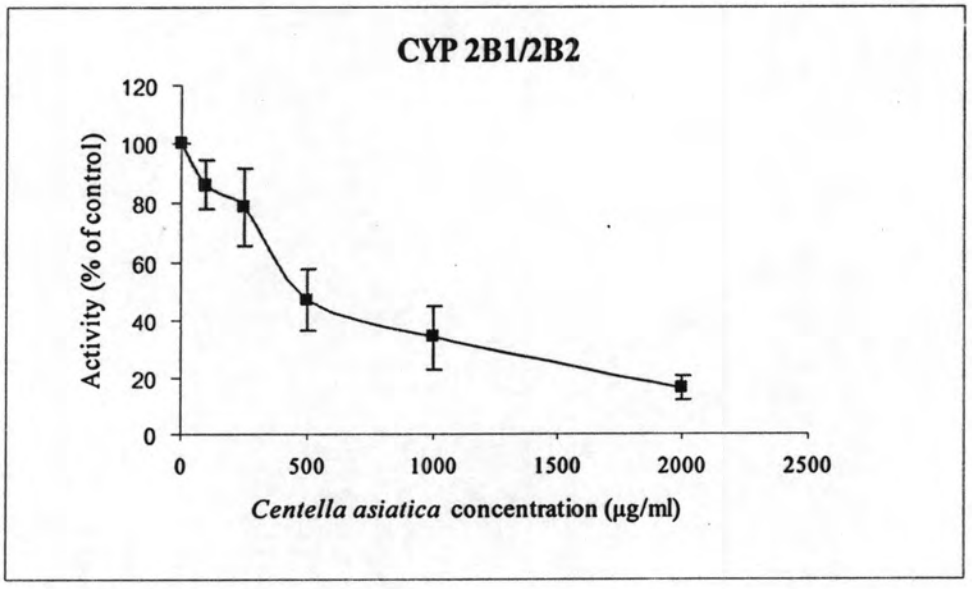


Figure 12 Effects of the standardized extract of C. *asiatica* on CYP2B1/2B2 in an *in vitro* study. The standardized extract of *C. asiatica* was co-incubated simultaneously with BR (A) and PR (B) under condition described in the Materials and Methods. The concentrations of the standardized extract of *C. asiatica* in the reaction mixture were 0,100, 250, 500, 1000 and 2000 µg/ml. IC_{50} value for BROD and PROD were 523 and 563 µg/ml respectively (n=4).

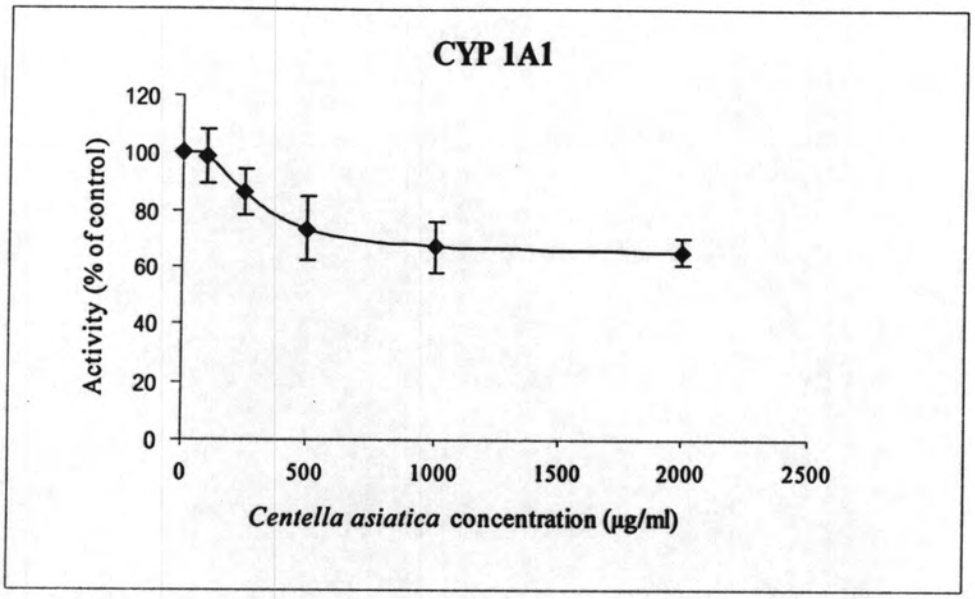


Figure 13 Effects of the standardized extract of *C. asiatica* on CYP1A2 in an *in vitro* study. The standardized extract of *C. asiatica* was co-incubated simultaneously with MR under the condition described in the Materials and Methods. The concentrations of the standardized extract of *C. asiatica* in the reaction mixture were 0,100, 250, 500, 1000 and 2000 µg/ml. IC_{50} value was > 1000 µg/ml (n=4).

Table 8 Effect of the standardized extract of *C. asiatica* on CYP1A1 in an *in vitro* study.

Concentrations of the standardized extract of *C. asiatica* in the reaction mixture (μg/ml)	CYP1A1 activities (pmol/mg protein/min)
0	685.67±80.10
100	748.36±58.12
250	750.27±45.08
500	794.49±41.23
1000	835.88±38.06
2000	866.55±33.84

Various concentrations of the standardized extract of *C. asiatica* were co-incubated simultaneously with ER under the condition described in the Materials and Mcthods. The concentrations of the standardized extract of *C. asiatica* in the reaction mixture were 0,100, 250, 500, 1000 and 2000 μg/ml. The data shown were mean ± SD of n=4.

Table 9 Effect of the standardized extract of *C. asiatica* on CYP2E1 in an *in vitro* study.

Concentrations of the standardized extract of *C. asiatica* in the reaction mixture (μg/ml)	CYP2E1 activities (nmol/mg protein/min)
0	0.297±0.036
100	0.286±0.038
250	0.275±0.031
500	0.281±0.021
1000	0.229±0.017
2000	0.237±0.005

Various concentrations of the standardized extract of *C. asiatica* were co-incubated simultaneously with aniline 4-hydroxylase under the condition described in the Materials and Methods. The concentrations of the standardized extract of *C. asiatica* in the reaction mixture were 0,100, 250, 500, 1000 and 2000 μg/ml. The data shown were mean ± SD of n=4.

Table 10 Effect of the standardized extract of *C. asiatica* on CYP3A in an *in vitro* study.

Concentrations of the standardized extract of *C. asiatica* in the reaction mixture (μg/ml)	CYP3A activities (nmol/mg protein/min)
0	2.463±0.567
100	2.359±0.546
250	2.279±0.488
500	2.313±0.707
1000	2.383±0.547
2000	2.220±0.490

Various concentrations of the standardized extract of *C. asiatica* were co-incubated simultaneously with erythromycin N-demethylase under the condition described in the Materials and Methods. The concentrations of the standardized extract of *C. asiatica* in the reaction mixture were 0,100, 250, 500, 1000 and 2000 μg/ml. The data shown were mean ± SD of n=4.