CHAPTER V

DISCUSSION AND CONCLUSION

This study was preformed primarily to investigate effects of the standardized extract of *C. asiatica* on hepatic CYP, by using *in vivo* and *in vitro* assays. Results from this study showed that standardized extract of *C. asiatica* did not modulate CYP1A1, 1A2, 2B1/2B2, 2E1 and 3A *in vivo*, in rats of both male and female. No induction and inhibition effect of the extract in the *in vivo* study indicated an advantage of the extract in terms of drug-drug interactions when the extract was administered concomitantly with other currently used medicines. Such medicines that are metabolized by these CYP isoforms are as following: propranolol, theophylline, tracine, verapamil etc., are metabolized by CYP1A2; bupropion, coumarins, cyclophosphamide etc., are metabolized by CYP 2B6 (CYP2B1/2B2 and human CYP2B6 share approximately 80% nucleotide sequence identity, Lewis, 1999); chlorzoxazone, ethanol, paracetamol are metabolized by CYP2E1; digoxin, meloxicam, tacrolimus, loratadine, methadone etc., are metabolized by CYP3A (Coleman, 2005 and Ekins & Wrighton, 1999).

Results from this *in vivo* study was consistent to the result reported earlier by Phongjit (2003). In the study of Phongjit (2003), 80% ethanolic extract of *C. asiatica* was given to male rats at doses of 250 and 1000 mg/kg/day for 30 days. No effects of the ethanolic extract of *C. asiatica* were found on CYP1A1, 1A2, 2B1/2B2, 2E1 and 3A at both dosages of the extract. In the present study CYP2B1/2B2 was shown to be inhibited by the standardized extract of *C. asiatica* in the *in vitro* study, with an IC_{50} of 523 μg/ml (as determined by BROD assay) and 563 μg/ml (as determined by PROD assay). A potent CYP 2B6 inhibitor, ticlopidine demonstrated an inhibitory effect clinically (Korhonen, et al., 2007), while in an *in vitro* study this compound possessed an IC_{50} of 0.084 μg/ml in this CYP isoform (Turpeinen, et al., 2005). The inhibitory effect of the standardized extract of *C. asiatica* found in this study may or may not be clinically significant, the issue of which needed to be clarified. Likewise, effect of this extract on CYP1A2 with an IC_{50} more than 1000μg/ml (2895 μg/ml) was rather high in comparison to furafylline, a potent inhibitor on CYP 1A2. Effect of this selective inhibitor of CYP1A2 was clinically significant compiling with an *in vitro*

study showing inhibitory effect with the IC_{50} of 0.72 μg/ml (Krippendorff, et al., 2007).

An inhibitory effect of this extract on CYP2B1/2B2 found in an *in vitro* study in which the extract was simultaneously added in to the reaction with BR or PR, a selective substrate of CYP2B1/2B2. However, no inhibitory effect was noted in the *in vivo* study. This finding suggested that some constituents in the extract could probably be a substrate these CYP isoformes. Thus, adding together with the selective substrate during performing the reaction showed a decreased rate of the selective substrate oxidation. In addition, inhibitory effect that was shown *in vitro* but not shown *in vivo* rather indicated that the inhibition was reversible. Thus, the inhibition effect could not be detected *in vivo* due to the recovery of the enzyme. Further study is needed to clarified regarding types of inhibition on these CYPs isoformes by the standardized extract of *C. asiatica.*

With regards to the drug-drug interaction issue, no induction effects of the standardized extract of *C. asiatica* was observed on CYP1A1, 1A2, 2B1/2B2, 2E1, 3A which normally bioactivate many procarcinogen, medicines and other environmental xenobiotics, offered an advantage of this extract in term of chemical bioactivation. Safety in term of no increase risk to toxicity, mutagenenis and/or carcinogenesis from other xenobiotics that are metabolic activated by these CYP isoforms is offered following long term use of the extract. Such xenobiotics, that are metabolic activated by CYP isoforms, investigated in this study are as following: Polycyclic aromatic hydrocarbon (PAHs) such as naphthalene, Benzo[a]pyrene etc., are bioactivated by CYP1A1; 2-amino-1-methyl-6-phenylimidzo [4,5-b]pyridine, 2-naphthylamine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone etc., are bioactivated by CYP1A2; nicotine are bioactivated by CYP2B6; benzene, styrene, acrylonitrile, vinyl carbamate, vinyl chloride are bioactivated by CYP2E1; aflatoxin B1, aflatoxin G1, sterigmatocystin, 7,8-dehydroxy-7,8-dihydrobenzo(a) pyrene are bioactivated by CYP3A (Coleman, 2005 and Schoedel et al, 2001).

In this study, Wistar rats of both sexes, male and female were use and the duration of treatment was 90 days. This was due to the fact that these groups of animals were designed for subchronic toxicity study of the standardized extract of *C. asiatica.* Thus, the experiment was designed according to the protocol suggested by WHO guideline (WHO, 1993). In addition to the subchronic toxicity data, the data on long term used of the extract obtained from the same group of experimental animals

were also beneficial in term of safety regarding drug-drug interaction and the possibility to increase/decrease risk to xenobiotic exposure. Doses of the standardized extract of *C. asiatica* used in this study were 10, 100 and 1000 mg/kg/day. The lowest dose (10 mg/kg/day) was the dose that was found to possess the beneficial effect of learning and memory enhancer in rats (มะยุรี ตันติสิระ และคณะ, 2550)

To investigate effects of the standardized extract of *C. asiatica* on hepatic CYP activities, selective substrate of the individual CYP were used. Rate of the selective substrate oxidation was considered to represent the corresponding CYP activity in hepatic microsome of rats treated with the extract. ER, MR and BP&PR have been proved to be selective substrate of CYP1A1, 1A2, 2B1/2B2, respectively (Burke and Mayer, 1974, Lubet et al. 1985). Aniline hydrochloride and erythromycin stearate have been suggested to be used as selective substrates of CYP2E1 (Schenkman et al., 1967) and CYP3A (Nash et al. 1953), respectively. Before using all oxidation reaction assays to determine the activity of CYP in the microsome samples, the assays were verified by performing linearity assay.

Linearity of the assays was shown by the coefficient of determination (r^2) between amount of microsomal protein and the absorbance of the product formed from the reaction. Correlation coefficient of assay for CYP1A1 (EROD), CYP1A2 (MROD), CYP2B1/2B2 (BROD, PROD), CYP2E1 (aniline 4-hydroxylase) and CYP3A (erythromycin N-demethylase) were 0.9942, 0.9924, 0.9955, 0.9837, 0.9920 and 0.9939, respectively.

In conclusion, subchronic effects of the standardized extract of *C. asiatica* on hepatic CYPs were investigated both *in vivo* and *in vitro*. In an *in vivo* study, both male and female rats were given orally with the standardized extract of *C. asiatica* at the doses of 10, 100 and 1000 mg/kg/day for 90 days, compared to the corresponding sex, control group given distilled water in the same manner. The results showed that the standardized extract of *C. asiatica* did not cause any changes on total CYP contents and the activities of CYP1A1, 1A2, 2B1/2B2, and 2E1 and 3A *in vivo*. Likewise, no effects of the extract on CYP1A1, 2E1 and 3A were shown in the *in vitro* study using rat liver microsomes. Decrease of CYP2B1/2B2 and CYP1A2 activities by the standardized extract of *C. asiatica* were shown in the *in vitro* study. The inhibitory effects were shown with the IC_{50} of 523 μg/ml (BROD), 563 μg/ml (PROD) for CYP2B1/2B2 and IC_{50} of more than 1000 μg/ml (2895 μg/ml) for

CYP1A2. Further study regarding types of inhibition as well as effects of the extract on other human CYP isoforms should be performed.