CHAPTER V

DISCUSSION AND CONCLUSION

This study was preformed primarily to investigate effects of *C. comosa* ethanolic extract on hepatic CYPs involving in metabolism of various drugs and metabolic activations of various mutagenic and/or carcinogenic xenobiotics. This would partly provide a preliminary information of drug-drug interactions of *C. comosa* as well as its potential either to afford antimutagenic/anticarcinogenic effects against xenobiotic-induced carcinogenesis or on the other hand, to increase risk of xenobiotic-induced mutagenesis/carcinogenesis. Drug-drug interaction potential of *C. comosa* would be indicated if this plant is taken concomitantly with other medicines which are metabolized by the CYP isoforms modulated by the plant. The study was performed in female Wistar rats which were given the ethanolic extract of *C. comosa* orally at dosages of 100, 250 and 500 mg/kg/day, for 30 consecutive days.

C. comosa is traditionally used for a wide range of indications, such as for postpartum uterine pain, enhancement of uterine involution, etc. Repeated administration of this plant is recommended for good effectiveness. Long term exposure of *C. comosa* may affect CYPs, the important phase I hepatic drug metabolizing enzymes, especially CYP isoforms that play an important role in drug metabolism as well as in chemical-induced toxicity, mutagenesis and/or carcinogenesis. These CYP isoforms include CYPs 1A1, 1A2, 2B1/2B2, 2E1 and 3A (Soucek and Gut, 1992).

Results from this study showed that these three dosages of *C. comosa* ethanolic extract did not changes hepatic microsomal total CYPs contents as well as the activities of CYPs 1A1, 1A2, 2E1 and 3A. No effects of *C. comosa* on those isoforms of CYP would be an advantageous characteristic of this extract in term of risks to chemical-induced toxicities, mutagenesis, and/or carcinogenesis as well as drug-drug interactions. No induction effects on these isoform of CYPs ruled out the potential increased risks of the extract on xenobiotic-induced toxicities, mutagenesis, and/or carcinogenesis. Examples of xenobiotics which are bioactivated by the individual CYPs isoform are as following:

benzo(a)pyrene, 7,12-dimethylbenz(a)anthracene, 2-naphthylamine, 2-acetylfluorene, N-methyl-4-aminoazobenzene, 6-nitrochrysene and aflatoxin B_1 are activated by CYP 1A1; 2-amino-3,4,8-trimethylimidazo[4,5-f]quinoxaline, 2-acetylfluorene, 2-aminofluorene and aflatoxin B_1 are activated by CYP 1A2; N,N'-nitrosodimethyllamine, N-nitroso-N-benzyl-N-methylamine, N-nitroso-N-diethylamine, nitrosodimethylamine, acetaminophen and benzene are activated by CYP 2E1; aflatoxin B_1, benzo(a)pyrene, 6-nitrochrysene, 2-amino-3,4,8-trimethylimidazo[4,5-f]quinoxaline and tris(2,3-dibromopropyl) are activated by CYP 3A (Soucek and Gut, 1992). No inhibition effects of *C. comosa* ethanolic extract on these CYP isoform, excluded an utilization of this aspect to explain the chemoprotective of this extract against procarcinogens bioactivated by these CYPs. Likewise, no effects of this extract on these CYPs excluded the possibilities of drug-drug interactions or drug-food interactions if *C. comosa* ethanolic extract is consumed concomitantly with any medicines that are metabolized by these CYPs. Examples of such therapeutic drugs that are metabolized by CYP 1A1 are warfarin, paracetamol; by CYP 1A2 are paracetamol, theophylline, tamoxifen, chlorpromazine; by CYP 2E1 are paracetamol, chlorzoxazone; by CYP 3A are clarithromycin, carbamazepine, erythromycin, etc (Lin and Lu, 1998).

Results from this study showed an increase activity of CYP2B1/2B2 by *C. comosa* administration at the dosages of 250 mg/kg/day and 500 mg/kg/day. And the induction was shown to be dose-related with a highly positive correlation to the doses of *C. comosa* administration. Induction effects of this extract on these isoforms of CYPs indicated potential increased risks of the extract on xenobiotic-induced toxicities, mutagenesis, and/or carcinogenesis. Examples of xenobiotics bioactivated by CYP 2B1/2B2 are aflatoxin B_1, benzo(a)pyrene, methylene chloroaniline, aminoanthracene and N,N'-nitrosodimethylamine (Soucek and Gut, 1992). Induction effects of this extract on CYP2B1/2B2 also indicated the possibilities of drug-drug interaction if *C. comosa* ethanolic extract is consumed concomitantly with other medicines that are metabolized by this isoform of CYP. Examples of such therapeutic drugs that are metabolized by CYP 2B1 are phenobarbital, cyclophosphamide; by CYP 2B2 are phenobarbital

(Berthou, 2001). CYP2B1/2B2 are not presented in human. Comparing the similarily of cDNA and sequences of the protein enzyme, rat CYP 2B1 is closely analogous to human CYP 2B6 (Yamano *et al.*, 1989; Soucek and Gut, 1992) which is expressed at very low level in human liver, approximately 0.2 % of total CYP (Redic and Di Carlo, 1997). CYP 2B6 is reported to play a role in the bioactivation reactions of 6-aminochrysene and 3-methoxy-4-aminoazobenzene (Gonzalez and Gelboin, 1994) and the metabolisms of cyclophosphamide, testosterone (Redic and Di Carlo, 1997). There is an evidence suggesting that CYP 2B6 may be of minimal functional significance in human liver and that variabilities exist across species in the inducibility of CYP 2B (Murray, 1999). Thus, induction of CYP2B1/2B2 by *C. comosa* administration in rat may or may not affect the metabolism or bioactivation of xenobiotics that are metabolized or bioactivated by CYP2B6 in human. Further study was suggested to explore the mechanism of induction of CYP2B1/2B2 by *C. comosa* ethanolic extract. Effect of this extract on other CYP isoforms that had not been studied was also suggested to be investigated.

This study provided an additional subacute (30 days) toxicity data for *C. comosa*. Results from this study showed that all three dose of *C. comosa* ethanolic extract used in this study did not affect body weight, food&water consumptions. In contrast, an increase of both liver weight and relative liver weight was observed in rats receiving the extract at 250 and 500 mg/kg/day as compared to the control group. The explanation for this increment might be find out if histopathological of the liver was explored whether it is due to an accumulation of fat in the liver or other causes. An induction hepatic drug metabolism enzymes (Williams, *et al.*, 2000), an increase blood flow to the liver and an inflammation of the liver associated with the accumulation of fat in the liver (California Pacific Medical Center, 2001), etc can be a contribution cause of this increase of liver weight. Results from this study showed that *C. comosa* given orally at 100, 250 and 500 mg/kg/day did not cause any toxic effects to the hematopoietic system and many important organs such as liver, kidneys as well as most serum electrolytes, carbohydrate and lipid metabolism. 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 those of the control group. However, the significantly higher ALP and potassium level were still within the normal range. The subacute toxicity data from this study was quite comparable to a previous study of Chivapat, *et al.* (2003). In that study, subchronic toxicity of *C. comosa* ethanolic extract was investigated by giving oral administration of 100, 200, 400 and 800 mg/kg/day of the extract to male and female rats for 90 consecutive days. They found that the extract did not affect growth and food consumption of rats. Decreases of hematocrit and hemoglobin in male rats receiving the highest dose of the extract were still within the normal range. Male rats treated with 800 mg/kg and female rats receiving 400 and 800 mg/kg of the extract had a significant increase of alkaline phosphatase level. However, the significantly higher ALP level were still within the normal ranges. Generally, an increase of serum ALP indicates an injury of bile duct epithelium which leads to liver cholestasis (Kerai, *et al.*, 2001). Therefore, these two consistant findings of an increase of serum ALP by *C. comosa* ethanolic extract indicated that liver function may be affected if this extract is administered at high dose or is given for long period of time. In this study, serum estradiol was determined in all rats. Estradiol concentrations of rats receiving the extract at the doses of 250 and 500 mg/kg were significantly higher than those of the control. The increase of estradiol concentrations in rats treated with *C. comosa* ethanolic extract were shown to be dose-related and showed a highly positive correlation between doses of administration and the serum estradiol level. Some constituents in *C. comosa* ethanolic extract may exert estrogenic activity which might be beneficial for an indication of hormone replacement therapy. However, toxicity study must be more extensively exercised if this extract will be used clinically for this indication.

In this study, *C. comosa* was extracted with 95% ethanol, the fraction which was shown to possess uterotrophic effect (อนุกูล สวัสดิ์พาณิชย์, 2537). Morever, ethanolic extract was closely similar to the way of using this plant traditionally. Before using this extract in the experiment, the extract was identified *via* a chemical identification. In this study, ethanolic extract of *C. comosa* was determined for 1,7 diphenyl-4,6-heptadiene-

3-ol which was of the active ingredient found in the extract using HPLC. Doses of *C. comosa* ethanolic extract used in this study was 100, 250 and 500 mg/kg/day. The dose of 100 mg/kg/day used in this study was the dosage regimen shown to decrease plasma triglyceride level and increase liver triglyceride content (Piyachaturawat *et al.*, 1997). The dosage of 250 mg/kg/day used in this study was shown to possess uterotrophic effect (Piyachaturawat *et al.*, 1995a). And, the dosage of 500 mg/kg/day used in this study was also the dosage regimen shown to possess uterotrophic effect and estrogenic-like action (Piyachaturawat *et al.*, 1995a, 1995b, 1998, 1999; อนุกูล สวัสดิ์พาณิชย์, 2537). This highest dose was also shown to possess antihypercholesterolemic effect (Piyachaturawat et al., 1997) as well as increase bile flow rate (Piyachaturawat *et al.*, 1996).

To investigate effects of *C. comosa* ethanolic extract on hepatic CYPs, specific substrate of the individual CYP was used and rate of specific substrate oxidation was determined to represent the corresponding CYP activity in hepatic microsomes of rats treated with the extract. ER, MR and PR&BR have been proved to be specific substrates of CYP 1A1 (Burke and Mayer, 1974), CYP 1A2 (Burke, *et al.*, 1985) and CYP 2B1&2B2 (Burke, *et al.*, 1985; Lubet, *et al.*, 1985), respectively. Aniline 4-hydroxylation was shown to represent CYP 2E1 activity (Schenkman, *et al.*, 1967) while erythromycin N-demethylation was classically used for determining CYP 3A activity (Nash, 1953; Friedli, 1992).

Hair loss occurred in the *C. comosa* treated rats. This effect has been reported to induced by chronic estrogen treatment (Biegel *et al.*, 1998; Gibson *et al.*, 1967). Although less studies were performed on estrogens than on androgens, prolonged intraperitoneal, subcutaneous implant or oral administration of estrogens has been shown to block hair growth in rats and mice (Smart *et al.*, 1999; Biegel *et al.*, 1998; Gibson *et al.*, 1967). Topical ICI 182, 780, a pure estrogen receptor antagonist, stimulates hair regrowth in male mice (Smart *et al.*, 1999). Hair follicle is a complex structure that is influenced by systemic factors including androgens, glucocorticoids

and estrogens. The estrogen receptor pathway within dermal papilla regulates the telogen-anagen transition of the hair follicle in CD-mice (Oh and Smart, 1996). Thus, hair loss found in *C. comosa* treated rats shown in this study was possibly attributed from estrogenic-like effect of *C. comosa*.

In conclusion, subacute (30 days) effects of *C. comosa* ethanolic extract on hepatic CYPs and clinical blood chemistry and hematology were investigated. Three doses (100, 250 and 500 mg/kg/day) of the extract were given orally to female rats for 30 days compared to control group given corn oil in the same maner. The results showed that *C. comosa* ethanolic extract did not affect rat hepatic microsomal total CYP contents and the activities of, CYP1A1, CYP1A2, CYP2E1 and CYP3A. The activities of CYP2B1/2B2 were significantly dose-dependent increased by *C. comosa* administration at the dosages of 250 and 500 mg/kg/day. Serum alkaline phosphatase and potassium levels were significantly increased in rats receiving the extract at 500 mg/kg/day. All three doses of *C. comosa* used in this study did not cause any serious toxicity to many important organs/systems such as liver, kidney, hematopoietic, electrolytes, as well as carbohydrate and lipid metabolism. Further studies on the effects of *C. comosa* at various doses, long term used as well as mechanism of induction of CYP2B1/2B2 was suggested. Effects of this compound on other isoforms of CYP should also be explored.