CHAPTER V

DISCUSSION AND CONCLUSION

This study was performed to investigate effect of *C. comosa* hexane and ethanolic extracts on hepatic CYP. As mentioned earlier, modulation of CYP (either inhibition or induction) by any compounds is associated with drug-drug interaction resulting in drug toxicity or on the other hand a decrease of drug efficacy (Yan and Caldwell, 2001). In addition, induction of some isoforms of CYPs by any compounds may lead to an increase risk of toxicity/mutagenesis/carcinogenesis induced by many chemicals/environmental toxicants which are bioactivated by that CYP isoform. On the other hand, inhibition of CYP by any compounds may lead to a decrease risk of (or a protection from) toxicity/mutagenesis/carcinogenesis induced by many chemicals/ many environmental toxicants which are bioactivated by that CYP isoform (Sato and Aoki, 2002). In order to develop *C. comosa* for a medicinal purpose associated with its estrogenic activity, effect of this plant extracts on various CYP isoforms that play an important role in drug metabolism and xenobiotic bioactivation should be investigated.

In this study, hexane and ethanolic extracts of *C. comosa* were given orally to male Wistar rats at the doses of 250 and 500 mg/kg/day for 30 days. The doses 250 and 500 mg/kg/day of hexane extract were shown to possess uterotrophic and estrogenic-like effect (Piyachaturawat et al., 1995a) whereas ethanolic extract at the doses of 250 and 500 mg/kg/day were shown to decrease plasma triglyceride level and increase liver triglyceride content (Piyachaturawat et al., 1997).

Regarding effect of *C. comosa* on hepatic CYPs, it was found that both hexane and ethanolic extracts of *C. comosa* given at both doses in this study, did not affect the activities of CYP1A2, CYP2E1 and CYP3A. No effects of *C. comosa* extracts on these isoforms rule out the possibilities of drug-drug interaction when *C. comosa* extracts are given concomitantly with other medicines that are metabolized by CYP1A2, CYP2E1 and CYP3A. In addition, these findings rule out the possibility of *C. comosa* extracts to increase/decrease risks to chemical-induced

toxicity/mutagenesis/carcinogesis from other environmental xenobiotics that are bioactivated by these CYPs during *C. comosa* repeated administration.

Normally, CYP3A is the most abundantly CYP (accounted for approximately 30-50% of the total CYP that is expressed in human liver and intestine (Gibson and Skette, 2001). Particularly, CYP3A4 catalyzes the metabolism of numerous commonly used drugs (Guengerich, 1999). Examples of medicines that are metabolized by CYP3A4 are acetaminophen, clarithromycin, codeine, cyclophosphamide, cyclosporine, dapsone, dextromethorphan, diazepam, diltiazem, erythromycin, felodipine, imipramine, lidocaine, losartan, lovastatin, midazolam, nifedipine, quinidine, ritronavir, sulfamethoxazole, tamoxifen, terfenadine, triazolam, troleandomycin, verapamil, warfarin, etc. (Wilkinson, 1996; Guengerich, 1999; Patt et al., 1992). The chemicals or procarcinogens that are bioactivated by CYP3A4 include aflatoxin B1, aflatoxin G1, 1-nitropyrene, 6-aminochysene, estradiol, etc. (Guengerich, 1992; Omiecinski et al., 1999; Lewis et al., 1998).

CYP1A2 is constitutive expressed in the liver with approximately 13% of the total CYP (Faber et al., 2005) and also induced by various exogenous compounds. This CYP isoform is responsible to metabolism of many currently medicines such as carbamazepine, paracetamol, caffeine, ondansetron, omeprazole, phenacetin, tacrine, tamoxifen, theophylline, propanolol, amitriptyline, etc. (Cupp and Tracy, 1998; Lin and Lu, 1998; Batt et al., 1992). Examples of chemicals and procarcinogens that are bioactivated by CYP1A2 are 4-aminobiphenyl, 2-naphthylamine, 2-aminofluorene, 3-methylcholantrene, arylamines, arylamide and etc. (Lewis et al., 1998; Guengerich, 1992; Butler et al., 1989; Kondraganti et al., 2002).

CYP2E1 is constitutive expressed in the liver with approximately 6.6 % (Yan and Caldwell, 2001). This CYP isoform play a role in metabolism of many common medicines such as acetaminophen, chlorzoxazone, ethanol, etc (Lin and Lu, 1998; Cupp and tracy, 1998). Examples of chemicals and procarcinogens that are bioactivated by CYP2E1 are benzene, styrene, acrylonitrile, vinyl carbamate, vinyl chloride, vinyl bromide, ethyl carbamate, trichloroethane, tetrachloromethane, chloroform, etc. (Lewis et al., 1998).

Both *C. comosa* hexane extract (given at the doses of 250 and 500 mg/kg/day) and ethanolic extract given at the dose of 250 mg/kg/day caused an increase of total CYP content and CYP1A1 activity. CYP1A1 catalytic activity was

increased 2.5, 2 and 2 times to the control group in rats treated with *C. comosa* hexane extract at the doses of 250 and 500 mg/kg/day and *C. comosa* ethanolic extract at the dose of 250 mg/kg/day, respectively. The induction of CYP1A1 activity by *C. comosa* was less than that found in the positive control group treated with β-NF at the dose of 80 mg/kg/day for 3 days, which increased CYP1A1 activity for 23 times to the corresponding control group. This characteristic of CYP1A1 induction by *C. comosa* seem to indicate that the extract might be a substrate of CYP1A1. Then following the extract administration, enzyme is induced so as to sufficiently play it role. However, at high dose of the extract administration, an increase of the enzyme is utilized to metabolize the constituents in the extract. So an increase of CYP1A1 activity following *C. comosa* extracts was less at the higher dose (500 mg/kg/day) of *C. comosa* extracts administration compared to the lower dose (250 mg/kg/day) of the extract administration. An increase of CYP1A1 activity by *C. comosa* is of interest regarding the concern of *C. comosa* administration that may cause an increased risk of human to several environmental-induced mutagenesis/carcinogenesis. Normally, CYP1A1 is predominantly expressed in extra-hepatic tissues particularly in human lung (Guengerich et al., 1992) and is present in low amounts in the non-induced liver. Due to the induction, hepatic and non-hepatic CYP1A1 can be increased several folds (Beresford et al., 1996; Zhang et al., 1997). CYP 1A1 is an important enzyme that play a key role in the metabolic activation of a large group of polycyclic aromatic hydrocarbons (PAHs) to biologically reactive metabolites that interact with DNA, resulting in carcinogenesis (Hammons et al., 1997; Brunoa and Njar. 2007). At the same time, CYP1A1 are also induced by the PAHs such as 3-methylcholanthrene, β-naphthoflavone, benzo[a]pyrene (Gibson and Skette, 2001; Kondraganti et al., 2002). CYP1A1 have been used as biomarkers of exposure to certain classes of contaminants in both aquatic and terrestrial species (Melancon, 2004). Normally, an induction of CYP1A1 involves a specialized cytosolic receptor called Ah receptor. Binding to the Ah receptor, the complex accompanying with other transcription factor such as Arnt, then translocate into the nucleus and activate gene transcription. Whether *C. comosa* extracts increase CYP1A1 via this mechanism need to be further investigated.

CYP2B1/2B2 activities were significantly increased in rats treated with both *C. comosa* hexane extract and ethanolic extract. This finding was consistent to the

result of Suknoy (2004). In that study an induction of CYP2B1/2B2 was found in hepatic microsomes of female rats given the crude ethanolic extract of *C. comosa* at 250 and 500 mg/kg/day for 30 days. Degree of CYP2B1/2B2 activity enhancement produced by *C. comosa* hexane extract is significantly higher than by the ethanolic extract. Corresponding with the finding that hexane extract of *C. comosa* exerted more estrogenic-like effect than the ethanolic extract, this seem to be consistent to the finding that compounds with estrogenic-like effect (such as estradiol, equilenin, diethyl stilbestrol, DDT etc.) always have an induction effect on these CYP isoforms (Jinno et al., 2006). However, CYP2B1/2B2 activities, which represented by BROD were increased 16, 24, 5 and 4 times in rats treated with *C. comosa* hexane extract at the doses of 250 and 500 mg/kg/day as well as *C. comosa* ethanolic extract at the doses of 250 and 500 mg/kg/day, respectively. The induction of CYP2B1/2B2 by *C. comosa* extracts was lower than that found in phenobarbital-induced rats, of which rate reaction of BROD was increased 41 times to the corresponding control. Likewise, the increments rate reaction of PROD in *C. comosa* treatment groups (14, 15 ,7 and 8 times for *C. comosa* hexane extract at the doses of 250, 500 mg/kg/day and *C. comosa* ethanolic extract at the doses of 250 and 500 mg/kg/day, respectively) were lower than that found in the phenobarbital-induced rats (42 times to the corresponding control group). Actually, CYP2B1/2B2 are not expressed in human liver. Amino acid sequence homology of human CYP2B6 is closest to rat CYP2B1/2B2. Even though CYP2B6 is expressed in human liver with a very small amount, it is also play an important role in metabolism of several drugs such as cyclosporine, cyclophosphamide, ifosfamide, diazepam, etc. (Omiecinski et al., 1998). CYP 2B6 also associates with the metabolic activation of procarcinogen such as 6-aminochrysene, styrene, etc. resulting in the chemical-induced carcinogenesis (Omiecinski et al., 1998). CYP2B6 expressing cells were found to be more sensitive than control cells to the cytotoxicity of cyclophosphamide, aflatoxin B_1 and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (Code et al., 1917). Therefore, the expected development of *C. comosa* hexane and ethanolic extracts for the medicinal or the food supplement purpose for human need to concern the possibility of drug-drug interaction if these extracts are given concomitantly with any medicines that are metabolized by this isoform of CYP. In addition, long-term use of the extracts need to be concerned in term of an increase risks to other chemical-induced carcinogenesis

resulting from an induction effect of these extracts on the enzyme that bioactivates those chemicals.

Regarding the subacute toxicity effect of *C. comosa* hexane and ethanolic extracts, rat clinical blood chemistry and hematology were investigated following 30 days of extract administration. In addition, body weight gain, food and water consumption were recorded during the treatment period while liver weights were recorded at the time of sacrification. The results showed that both *C. comosa* hexane and ethanolic extracts given at 250 mg/kg/day or 500 mg/kg/day caused significant lower body weight gain as compared to rats in the control group. Decrease of body weight gain from *C. comosa* extract administration was not due to the decrease of food and water consumption because these two parameters were not significantly different from the control group. The etiology under this decrease of body weight gain from *C. comosa* administration need to be investigated. Percent relative liver weight (g of liver/100 g of body weight) of rats only in the group that received *C. comosa* hexane extract (not in the group that received *C. comosa* ethanolic extract) was significantly higher than that of the control rats. Cause of this increment of liver weight was not investigated in this study. The more induction of hepatic enzymes (such as CYP2B1/2B2) from *C. comosa* hexane extract than the ethanolic extract seem to be corresponded with the higher liver weight of rats in the hexane extract group than that of rats in the ethanolic extract group.

Effects of *C. comosa* hexane and ethanolic extracts on clinical blood chemistry and hematology were consistent with the previous study of Suknoy (2004) and Chivapat et al. (2003). Both extracts of the *C. comosa* at the dosages and duration used in this study did not cause any harmful effects on hematopoetic system, functions of the liver and kidney, serum electrolytes as well as the metabolism of carbohydrate. Beneficial effect of *C. comosa* extracts on metabolism of lipid was shown by a decrease of serum cholesterol from the hexane extract at a dose of 500 mg/kg/day and decrease of triglyceride level from the ethanolic extract at both doses given but only at 500 mg/kg/day of the hexane extract. Serum triglyceride lowering-effect of the ethanolic extract of *C. comosa* found in this study was consistent with the results reported earlier by Chivapat et al. (2003) that used the crude ethanolic extract of this plant. Furthermore reduction of serum cholesterol and triglyceride by *C. comosa* extracts was consistent to the result of Piyachaturawat et al. (1999), who

reported the plasma cholesterol lowering effect of *C. comosa* extract in hypercholesterolaemic hamsters. The results in that study showed that intragastric administration of ethyl acetate extract of *C. comosa* caused a decreased of plasma cholesterol and triglyceride level with a dose-dependent manner. In addition, cholesterol and triglyceride lowering effects of *C. comosa* in the present study was consistent to the effects of phloracetophenone in hypercholesterolemic hamsters which reported by Piyachaturawat et al. (2002). In that study phloacetophenone caused a decrease in plasma cholesterol, triglyceride, VLDL and LDL-C level in hypercholesterolemic hamsters. Actually, rats seem not to be a good animal model for studying a lipid-lowering effect of the compounds. As using hamsters for study this effect by *C. comosa* extracts, beneficial effects of these extracts were shown by an increasing of HDL-C and the lowering serum lipid parameters such as total cholesterol, triglyceride , VLDL and LDL-C (Piyachaturawat et al., 1999b; 2002a).

In conclusion, *C. comosa* hexane and ethanolic extracts, given to male Wistar rats at doses of 250 and 500 mg/kg/day for 30 days, did not cause any changes of CYP1A2, CYP2E1 and CYP3A activities. *C. comosa* ethanolic extract caused an increase of CYP1A1 activity only when the extracts were given at 250 mg/kg/day but not at 500 mg/kg/day whereas both doses of *C. comosa* hexane extract significant increased CYP1A1 activity. Both of the extracts caused a dose-dependent increase of CYP2B1/2B2 activities with the degree of enhancement was significantly higher by the hexane extract than by the ethanolic extract. Modulation of CYP1A1 and CYP2B1/2B2 activities provided a preliminary data of *C. comosa* hexane and ethanolic extracts regarding the drug-drug interaction and a possibility to increase risks to chemical-induced toxicity/mutagenesis/carcinogenesis during repeated administration of the extracts. In addition, subacute toxicity of *C. comosa* hexane and ethanolic extracts was also investigated by determination of clinical blood chemistry and hematology. It was shown that both hexane and ethanolic extracts of *C. comosa* given at both dosage regimens in this study did not cause any harmful effects on the function of importants organs or systems such as liver, kidney, serum electrolytes, hematopoietic system and metabolism of carbohydrate and lipid. Further study should be explored regarding the mechanism of induction of *C. comosa* extracts on CYP1A1 and CYP2B1/2B2. Whether or not the major active compounds found in these extracts such as diarrylheptanoids in the hexane extract or phloracetophenones in the

ethanolic extract play a role in these induction effect of *C. comosa* need to be clarified.