CHAPTER V

DISCUSSION AND CONCLUSION

Recently, dietary supplements with an inhibition property on mutagenesis and/or carcinogenesis are particularly of interested since they may be valuable for human chemoprevention. Many compounds with antimutagenic and anticarcinogenic properties are found naturally in plants such as fruits, vegetables, spices, and herbs (Wattenberg, 1983; Hayatsu *et al.*, 1988; Ferguson, 1994). *M. loriformis* is a medicinal plants that are used traditionally by several patients suffering from various types of cancer. It decreases some side effects caused by radiotherapy and chemotherapy in the process of cancer treatment. However, their mechanism of action have not yet been clarified. Generally, several mechanisms of chemopreventive effects are proposed such as antioxidant effect, prevention of carcinogen-binding to DNA, inhibition of DNA-adduct formation, enhancement of level of DNA repairment, etc (Boone *et al.*, 1990; Colic and Pavelic, 2000; Kakizoe, 2003). In addition, modulation of drug metabolizing enzymes, especially an inhibition of enzymes in phase I (i.e. CYP), enzymes responsible for the formation of reactive carcinogenic metabolites, or an induction of detoxification enzymes in phase II are also play a significant role in chemopreventive effect of many naturally compounds (Percival, 1997; Kakizoe, 2003).

This study focused on the effect of *M. loriformis* ethanolic extract on hepatic CYPs particularly the isoforms that are responsible for xenobiotic bioactivation such as CYP 1A1, 1A2, 2B1/2, 2E1, and 3A. Induction of these isoforms indicated the potential risks to chemical-induced toxicities, mutagenesis, and carcinogenesis of this plant if patients received chronically. In the other hand, antimutagenic and/or anticarcinogenic potential may be indicated if this plant possessed inhibition effects on these CYPs. In addition, cancer patients are normally polydrug therapy, modulation of hepatic CYPs by *M. loriformis* should be concerned in term of drug-drug interaction. In general, *M. loriformis* is taken traditionally to treat and relieve ailment of several types of cancers in forms of either pressed juice from fresh *M. loriformis* or powder of dried *M. loriformis* in capsules. In this study, dried powder of *M. loriformis* was extracted with 80% ethanol, the fraction of which was reported to be pharmacologically active in term of

antimutagenesis and chemopreventive effects (Vinitketkumnuen *et al.*, 1996; Intiyot *et al.*, 2002). Eighty percent ethanolic extract also provided more appropiate form of *M. loriformis* to administered to animal than using the dried powder of this plant. Eighty percent ethanolic extract of *M. loriformis* used in this study was expected to contain both important polar and non polar constituents in this plant despite somewhat different constituents as in pressed juice of fresh *M. loriformis* or dried *M. loriformis* powder, the forms of which were used traditionally. In the previous study, ratio of fresh whole plant of *M. loriformis* to dried powdered was 16.67:1 (Jiratchariyakul *et al.*, 1997). In this study, four kilograms dried powder of *M. loriformis* was extracted with 80% ethanol. Percentage yield of the extract was 16.34% w/w of the dried *M. loriformis* powder. Therefore, the doses of the extract 0.1 and 1.0 g/kg/day used in this study were equivalent to 10.2 g/kg/day (or 510 g/50 kg/day) and 102.0 g/kg/day (or 5100 g/50 kg/day) of fresh *M. loriformis*. As compared to the dosage used in human which is about 100-120 g/50 kg/day, doses of 0.1 and 1.0 g/kg/day used in this study was estimated to be five times and fifty times, respectively, of the dose suggested for human. Preliminary identification tests including color reaction tests and thin layer chromatography were performed according to the method suggested by Jiratchariyakul W. and Soonthornchareonnon N. (1995) before the extract was given to the animals. The results showed that *M. loriformis* ethanolic extract used in this study include phenolic compounds, phytosterols (i.e β-sitosterol, sitosteryl glucoside), steriodal compound (i.e. glycosphingolipid) which possessed similar characteristic pattern of the constituents conformed to the study reported by Jiratchariyakul W. and Soonthornchareonnon N. (1995). Two doses (0.1 and 1.0 g/kg/day) used in this study were the dosage regimens, shown to significantly inhibit azoxymethane-induced aberrant crypt focus formation in rat colon (Intiyot *et al.*, 2002).

Results from this study showed that rats given *M. loriformis* ethanolic extract at doses of 0.1 and 1.0 g/kg/day for 30 days did not modify the activity of CYP1A1, 1A2, 2B1/2, 2E1 and 3A. Lack of induction effects on these CYP isoforms suggesting beneficial characteristics of *M. loriformis* in the aspect of no potential to increase risks of chemical-induced toxicities, mutagenicities and/or carcinogenicities following repeated consuming of this plant. Chemicals/procarcinogens that are bioactivated by CYP1A1

including polycyclic aromatic hydrocarbon (PAHs) such as benzo(a)pyrene, 6-nitrochrysene, etc; by CYP1A2 including 2-acetylfluorene, 2-aminoanthracene, 2-naphthylamine, etc; by CYP2B1/2 including aflatoxin B_1, benzo(a)pyrene, 3-methylcholanthrene, etc; by CYP2E1 including benzene, carbon tetrachloride, chloroform etc. and by CYP3A including aflatoxin B_1, aflatoxin G_1, benzo(a)pyrene etc. (Soucek and Gut, 1992). Moreover no induction and inhibition of these CYP isoforms which are normally responsible for metabolisms of many therapeutic drugs, would be an advantageous of this plant in term of drug-drug interaction if concomitant administrations of this plant and the interacting drugs occur. The examples of drugs metabolized by CYP 1A1 are R-warfarin, amiodarone, etc; by CYP1A2 are acetaminophen, phenacetin, etc; by CYP2B6 (human CYP which is homology to rat CYP2B1/2) are cyclophosphamide, etc; by CYP2E1 are acetaminophen, chlorzoxazone etc. and by CYP3A are erythromycin, omeprazole, etc (Rendic and Di Carlo, 1997). No inhibition effects of *M. loriformis* ethanolic extract on the important xenobiotic-bioactivating CYP isoforms found in this study, exclude the possibilities to explain the chemopreventive, antimutagenic and/or anticarcinogenic effects of this plants. Effect of this extract on the phase II detoxification enzymes should be further studied to investigate if this extract modulate these enzymes resulting in the beneficial pharmacological effects mentioned above.

Acute and subchronic (3 months) toxicity studies of *M. loriformis* were performed in rats in form of pressed juice from fresh *M. loriformis* (พิมลวรรณ ทัพยุทธพิจารณ์ และคณะ, 2533; พิมลวรรณ ทัพยุทธพิจารณ์ และคณะ, 2534) at doses of 5, 10, 20 and 30 g/250 g body weight/day for acute toxicity and the doses of 0.7, 1.75, 3.5 g/250 g body weight/day for subchronic toxicity. No any sign of toxicity were observed. This study provided an additional subacute (1 month) toxicity data for *M. loriformis* ethanolic extract in rats at doses 0.1 and 1.0 g/kg body weight/day of *M. loriformis* ethanolic extract which were equivalent to 2.55 and 25.50 g/250 g body weight/day of fresh *M. loriformis*. Despite somewhat different in dosage regimen and preparation form of *M. loriformis* among the studies, results from this study supported the previous studies of no apparent toxicity of this plant in rats. However, species variation in drug metabolism

should be taken into consideration when extrapolating toxicity data of *M. loriformis* in animals to humans.

In conclusion, subacute effects of *M. loriformis* ethanolic extract on hepatic CYPs and clinical blood chemistry were studied in male Wistar rats. Two doses (0.1 and 1.0 g/kg/day) of the extract were given orally to rats for 30 days compared to the control group given distilled water in the same manner. The results showed that *M. loriformis* ethanolic extract caused no significant effect on total CYP contents and the activities of CYP 1A1, 1A2, 2B1/2, 2E1 and 3A. *M. loriformis* ethanolic extract did not produce any change of following clinical blood chemistry and hematology: AST, ALT, ALP, total bilirubin, direct bilirubin, BUN, SCr, total cholesterol, TG, LDL-C, HDL-C, glucose, sodium, potassium, chloride, hemoglobin, hematocrit, platelet count, WBC count, RBC count, % differential WBCs, RBC indices (MCV, MCH, MCHC), and RBC morphology. Further studies on the effects of this plant on human hepatic CYPs, hepatic phase II enzymes and human clinical blood chemistry are suggested.