CHAPTER V

DISCUSSION AND CONCLUSION

Anticancer action has been demonstrated by several natural compounds with multifunctional activities. Antioxidant properties, nitric oxide synthase inhibition (Colic M., Pavelic K., 2000), inhibition of phase I enzymes while enhancing of phase II enzymes, enhancing of DNA repair enzyme activities, etc. (Wang and SU, 2001) are normally proposed to explain the antimutagenic and/or anticarcinogenic effects of these compound. Results from this study showed that *M. citrifolia* fruit extract significantly decrease CYP1A1 activity at 1200 mg/kg/day but not at a dosage of 600 mg/kg/day. Due to high interanimal variations of the CYP1A1 activity results, significant inhibition effect on this CYP isoform was not observed at the latter dosage regimen despite an obvious tendency of inhibition. Since CYP1A1 plays a key role in activation of procarcinogens mainly in a class of polycyclic aromatic hydrocarbons (PAHs). The inhibition effect of *M. citrifolia* fruit extract on CYP1A1 found in this study is thus a subject of interest. This effect may in part explain the findings of Wang and Su (2001) that demonstrated the significant reduction of DMBA-DNA adduct formation in rats and mice *in vivo* following 7–day administration of *M. citrifolia* fruit juice in drinking water. In an *in vitro* study, they found that *M. citrifolia* fruit juice possessed strong antioxidant activities, the characteristic that they proposed that it may contribute to the cancer preventive effect found *in vivo* (Wang and Su, 2001). An inhibition effect of *M. citrifolia* fruit extract on CYP1A1 found in this study may be partly or additionally explained the cancer preventive effect of this plant besides its antioxidant properties proposed earlier. On this regard, *M. citrifolia* fruit is likely beneficial for cancer prevention against xenobiotics bioactivated by CYP1A1 such as many environmental PAH pollutants. However, inhibition effect of this extract on CYP1A1 which is responsible for metabolism of many drug such as *R*-warfarin, amiodarone, etc. (Frederick, 1997) should be concerned in term of drug-drug interaction if concomitant administrations of this extract and the interacting drugs occur.

No changes of CYP1A2, CYP2B1&2B2, CYP2E1 and CYP3A were observed following both doses of *M. citrifolia* fruit extract used in this study. No induction effects on these CYP isoforms are beneficial characteristics of *M. citrifolia* in an aspect that no potential increase risks of chemical-induced toxicities, mutagenicities and/or carcinogenicities following repeated consuming of this fruit extract. Such chemicals/procarcinogens that are bioactivated by CYP1A2 include 2-acetylfluorene, 2-aminoanthracene, 2-naphthylamine, etc; by CYP2B1&2B2 include aflatoxin B_1, benzo(a)pyrene, 3-methylcholanthrene, etc; by CYP2E1 are benzene, carbon tetrachloride, chloroform etc. and by CYP3A are aflatoxin B_1, aflatoxin G_1, benzo(a)pyrene etc. (Soucek and Gut, 1992). Moreover, no modulation of these CYP isoforms which are normally responsible for metabolisms of many clinical drugs, would be an advantageous of this fruit extract in term of drug-drug interaction if concomitant administrations of this fruit extract and the interacting drugs occur. The examples of drugs that are metabolized by CYP1A2 are acetaminophen, phenacetin, etc; by CYP2B1&2B2 are phenobarbital etc; by CYP2E1 are acetaminophen, chlorzoxazone etc. and by CYP3A are erythromycin, omeprazole, etc. (Rendic and Di Carlo, 1997)

Following both doses of *M. citrifolia* fruit extract used in this study, no subacute toxicities were observed on many important organs/systems such as liver, kidney, blood system, electrolytes as well as lipid and carbohydrate metabolisms. These were indicated by the results of hematology and clinical blood chemistry. In addition, rats in both groups treated with *M. citrifolia* fruit extract did not demonstrate any adverse effects on body weight, relative liver weight, food and water consumptions. These results are consistent to the results reported recently by Glerup (2001). In that study, they performed a 13-week oral toxicity study in rats using a commercial *M. citrifolia* fruit juice at various daily doses (0.4, 4, 8, 50 and 80 ml/kg). The results in that study showed that all groups showed no treatment related differences in body & organ weight, food consumption, clinical examination, blood chemistry, hematology and histological tissue examination (Glerup, 2001; Wang *et al*, 2002). The actual daily dose recommended of that commercial *M. citrifolia* fruit juice

for human is approximately 0.5 ml/kg which is in the range of doses that did not demonstrate any significant adverse effects in animals reported by Glerup (2001). However, species variation in drug metabolism should be concerned when extrapolating toxicity data of animals to humans.

Doses of *M. citrifolia* fruit extract used in this study were 600 and 1200 mg/kg/day. The former dosage regimen was estimated corresponding to the dosage of *M. citrifolia* fruit juice that demonstrated a cancer preventive effect reported by Wang and Su (2001). In that study, rats and mice were given 10% commercial *M. citrifolia* fruit juice in drinking water for 7 days before administration of DMBA on the 8^{th} day. The results showed that DMBA-DNA adduct formations were reduced in several organs of both mice and rats as compared to the corresponding control groups pretreated with water instead of *M. citrifolia* fruit juice. The higher dosage regimen (1200 mg/kg/day) was also added in this study in order to investigate the dose-related effects of interest.

M. citrifolia fruit extract used in this study was quantitated for carbohydrate content since this fruit was mainly composed of polysaccharides accompanying with the findings that the extract possessed immunomodulator and anti-tumor/anticancer effects (Hirazumi, 1999). Immunomodulatory polysaccharides are currently emerging as promising immunotherapeutic agents in the treatment of cancer (Wong *et al.*, 1994). Preclinical studies of several polysaccharides isolated from higher plants, mushrooms and seaweeds have demonstrated antitumour activity against transplantable tumors in mice (Sakagami *et al.*, 1987; Yamada *et al.*, 1990; Muller *et al.*, 1989; Tsukagoshi *et al.*, 1984; Chihara, 1991; Furusawa and Furusawa *et al.*, 1985; Furusawa *et al.*, 1992; Furusawa *et al.*, 1995; Yamamoto *et al.*, 1974). Result from this study showed that *M. citrifolia* fruit extract contained 0.47 ± 0.01 mg of carbohydrate per mg of the extract. This indicated that the extract was primarily composed of carbohydrate. This result was quite consistent to the finding reported earlier by Hirazumi and Furusawa, 1999.

In conclusion, subacute effects of *M. citrifolia* fruit extract on hepatic CYPs and clinical blood chemistry were studied in male Wistar rats. Two doses (600 and 1200 mg/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. citrifolia* significantly decreased CYP1A1 activity at a dosage of 1200 mg /kg/day but not at a dosage of 600 mg/kg/day. No changes of CYP1A2, CYP2B1&2B2, CYP2E1 and CYP3A activities were observed following both dosages of the extract. Likewise, no subacute toxicities were observed on many important organs/systems at both doses of the extract. Inhibitory effect of *M. citrifolia* fruit extract on CYP1A1 may partly explain the antimutagenic/anticarcinogenic effects of this plant. Further studies on the effects of this fruit extract on human hepatic CYPs and human clinical blood chemistry were suggested. Mechanism of CYP1A1 inhibition by the extract should be further clarified. Effect of *M. citrifolia* fruit extract on Phase II enzymes that might contribute to the cancer preventive effect of this plant should also be investigated.