CHAPTER V

DISCUSSION AND CONCLUSION

Results from this study showed that rats given *H. sabdariffa* aqueous extract at doses of 250 and 1,000 mg/kg/day for 30 days did not modify the activity of CYP 1A1, 1A2, 2B1/2, 2E1 and 3A. Since these CYP isoforms play a significant role in chemical bioactivation, no induction effects on these CYPs suggested beneficial characteristics of *H. sabdariffa* in the aspect of no potential to increase risks of chemical-induced toxicities, mutagenicities and/or carcinogenicities. Procarcinogens that are bioactivated by CYP 1A1 include environmental polycyclic aromatic hydrocarbon (PAHs), such as benzo(a)pyrene, 7, 12-dimethylbenz(a) anthracene, 6-nitrochrysene, etc.; by CYP 1A2 include 2-acetylaminofluorene, 2-aminofluorene, aflatoxin B_1, 2-aminoanthracene, 2-naphthylamine, etc.; by CYP 2B1/2 include aflatoxin B_1, benzo(a)pyrene, 3-methylcholanthrene, etc.; by CYP 2E1 include acrylonitrile, benzene, carbon tetrachloride, chloroform, trichloroethylene, etc., and by CYP 3A include aflatoxin B_1, aflatoxin G_1, benzo(a)pyrene, 6-aminochrysene, etc. (Soucek, P., and Gut, I., 1992). Moreover no modulation of these CYP isoforms which are normally responsible for metabolisms of many therapeutic drugs, would be an advantageous of *H. sabdariffa* in term of drug-drug interaction if this plant extract is concomitantly administered with the interacting drugs. The examples of drugs that are metabolized by CYP 1A1 are R-warfarin, amiodarone, etc.; by CYP 1A2 are acetaminophen, amitriptyline, theophylline, etc.; by CYP 2B1&2B2 are phenobarbital, pentobarbital, etc.; by CYP 2E1 are acetaminophen, chlorzoxazone, etc., and by CYP 3A are erythromycin, terfenadine, omeprazole, etc. (Parkinson, A., 2001; Rendic, S., and Di Carlo, F.J., 1997). *H. sabdariffa* was reported to have antimutagenic effect *in vitro* against PhIP and other heterocyclic amines as well as *in vivo* against ACF formation which was induced by AOM and PhIP in colon of F344 rats (Chewonarin, T., *et al.*, 1999). PhIP, heterocyclic amine and AOM are bioactivated by CYP 1A2 and CYP 2E1, respectively (Gonzalez, F.J. and Gelboin, H.V., 1994; Ioannides, C., 1996). No inhibitory effects of *H. sabdariffa* aqueous extract on CYP 1A2 and CYP 2E1 found in this study, excluded the possibilities of using this effect to explain the antimutagenic effects of this plant against those procarcinogens reported earlier. Effect of this

extract on phase II detoxification enzymes which may explain these antimutagenic effects is interesting to be further investigated.

The hematological and clinical blood chemistry data obtained from this study provided a preliminary data for subacute toxicity of *H. sabdariffa*. This is the first report of subacute toxicity study of *H. sabdariffa* aqueous extract in rats. Orisakwe, O.E. and collaborates (2003, 2004) performed an acute toxicity study and subchronic toxicity study. Regarding the subchronic study, they administered *H. sabdariffa* aqueous extract to rats for 3 months at the doses of 1.15, 2.30 and 4.60 g/kg/day. All of doses were higher and the duration of treatment were much longer than this study. Therefore, it would not be surprised that they found toxic effect of the extract such as the toxic effects on kidney and male reproductive organs while this study (administration the extract at 0.25 and 1.00 g/kg/day for 30 days to rats) did not find any toxic effects on kidney and several important organs/systems despite without an investigation of the male reproductive system. Difference between these two studies indicated that *H. sabdariffa* aqueous extract was quite safe if administered at appropriate doses and for limited duration of times. Therefore, extrapolation this data to human provided a precaution that consuming at doses higher than 1 g/kg/day or administration for long period of time should be avoided. In this study, effect of *H. sabdariffa* aqueous extract on lipid metabolism was quite different from the study of Hirunpanich, V (2001). In the study of Hirunpanich, V (2001), they found that *H. sabdariffa* aqueous extract at doses of 500 and 1,000 mg/kg/day given to male hypercholesterolemic SD rats for 6 weeks, could reduce serum cholesterol, TG and LDL-C. In contrast, effects of *H. sabdariffa* aqueous extract on these lipid parameters were not found in this study. Lipid-lowering effect of *H. sabdariffa* aqueous extract might exhibit only in the hypercholesterolemic condition or the different result might be due to the different duration of treatment (6 weeks vs 4 weeks), the different of strain of rats (SD rats vs Wistar rats) or some other reasons.

In this study, dried calyx of *H. sabdariffa* was extracted with hot water. The solution was then evaporated, freeze-dried and the dried residue was ground to fine powder which was redissolved with water before administration orally to the animals. This aqueous extract fraction of *H. sabdariffa* is closely similar to the way that used traditionally for antihypertensive and antilipidimic effects and similar to the way that people prepare *H. sabdariffa* for using as daily

beverage. Generally, the daily dose recommended of dried calyx of *H. sabdariffa* for humans is approximately 9 g/day (พร้อมจิต ศรลัมพ์, 2532; สุนทรี สิงหบุตรา, 2540; Boonyapraphastsara, N., 1987). As compared to the dosage used in humans, doses of 250 and 1,000 mg/kg/day used in this study were estimated to be 1.95 times and 7.81 times, respectively, of the doses recommended for humans. A criteria for choosing the dosage regimen of the extract used in this experiment was using the doses that possess pharmacological effects without severe toxic effects and administration by the practical route. Doses of *H. sabdariffa* aqueous extract used in this study were 250 and 1,000 mg/kg/day. The former dosage regimen was found to possess antihypertensive effect in SD rats (Odigie, I.P., *et al.*, 2003). This dose was also shown to exhibit gastroprotective effect against indomethacin, ethanol and water immersion restraint stress (Rujjanawate, C., *et al.*, 2000). The dosage of 1,000 mg/kg/day was shown to demonstrate lipid-lowering effect in hypercholesterolemic rats (Hirunpanich, V., 2001). Also, these two doses of *H. sabdariffa* aqueous extract have not been reported to possess severe toxic effects in rats.

H. sabdariffa aqueous extract used in this study was quantitated for total phenolic compounds. In this study, *H. sabdariffa* aqueous extract contained total phenolic compounds 3.874 % w/w of the extract (0.958 % w/w of the dried calyx of *H. sabdariffa*). The study of Duh, P.D., and Yen, G.C. (1997) found that the percentage of total phenolic compounds in dried calyx of *H. sabdariffa* was 1.44 % w/w while the percentage of total phenolic compounds in dried calyx of *H. sabdariffa* found in this study was 0.958% w/w. Somewhat difference of the total phenolic compounds in dried calyx of *H. sabdariffa* between studies could be explained by the different extraction procedure and different source of the calyx of *H. sabdariffa*.

H. sabdariffa aqueous extract was given to rats for 30 days, the duration of which was sufficiently enough to induce CYPs. At the same time, subacute toxicity data could also be obtained from hematological and clinical blood chemistry parameters. The model used for studying effects of *H. sabdariffa* aqueous extract on hepatic CYP in this study was the *in vivo* model but detecting the activities of CYP *in vitro* so taken together, we may call as the *ex vivo* model. The advantage of using this model was that we could investigate both enzymes induction and inhibition effects at the same time, limitation in this model was that we could detect only

irreversible inhibition mechanism. In addition, the microsomal fraction isolating from rat liver can be stored at -80 °C for quite long period of time (about 1 year) (Lake, B.G., 1987).

In conclusion, subacute effects of *H. sabdariffa* aqueous extract on hepatic CYPs and clinical blood chemistry were studied in male Wistar rats. Two doses (250 and 1,000 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 *H. sabdariffa* aqueous extract caused no significant effect on total CYP contents and the activities of CYP 1A1, 1A2, 2B1/2, 2E1 and 3A. *H. sabdariffa* aqueous extract did not change clinical blood chemistry and hematology. These results suggested that no drug-drug interactions and the possibility to increase risks of chemical-induced toxicity, mutagenicity and/or carcinogenicity from the compounds that are metabolized or bioactivated by the CYP isoforms investigated in this study if they are given concomitantly with *H. sabdariffa* aqueous extract. In addition, *H. sabdariffa* aqueous extract at both doses used in this study caused no harmful effects on several important organs/systems such as liver, kidney, blood system, electrolytes as well as lipid and carbohydrate metabolisms. Further studies on the effects of this extract on human hepatic CYPs, hepatic phase II enzymes and human clinical blood chemistry were suggested.