CHAPTER I

INTRODUCTION

Hibiscus sabdariffa Linn. is a medicinal plant in family Malvaceae. It is natively grown in tropical Africa but today grows throughout many tropical climates. It has been called in Mexico as Jamaica roselle, in Switzerland as Karkade roselle, and in England as Roselle or Jamican roselle. It is also widely cultivated in Egypt, Sudan, and Thailand. This plant has been known in Thailand as Krachiap, Krachiap-daeng, Krachiap-prieo (Central), Phak-Kheng-Kheng (North), Som-poo (Mae-Hong Son), Som-por-dee (Northeast) (Boonyapraphastsara, N., 1987; Farnsworth, N.R., and Bunyapraphatsara, N., 1992; Marderosian, A.D., and Beutler, J.A., 2002).

Used part of *H. sabdariffa* is dried red calyx. It is claimed as a Thai traditional medicine used as diuretic, hypocholesterolemic agent, antihypertensive agent, mucolytic agent, intestinal peristalsis stimulation, bile acid secretion enhancement, mild laxative and as refreshing beverages (พร้อมจิต ศรลัมพ์, 2532; สุนทรี สิงหบุตรา, 2540; Boonyapraphastsara, N., 1987).

H. sabdariffa contains many chemical constituents such as *organic acids*: citric acid, hydroxycitric acid lactone (hibiscus acid), malic acid, tartaric acid; *phenolic compounds*: protocatechuic acid (PCA); *flavonoid derivatives*: gossypetin-3-glucoside, gossypetin-8-glucoside, gossypetin-7-glucoside, quercetin; *anthocyanins*: hibiscin, gossypetin (3, 5, 7, 8, 3, 4-hexahydroxy flavone), cyanidin-3-ß-D-glucoside, cyanidin-3, 5-diglucoside, cyanidin-3-monoglucoside, cyanidin-3-sambubioside, sabdaretin, hibiscetin, delphinidin, delphinidin-3-*O*-ß-D-glucoside, delphinidin-3-sambubioside (สุนทรี สิงหบุตรา, 2540; Boonyapraphastsara, N., 1987; Farnsworth, N.R. and Bunyapraphatsara, N., 1992; Marderosian, A.D. and Beutler, J.A., 2002; Subramanian, S.S. and Nair, A.G.R., 1971; Wang, C.J., *et al.* 2000).

Pharmacological effects: *H. sabdariffa* aqueous extract was shown to possess antihypertensive effect when the extract was given at 250 mg/kg/day, p.o. for 8 weeks to hypertensive rats (Odigie, I.P., *et al.*, 2003). Single intravenous administration of the extract at 62.5, 125 and 250 mg/kg caused hypotension and bradycardia in normotensive rats (Tiamjan, R., 1999). Lipid-lowering effects of the dried calyx aqueous extract of *H. sabdariffa* was shown at the doses of 500 and 1,000 mg/kg/day given orally for 6 weeks to hypercholesterolemic rats. The effects were shown by a decrease of serum cholesterol, triglycerides and low density

lipoprotein-cholesterol (Hirunpanich, V., 2001). The same results were also found in hypercholesterolemic rabbits giving the extract at 0.5 and 1.0 % in the high cholesterol diet for 10 weeks (Chen, C.C., *et al.*, 2003). *Hibiscus* anthocyanins was also shown to possess antioxidative effects *in vitro* using the model of *tert*-butyl hydroperoxide (*t*-BHP) induced cytotoxicity in rat primary hepatocytes at the concentrations of 0.1 and 0.2 mg/ml and *in vivo* study using *t*-BHP induced hepatotoxicity in rats at the dosages of 100 and 200 mg/kg/day, p.o. for 5 days (Wang, C.J., *et al.*, 2000). Neurological effects of *H. sabdariffa* aqueous extract was shown in mice given single intraperitoneal administration of the extract at 100, 200, and 400 mg/kg, resulting in a dose-dependent decrease of spontaneous motor activity and a prolonged barbiturate sleeping time (Amos, S., *et al.*, 2003). Ethanolic extract of *H. sabdariffa* was shown to possess antimutagenic and chemopreventive effects both *in vitro* and *in vivo* (Chewonarin, T., *et al.*, 1999).

Toxicological effects: Medium lethal dose (LD_{50}) of *H. sabdariffa* aqueous extract is 5 g/kg. Subchronic toxicity study of the extract was performed in rats given the extract at doses of 1.15, 2.30, 4.60 g/kg for 12 weeks. Major toxicity was found in kidney as shown with tubular necrosis as well as in the reproductive system of male rats (Orisakwe, O.E., *et al.*, 2003 and 2004).

Since calyx of *H. sabdariffa* has been widely used as a daily beverage, repeated exposure is potentially occurred. Repeated exposure to any xenobiotics often leads to modulation of hepatic drug metabolizing enzymes. Recently, *H. sabdariffa* is chosen as one of the herbal medicine of interest to be develop into national champion products of Thailand due to its well-reported pharmacological actions. However, effects of *H. sabdariffa* on hepatic drug metabolizing enzymes have never been investigated. Therefore, this study was performed to investigate subacute (30 days) effects of *H. sabdariffa* aqueous extract on some isoform of CYPs involved in drug metabolism and mutagenic/carcinogenic bioactivation such as CYP 1A1, 1A2, 2B1/2, 2E1 and 3A using an *ex vivo* study in rats. Results from this experiment would be a beneficial information for prevention of drug-drug interactions. In addition, modulation of these bioactivating CYP isoforms would provide an information for *H. sabdariffa* to potentially decrease and/or increase xenobiotic-induced toxicity, mutagenesis and/or carcinogenesis. Inhibition of CYPs may partly give an explanation for the chemopreventive effect of this extract.

In contrast, if *H. sabdariffa* aqueous extract induces these CYPs, chronic exposure to *H. sabdariffa* may increase risks of xenobiotic-induced toxicity, mutagenesis and/or carcinogenesis. Moreover, effects of *H. sabdariffa* aqueous extract on clinical blood chemistry and hematology were also determined so as to primarily investigate subacute toxicity of this extract on important organs/systems such as liver, kidney, electrolytes as well as carbohydrate and lipid metabolisms.

Hypothesis

Subacute exposure of *H. sabdariffa* aqueous extract caused an induction and/or inhibition on hepatic microsomal CYPs as well as changes of hematology, clinical blood chemistry in rats.

Anticipated benefit from the study

Results from this study would be a preliminary data whether subacute exposure of *H. sabdariffa* aqueous extract induces and/or inhibits CYP isoforms involving in drug metabolism and in bioactivation reactions of various drugs, chemicals as well as environmental toxicants. This would be useful for considering the possibility of drug-drug interactions if this plant extract is taken simultaneously with other medicines. In addition, this would be useful to estimate the possibility of *H. sabdariffa* aqueous extract to increase and/or decrease risks of chemical induced toxicities, mutagenicities and/or carcinogenicities. Moreover, effects of *H. sabdariffa* aqueous extract on clinical blood chemistry and hematology would provide a preliminary subacute toxicity data of this plant extract in rats.

Study design and process

1. Preparation of *H. sabdariffa* aqueous extract and chemical identification tests
2. An *ex vivo* study
    - 2.1 Animal treatment for 30 days
    - 2.2 Blood collecting
    - 2.3 Determination of clinical blood chemistry and hematology
    - 2.4 Preparation of liver microsomes
    - 2.5 Determination of microsomal protein concentrations, total CYP contents and CYP activities

3. Data collecting and analysis

4. Writing a thesis