CHAPTER I

INTRODUCTION

Morinda citrifolia Linn., called in Thai as "Yor" is a medicinal plant in Rubiaceae family and commonly found in certain tropical countries. The Morinda plant has many different names in different countries such as "Yor-bann" or "Mataasuea" in Thailand, "Indian Mulberry" in India, "Ba-ji-tian" in China, "Nono" in Tahiti and "Noni" in Hawaii etc. (Wang and Su, 2001). Various parts of this plant have been in traditional medicine. Root can be for treatment of constipation. Leave is recognized as a medication for relieving diarrhea, fever, flatulence, chronic wound and arthritis. Fruit may be accounted for preventing flatulence, treating gingivitis, relieving sore throat, emmenagogue. Seed can be used as a laxative agent while bark as an astringent or antipyretic etc. (มงคล แก้วเทพ, 2544). Its fruit is recommended as an antiemetic agent for the primary health care in Thai traditonal medicine (รุจินาถ อรรถสิษฐและคณะ, 2530). Fruit comprises the following constituents: asperuloside (Inouye *et al.*, 1988) which is one of monoterpene substances; β - carotenes (Aalbersberg *et al.*, 1993) and polysaccharides (Hirazumi and Furusawa, 1999). In addition, volatile agents that are generally found in ripe fruits are carboxylic acids such as octanoic acid, hexanoic acid and decanoic acid (มงคล แก้วเทพ, 2544).

Pharmacological investigations demonstrated that fruit of *M. citrifolia* exerted anti-emetic action in clinical trial (วิชัย เอกพลากรและคณะ, 2530). In an animal studies, *M. citrifolia* exhibited anti-tumor/anticancer and immunomodulator activities (Hirazumi *et al.*, 1994; Hirazumi *et al.*, 1996; Hirazumi, 1997; Hirazumi and Furusawa, 1999; Wang and Su, 2001). Ethanol-precipitated fraction (EtOH-ppt) of *M. citrifolia* fruit exerted anti-tumor activity against Sarcoma 180 in mice following an intraperitoneally injection at a dosage of 500 mg/kg 24 hours after an inoculation of the tumor (Hirazumi *et al.*, 1996).

Likewise, anti-tumor activity against Lewis lung carcinoma (LLC) was also indicated when 15 mg of the EtOH-ppt was injected intraperitoneally to mice for 4-5 days. The EtOH-ppt, which contained a polysaccharide-rich substance, possessed an anti-tumor activity while the ethanol soluble did not. Concomitant administration

the EtOH-ppt fraction with immunosuppressive agent, 2-chloroadenosine (macrophage inhibitor) or cyclosporine (T-lymphocyte inhibitor) to mice resulted in diminishing the anti-tumor activity, thereby substantiating an immunomodulatory mechanism (Hirazumi *et al.*, 1994). Subsequently, Hirazumi and Furusawa (1999) found that the EtOH-ppt could induce several cytokines that were cytotoxic to tumor cells. Those cytokines included tumor necrosis factor-α (TNF- α), interleukin1β (IL-1β), IL-12p70, IL-10 and interferon-γ (IFN-γ) as well as nitric oxide (NO). Wang and Su (2001) found that *M.citrifolia* fruit juice was able to significantly reduce the 7,12-dimethylbenz(a)anthracene (DMBA)-DNA adduct formation in rats and mice *in vivo*. And an *in vitro* study, they found that *M. citrifolia* fruit juice possessed strong antioxidant activities, the characteristic that may contribute to the cancer preventive effect of this plant.

So far, the influence of *M.citrifolia* fruit extract on hepatic cytochrome P450 (CYP) has never been investigated, especially CYP isoforms that play an important role in chemical-induced toxicity, mutagenesis and/or carcinogenesis. Such isoforms of CYP that bioactivate various xenobiotic compounds to toxic metabolites, mutagens and/or carcinogens include CYPs1A1, 1A2, 2B1, 2B2, 2E1, 3A4 etc. (Soucek and Gut, 1992).

Modulation of these isoforms of CYP by any compounds would provide a beneficial mechanistic explanation for a potential decrease and/or increase risk of these compounds on chemical-induced toxicity, mutagenicity and/or carcinogenicity. Inhibition effects of *M. citrifolia*, if existing, on these CYPs would partly explain the chemopreventive effects of this plant reported previously. In contrast, if *M. citrifolia* fruits possessed induction effects on any of these CYP isoforms, consuming of these fruits for a long period of time should be concerned.

A few data have been reported regarding the toxicity study of *M. citrifolia* (Nakanishi *et al.*, 1965; Dhawan *et al.*, 1977 and Younos *et al.*, 1990). Some acute and subchronic toxicity tests were performed on various parts of this plant, such as root, (Younos, Rolland and Fleurentin *et al.*, 1990), leaves (Nakanishi, Sasaki and Kiang *et al.*, 1965), and over-ground parts (Dhawan *et al.*, 1977 and Nakanishi *et al.*, 1965). There is no toxicity data of *M. citrifolia* fruit. Only one case study was reported

regarding a patient with chronic renal insufficiency and self-medicated with an alternative medicinal product known as *M. citrifolia* juice, presented to the clinic with hyperkalemia despite having low-potassium diet (Mueller *et al.*, 2000).

Therefore, the objectives of this study were to investigate subacute effects of *M. citrifolia* fruit extract on some CYP isoforms involving in metabolic activation of chemical mutagen and/or carcinogens such as CYP1A1, CYP1A2, CYP2B1, CYP2B2, CYP2E1 and CYP3A using an *ex vivo* study in rats. Moreover, effects of *M. citrifolia* fruit extract on several clinical blood chemistry were also determined so as to investigate the effects of this fruit on several important organs/systems such as liver, kidney, blood system, electrolytes as well as lipid and carbohydrate metabolisms.

Hypothesis

Subacute exposure of *M. citrifolia* fruit extract causes an induction and/or inhibition of hepatic microsomal CYP as well as changes in clinical blood chemistry.

Study design and process

1. Preparation and identification of *M. citrifolia* extract
2. Animal treatment
3. Blood sampling
4. Determination of clinical blood chemistry and hematology
5. Preparation of liver microsomes
6. Determination of total CYP and CYP activities
7. Data analysis and thesis writing

Benefit gained from the study

Results from this study would be a preliminary data of whether subacute exposure of *M. citrifolia* fruit extract induces and/or inhibits CYP isoforms involving in various bioactivating reactions of drugs, chemicals as well as environmental toxicants. This would be useful to estimate the possibility of *M.citrifolia* fruit to increase and/or decrease risks of chemical-induced toxicities, mutagenicities and/or carcinogenicities. In addition, effects of *M. citrifolia* fruit on clinical blood chemistry would be a preliminary subacute toxicity data of this plant in rats.