CHAPTER I

INTRODUCTION

Murdannia loriformis (Hassk.) Rolla Rao et Kammathy commonly called in Thai as "Ya Pak King" or "Angel Grass", is a medicinal plant in family Commelinaceae. *M. loriformis* is originally grown in south Chinese. Ancient Chinese have used *M. loriformis* as a remedy for throat infections, colds and bronchitis. In Thailand, *M. loriformis* is used as a traditional medicine for cancers of several origins. Some patients use *M. loriformis* combined with other chemotherapy for relief side effects of drug. *M. loriformis* is widely used for treatment of many kinds of cancers such as lung, stomach, uterus, liver, white blood cell, intestine, urinary bladder and bone, etc. About 100-120 g of fresh aerial parts of *M. loriformis* are ground with 4 tablespoons of water and the obtained juice is filtered. Patients are suggested to take the filtrate orally twice a day, before breakfast and evening meal (วีณา จิรัจฉริยากูล, 2542).

Chemical constituents found in *M. loriformis* include amino acids: L-phenylalanine; phenolic compounds: syringic acid; flavonoids: isovitexin, chalconoids; phytosterols: β-sitosterol, sitosteryl glucoside (3-β-D-glucopyranosyl-24ξ-ethyl-cholest-5-ene); glycosphingolipids: 1-β-O-D-glucopyranosyl-2-(2′-hydroxy-6′-cosamide)-sphingosine; and digalactosyl diglyceride (วีณา จิรัจฉริยากูล, 2536; Jiratchariyakul W., 1997; Narintorn A., 1999).

Toxicological effects: Acute toxicity study was performed using fresh juice of *M. loriformis* at doses of 5, 10, 20 and 30 g/250 g body weight, given orally once to rats. No abnormal symptoms were observed during 24 hours through 14 days after feeding. Clinical blood chemistry and histopathological findings of lungs, kidneys, spleens, livers and testis/ovaries were also within normal limits (พิมลวรรณ ทัพยุทธพิจารณ์ และคณะ, 2533). Subchronic toxicity was investigated using fresh juice of *M. loriformis* at doses of 0.7, 1.75, 3.5 g/250 g body weight, given orally to rats every day for 3 months. No abnormal symptoms were observed. Clinical blood chemistry and histopathological findings of lungs, kidneys, spleens, livers and testis/ovaries were also within normal limits (พิมลวรรณ ทัพยุทธพิจารณ์ และคณะ, 2534).

Pharmacological effects: *M. loriformis* extract was studied for anticarcinogenic effect. It was shown to contain glycosphingolipids (1-β-O-D-glucopyranosyl-2-(2´-hydroxy-6´-cosamide)-sphingosine) which possessed moderate cytotoxic activity (ED_{50} less than 10 μg/ml) against human colon carcinoma (SW 620) and human breast cancer (BT 474) cell lines *in vitro* (Jiratchariyakul *et al.*, 1998). In contrast, a study on anti-proliferative and cytotoxic on promyelocytic leukemia (HL 60), T-cell leukemia (Molt 4), B-cell leukemia (Daudi), monocytic leukemia and erythro-leukemia (K562), water and 80% ethanol extract of *M. loriformis* did not demonstrated any antiproliferative and cytotoxic effects on all types of leukemic cell lines tested (สาคร พรประเสริฐ, 2544). One previous study found that ethanolic extract of *M. loriformis* inhibited mutagenesis induced by alfatoxin B_1 (AFB_1), 2-amino-6-methyldipyrido[1,2-a:3',2'-*d*]imidazole (Glu-P-1), 2-aminodipyrido[1,2-*a*:3',2'-*d*]imidazole (Glu-P-2), 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole (Trp-P-1) and 3-amino-1-methyl-5H-pyrido[4,3-b]indole (Trp-P-2) *in vitro* as determined by Ames' test. This study also found that the extract induced DT-diaphorase activity in murine hepatoma cell lines (Vinitketkumnuen *et al.*, 1996). DT-diaphorase is an enzyme responsible for detoxification of a number of natural and synthetic compounds, including quinones and their derivatives. Moreover, DT-diaphorase protects cells against oxidative stress (Lind *et al.*, 1990). Regarding the chemopreventive effect, Intiyot *et al.*, (2002) found that ethanol extract of *M. loriformis* at doses of 0.1-1.0 g/kg significantly inhibited azoxymethane induced aberrant crypt focus formation both in the initiation stage (21-51%) and post-initiation stage (12-27%) in rat colon. So far, the mechanism of anticarcinogenic and/or chemopreventive effects of *M. loriformis* has not yet been clarified. Antimutagenic effect of the plant extract may be one of the explanation for these effect (Ramel *et al.*, 1986; Weinstein, 1991; Ferguson, 1994). Several mutagens and/or carcinogens require metabolic activation prior to initiate mutagenesis and/or carcinogenesis (Gonzalez and Gelboin, 1994). Subsequently, detoxification take place to eliminate toxic intermediate from the body. Cytochrome P450 (CYP) is an important enzyme of phase I drug metabolism. CYP isoforms in family 1, 2, and 3 play an important role in bioactivation of various xenobiotic compounds to toxic metabolites, mutagens, and/or carcinogens (Soucek and Gut, 1992). In this study,

effects of *M. loriformis* ethanolic extract on some isoforms of CYPs involved in mutagenic/carcinogenic bioactivation such as CYP 1A1, 1A2, 2B1/2, 2E1 and 3A were investigated by using *ex vivo* rat model. Inhibition of CYPs may partly give an explanation for the chemopreventive effect of this extract. In contrast, if *M. loriformis* ethanolic extract induces on CYPs, chronic exposure to *M. loriformis* may increase risks of xenobiotic-induced carcinogenesis. In addition, *M. loriformis* is currently used as a traditional medicine in patients of different types of cancers simultaneously with other chemotherapy (สุภาภรณ์ ปิติพรและสุดใจ พรหมเกิด, 2545). Results from this experiment would be a beneficial information for prevention of drug-drug interactions. Moreover, effects of *M. loriformis* ethanolic extract on clinical blood chemistry were also determined so as to primarily investigate subacute toxicity of this extract on important organs/systems such as liver, kidney, blood system, electrolytes as well as carbohydrate and lipid metabolism.

Hypothesis

Subacute exposure of *M. loriformis* ethanolic extract caused an induction and/or inhibition on hepatic microsomal CYPs as well as changes of clinical blood chemistry in rats.

Anticipated benefit from the study

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

Study design and process

1. Preparation of *M. loriformis* ethanolic extract and chemical identification tests
2. An *ex vivo* study

2.1 Animal dosing 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